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Application Number: 14555102 Document Date: 11/26/2014

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CYTEK V. BECKMAN
IPR2025-01319
BECKMAN 2015

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Yong Qin CHEN

Serial No.: not yet assigned

Filed: November 26, 2014

For: FLOW CYTOMETER

Group Art Unit: not yet assigned

Examiner: not yet assigned

Atty. Dkt. No.: BECC.P0002US

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

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11/26/14
Date

Gina N. Shishima

PRELIMINARY AMENDMENT

Commissioner:

Applicant respectfully submits this Preliminary Amendment in the above-referenced case. Consideration of this case in view of the amendments made herein is respectfully requested.

Amendments to the Claims being on page 2.

Remarks begin on page 7.

AMENDMENT

AMENDMENT TO THE CLAIMS

The listing of claims will replace all prior versions, and listing of claims in the application.

LISTING OF CLAIMS

1-363. (Canceled)

364. (New) A flow cytometer having a wavelength division multiplexer (WDM), the WDM comprising:

an extended light source providing light that forms an object;

a collimating optical element that captures light from the extended light source and projects a magnified image of the object as a first light beam; and

a first focusing optical element configured to focus the first light beam to a size smaller than the object of the extended light source to a first semiconductor detector.

365. (New) The flow cytometer of claim 364, wherein the first focusing optical element is configured to focus the first light beam to a size smaller than 1 mm in diameter.

366. (New) The flow cytometer of claim 364, further comprising an image relay optical element arranged to receive a color band of interest of the first light beam, the image relay optical element configured to project a second image as a second light beam, wherein the second light beam is substantially a unit magnification of the first light beam.

367. (New) The flow cytometer of claim 366, wherein the image relay optical element is one of a refractive optical device and a concave mirror.

368. (New) The flow cytometer of claim 366, wherein the image relay optical element is a concave mirror having a radius of curvature approximately equal to the distance between the collimating optical element and the magnified image projected thereby.

369. (New) The flow cytometer of claim 366, further comprising a second focusing optical element configured to focus the second light beam to a size smaller than the object of the extended light source to a second semiconductor detector.

370. (New) The flow cytometer of claim 369, wherein the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the image relay optical element and the second focusing optical element.

371. (New) The flow cytometer of claim 366, wherein the image relay optical element is a concave shaped dichroic filter.

372. (New) The flow cytometer of claim 366, further comprising a dichroic filter disposed between the collimating optical element and the first focusing optical element.

373. (New) The flow cytometer of claim 372, wherein the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the collimating optical element, the dichroic filter, and the image relay optical element.

374. (New) The flow cytometer of claim 372, wherein the dichroic filter is bonded to a filter holder such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder.

375. (New) The flow cytometer of claim 374, wherein the reference surface of the filter holder rests against an optically flat surface of a reference block included in the WDM, thereby providing consistent optical alignment when installing the dichroic filter into the WDM.

376. (New) The flow cytometer of claim 364, wherein the object is formed by one of light passing through a pinhole and light emitted from a facet of a multimode optical fiber.

377. (New) The flow cytometer of claim 364, wherein the first light beam focused by the first focusing optical element is approximately the same size as the effective size of the collimating optical element.

378. (New) The flow cytometer of claim 364, wherein the size of the magnified image of the object is less than approximately ten times the size of the object of the extended light source.

379. (New) The flow cytometer of claim 364, further comprising a composite microscope objective to direct light emitted by a particle in a flow channel in a viewing zone of the composite microscope to the extended light source, the particle illuminated by an illumination light, the composite microscope objective including,

a spherical mirror located on a first side of the viewing zone, the spherical mirror configured to reflect the emitted light, and,

an aberration corrector plate located on a second side of the viewing zone opposite the first side, the aberration corrector plate configured to reduce optical aberrations in the reflected light caused by the spherical mirror.

380. (New) The flow cytometer of claim 379, further comprising a fluidic system configured to supply liquid sheath to the flow channel, the fluidic system having a T-coupling arrangement configured to receive the liquid sheath from a reservoir and to direct a first fraction of the liquid sheath back to the reservoir via a bypass conduit and a second fraction of the liquid sheath to the flow channel.

381. (New) The flow cytometer of claim 379, further comprising a peristaltic pump configured to supply liquid sample to the flow channel, the peristaltic pump including a pump housing having a arcuate curved track formed therein, a plurality of rollers, and a compressible tube sandwiched between the rollers and the arcuate curved track, the arcuate curved track further including at least one recess section located between at least two pumping sections along the arcuate curved track.

382. (New) The flow cytometer of claim 379, further comprising a beam compressing optical element configured to compress the illumination light along an axis perpendicular to the direction of the flow channel.

383. (New) A flow cytometer comprising:

a viewing zone where a particle in a flow stream is illuminated by light; and

a composite microscope objective including,

a concave mirror configured to gather light scattered from or fluoresced by the illuminated particle and to reflect the light back towards the viewing zone, and

an aberration corrector plate configured to reduce optical aberrations in the reflected light caused by the concave mirror.

384. (New) A flow cytometer having a fluidic system, the fluidic system comprising:

a liquid pump for supplying liquid drawn from a reservoir; and

a T-coupling having at least one inlet and two outlets,

the inlet of the T-coupling receiving the liquid from the liquid pump;

a first fraction of the liquid received by the inlet flowing via a first one of the outlets and via a bypass conduit back to the reservoir; and

a second fraction of the liquid received by the inlet flowing via a second one of the outlets and via a particle filter to the outlet of the fluidic system.

385. (New) A flow cytometer having a peristaltic pump, the peristaltic pump comprising:

a pump housing having an arcuate curved track formed therein that extends between a pump inlet and a pump outlet;

a plurality of rollers that are attached to a rotor, the rollers having a substantially equal angular spacing between each pair of immediately adjacent rollers, the rotor being rotatable together with the rollers attached thereto inside the pump housing; and

a compressible tube sandwiched between the rollers and the arcuate curved track of the pump housing, the arcuate curved track including,

an exit section wherein, as one of the plurality of rollers rolls through the exit section, the compressible tube adjacent to the roller progressively expands from fully closed at a beginning of the exit section to fully open at the pump outlet where the roller breaks contact with the compressible tube;

at least two pumping sections along the arcuate curved track between the pump inlet and the pump outlet wherein the compressible tube is compressed to fully closed by at least one of the plurality of rollers; and

a recess section located between the at least two pumping sections, wherein the compressible tube at the recess section is not fully closed.

386. (New) A flow cytometer having a laser diode (LD) system, the LD system comprising:

a LD for emitting a diverging beam of light from an edge thereof, the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis;

a collimating lens for converting the diverging beam of light emitted from the LD into a collimated elliptical beam of light, wherein the minor axis of the collimated elliptical beam of light is oriented parallel to a direction in which particles pass through a viewing zone;

a beam compressing optical element for reducing the size of the elliptical beam of light at the viewing zone whereby a width of the elliptical beam of light oriented perpendicular to the direction in which the particles pass through the viewing zone is less than a width of a liquid sheath flow; and

a cylindrical focusing element positioned adjacent to the viewing zone with an axis of the cylindrical focusing element being oriented perpendicular to the direction in which the particles pass through the viewing zone whereby the minor axis of the elliptical beam of light becomes focused at the viewing zone; and the size of the major axis of the elliptical beam of light at the viewing zone remains essentially unchanged.

REMARKS

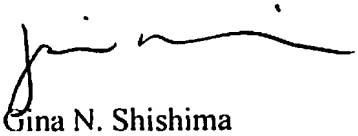
The claims have been amended without prejudice or disclaimer to manage the claims fees. Claims 1-363 have been canceled. Claims 364-386 have been newly added. Claims 364-386 are pending. Applicant retains the right pursue the subject matter as originally claimed at a later stage in this application or in future applications.

The filing fee has been calculated after amendment of the claims by the preliminary amendment. No new matter is added by entry of this preliminary amendment.

It is believed that no fee is due with this communication, however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed document, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski Deposit Account No. 50-1212/DANA.P0002US.

The Examiner is invited to contact the undersigned attorney with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



Gina N. Shishima
Reg. No. 45,104
Attorney for Applicant

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Date: 11/24/14

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Filer:	Gina N. Shishima/Ben Horton
Attorney Docket Number:	BECC.P0002US

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Pages:				
Utility Appl Size fee per 50 sheets >100	1081	1	400	400
Claims:				
Claims in Excess of 20	1202	3	80	240
Independent claims in excess of 3	1201	2	420	840

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous-Filing:				
Late Filing Fee for Oath or Declaration	1051	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**3220**

Electronic Acknowledgement Receipt

EFS ID:	20814146
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	32425
Filer:	Gina N. Shishima/Ben Horton
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US
Receipt Date:	26-NOV-2014
Filing Date:	
Time Stamp:	16:33:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$3220
RAM confirmation Number	5214
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Application Data Sheet	BECC002US_ADS.pdf	1566112 6632f8dca01dfbb656a30a2244fdda7e7fc2041f	no	7
Warnings:					
Information:					
2		BECC002US_SPEC.pdf	503181 5a1509b3e22085c17e748d3cd3ebc2af84e3f6c3	yes	138
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Specification		1	91	
	Claims		92	137	
	Abstract		138	138	
Warnings:					
Information:					
3	Drawings-other than black and white line drawings	BECC002US_FIGS.pdf	528774 9d51624c46c158dcd843a537a94313dc6f37c90a	no	35
Warnings:					
Information:					
4	Preliminary Amendment	BECC002US_PRELIM.pdf	248614 7a4ccfb364e7da08624dbd4f99b3e6fe794f3efa	no	7
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	41304 4c8b2e7215f64f1db5701edfa85725ab95b1ce30	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	2887985		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US
	Application Number	
Title of Invention	FLOW CYTOMETER	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Yong	Qin	Chen	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	San Jose	State/Province	CA	Country of Residence i	US

Mailing Address of Inventor:

Address 1	4097 Cortona Court		
Address 2			
City	San Jose	State/Province	CA
Postal Code	95135	Country i	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	32425		
Email Address	aoipdocket@nortonrosefulbright.com	Add Email	Remove Email

Application Information:

Title of the Invention	FLOW CYTOMETER		
Attorney Docket Number	BECC.P0002US	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	35	Suggested Figure for Publication (if any)	

Filing By Reference :

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US
	Application Number	
Title of Invention	FLOW CYTOMETER	

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	32425		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	61/911859	2013-12-04
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation in part of	PCT/US2013/043453	2013-05-30
Prior Application Status	Expired		Remove

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US
	Application Number	
Title of Invention	FLOW CYTOMETER	

Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/653245	2012-05-30
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/653328	2012-05-30
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/715819	2012-10-18
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/715836	2012-10-19
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/816819	2013-04-29
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.			Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) [i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country [i]	Filing Date (YYYY-MM-DD)	Access Code [i] (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.			Add

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US
	Application Number	
Title of Invention	FLOW CYTOMETER	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US
	Application Number	
Title of Invention	FLOW CYTOMETER	

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

(•) Assignee	( ) Legal Representative under 35 U.S.C. 117	( ) Joint Inventor
( ) Person to whom the inventor is obligated to assign.	( ) Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. [X]

Organization Name	IRIS INTERNATIONAL, INC.		
Mailing Address Information:			
Address 1	9172 Eton Avenue		
Address 2			
City	Chatsworth	**State/Province**	CA
Country [i]	US	Postal Code	91311
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

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Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US
	Application Number	
Title of Invention	FLOW CYTOMETER	

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

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NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications

Signature	/Gina Shishima/			Date (YYYY-MM-DD)	2014-11-26
First Name	Gina	Last Name	Shishima	Registration Number	45104

Additional Signature may be generated within this form by selecting the Add button. Add

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FLOW CYTOMETER

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority of U.S. Provisional Patent Application Serial No. 61/911,859 entitled "Flow Cytometer," filed on December 4, 2013. This application is also a continuation-in-part of International Patent Application Serial No. PCT/US2013/043453 entitled "Flow Cytometer," filed on May 30, 2013, which claims the benefit of priority under 35 U.S.C. 119 to U.S. Provisional Patent Application Serial No. 61/653,245 entitled "Pulseless Peristaltic Pump," filed on May 30, 2012, U.S. Provisional Patent Application Serial No. 61/653,328 entitled "Composite Microscope Objective with a Dispersion Compensation Plate," filed on May 30, 2012, U.S. Provisional Patent Application Serial No. 61/715,819 entitled "Wavelength Division Multiplexing for Extended Light Source," filed on October 18, 2012, U.S. Provisional Patent Application Serial No. 61/715,836 entitled "Diode Laser Based Optical Excitation System," filed on October 19, 2012, and U.S. Provisional Patent Application Serial No. 61/816,819 entitled "A Simple Fluidic System for Supplying Pulsation Free Liquid to Flow Cell," filed on April 29, 2013. All of the above-identified applications are incorporated herein by reference in their entirety.

BACKGROUND

Technical Field

The present disclosure relates generally to the technical field of flow cytometry and, more particularly, to the structure and operation of an improved flow cytometer together with various individual subassemblies included therein.

Background

Flow cytometry is a biophysical technique employed in cell counting, sorting, biomarker detection and protein engineering. In flow cytometry, cells suspended in a stream of liquid pass through an electronic detection apparatus. Flow cytometry allows simultaneous multiparametric analysis of physical and/or chemical characteristics of up to thousands of cells per second.

Flow cytometry has various applications including in the fields of molecular biology, pathology, immunology, plant biology and marine biology. Flow cytometry also has broad application in medicine (especially in transplantation, hematology, tumor immunology and chemotherapy, prenatal diagnosis, genetics and sperm sorting for sex preselection). In marine biology, the autofluorescent properties of photosynthetic plankton can be exploited by flow cytometry in characterizing abundance and community composition. In protein engineering, flow cytometry is used in conjunction with yeast display and bacterial display to identify cell surface-displayed protein variants with desired properties. A common variation of flow cytometry is physically sorting particles based on their properties thereby purifying a population of interest.

SUMMARY OF DISCLOSURE

The present disclosure provides an improved flow cytometer together with various improved components included therein, as well as component groups with interacting components.

In certain embodiments, the present disclosure provides a simple and reliable diode laser based optical system capable of delivering a focused laser beam of elliptical cross section with a Gaussian like intensity distribution along its minor axis and a width along major axis optimized for flow cytometric applications.

In certain embodiments, the present disclosure provides an imaging quality microscope objective that is easy to manufacture and has long working distance, large numerical aperture, large field of view and minimal chromatic aberration.

In certain embodiments, the present disclosure provides a simple fluidics system for flow cytometers that is not only reliable, compact and easy to manufacture, but also capable of supporting velocity critical applications such as in instruments with multiple spatially separated excitation laser beams or in droplet sorters.

In certain embodiments, the present disclosure provides a simple design for a peristaltic pump providing a pulseless liquid flow.

In certain embodiments, the present disclosure provides a peristaltic pump with minimal pulsation.

In certain embodiments, the present disclosure provides a peristaltic pump that is simple to manufacture and operate.

In certain embodiments, the present disclosure provides a device capable of collimating a light beam from an extended light source over an extended distance without significantly expanding the beam diameter.

In certain embodiments, the present disclosure provides a Wavelength Division Multiplexing (WDM) system to separate a light beam into multiple colored bands. The WDM system may be compatible with low noise semiconductor detectors. In addition, due to the diversity of fluorescent probes, the WDM system may be reconfigurable.

According to an exemplary embodiment, a flow cytometer may include:

1. a Laser Diode (LD) based optical subsystem for impinging a beam of light upon particles passing through a viewing zone;

2. a composite microscope objective for gathering and imaging light scattered from or fluoresced by particles passing through the viewing zone;

3. a fluidic subsystem for supplying a liquid sheath flow to the viewing zone;

4. a peristaltic pump for injecting a liquid sample flow carrying particles that pass together with the liquid sheath flow through the viewing zone;

5. a multimode optical fiber that receives scattered and fluoresced light from the viewing zone that the composite microscope objective gathers images; and

6. a wavelength division multiplexer for optically separating light received via the optical fiber into color bands.

According to an exemplary embodiment, the LD based optical subsystem for illuminating particles passing through the flow cytometer's viewing zone may generally include:

1. a laser diode oriented with its slow axis parallel to the direction of flow;

2. a collimating lens that converts the diverging beam from the LD into a collimated beam of elliptical shape with its major axis perpendicular to the flow;

3. a focusing lens system that reduces the laser beam at the viewing zone to an optimal width in the direction perpendicular to the flow; and

4. finally a high power cylindrical focusing element placed in the proximity of the viewing zone with its axis perpendicular to the direction of flow.

The high power cylindrical focusing element may transpose the far field profile of the LD along its slow axis to its Fourier conjugate at the viewing zone along the direction of flow, while maintains the transverse beam profile, such that the laser beam profile at the viewing zone is optimal for flow cytometric applications.

According to an exemplary embodiment, the composite microscope objective may generally include:

1. a concave spherical mirror;

2. a transparent aberration compensation plate with the flow cytometer's a viewing zone being located between the mirror and the plate. Scatter and fluorescence light emitted from particles in the viewing zone is collected by the mirror and reflected back toward the compensation plate. Optical aberrations originating from the mirror are significantly reduced after light passes through the compensation plate. In one embodiment of the present disclosure, the viewing zone may be located inside a flow cell provided by rectangular glass cuvette with a small rectangular channel through which a particle carrying liquid flows. The concave mirror may be made of an optically transparent material, such as glass or optical quality plastics, of plano-convex shape with a highly-reflective coating on the convex side for internal reflection. The plano-side of the mirror may be either gel-coupled or bonded to one side surface of the cuvette. The plano-aspheric compensation plate may be made of a transparent material, such as glass or optical quality plastics, with the plano side gel-coupled or bonded to the opposite side of the cuvette. The plano-convex shaped mirror and the aspheric compensation plate may also be formed integrally with the cuvettte. In yet another embodiment of the present disclosure, the viewing zone may be in a jet stream with both the concave mirror and the compensation plate being free standing from the viewing zone, and the mirror may be a front surface concave mirror.

According to an exemplary embodiment, the fluidic system may generally include a sheath liquid reservoir from which a liquid pump draws sheath liquid. Sheath liquid then

flows from the liquid pump to an inlet of a T-coupling. One outlet arm of the T-coupling connects to a bypass that returns a fraction of the pumped sheath liquid back to the sheath liquid reservoir with the returned sheath liquid flowing into air within the sheath liquid reservoir. A second outlet arm of the T-coupling connects to a sheath route that includes a reservoir capsule followed by a particle filter and then the flow cell. The sheath liquid exiting the flow cell then goes to the waste tank. The fluidic resistance along the bypass is designed to be lower than the fluidic resistance along the sheath route. Consequently, only a small fraction of the sheath liquid goes through the flow cell. Note that typical sheath flow rate in flow cytometric applications is a few tens of milliliter per minute. The bypass therefore permits using higher flow rate liquid pumps that not only are much less expensive and more reliable, but also operates at higher pulsation frequency which is much easier to attenuate. Since the exit of the bypass route connects to air, it also serves as a large fluidic capacitor for significantly reducing pulsation in sheath liquid flowing along the sheath route. During operation, the inlet portion of the filter cartridge is filled with air. Therefore, the filter cartridge also serves as a fluidic capacitor, for further reducing pulsation in the sheath liquid at the flow cell to negligible level. Due to the large fluidic resistance at the flow cell, the air trapped near the inlet of the filter cartridge becomes compressed. If the liquid pump is turned off, the compressed air in filter cartridge being pushed back towards the sheath liquid reservoir becomes stored in the reservoir capsule whose size is chosen to prevent the trapped air from reaching the T-coupling.

According to an exemplary embodiment, the peristaltic pump may generally include a plurality of rollers located at the periphery of a rotor that moves the rollers circularly inside a housing's arcuate curved track and a compressible tube that the rollers compress against the track. In one embodiment of the present disclosure, the track of the peristaltic pump's housing may have one recess so the compressible tube is progressively decompressed to full expansion then compressed to full closure every time one of the rollers moves past the recess. The location and shape of the recess maintains the total volume of liquid within the compressible tube from the recess to the pump's outlet substantially invariant. The effect of tube expansion as a roller moves past the pump's outlet is compensated by the tube compression when a different other roller immediately upstream of the pump outlet moves into the recess' compressing section. In another embodiment of

the present disclosure, the track of the pump housing may contain a plurality of recesses, providing for a plurality of roller upstream of the pump outlet to progressively modify the tube compression in multiple sections along the compressible tube. The locations and shapes of the plurality of recesses are designed such that the modification of tube compression at these sections substantially compensates the effect due to the tube expansion near the pump outlet. In yet another embodiment of the present disclosure, the compressible tube is kept fully closed underneath the roller except in the inlet and exit sections. A variable speed motor may be used to drive the pump. When a roller reaches the exit section, the motor's rotation may programmatically speed up to compensate for the tube's expansion.

According to an exemplary embodiment, a wavelength division multiplexer ("WDM") may include at least two optical elements. The first optical element collimates a beam of light received from an extended light source, such as the light from a pinhole or from a multimode optical fiber. The first optical element magnifies the extended light source, for example, as defined by the pinhole, or the core of the multimode optical fiber, to an image having a size similar to the effective cross section of the first optical element thereby creating a collimated light beam between the first optical element and its image. A second optical element is positioned near the image, and relays the first optical element with unit magnification down the optical path. In this way, the second optical element effectively doubles the collimated path length. Additional optical elements in the same 1:1 image relay configuration may also be included to further extend the collimated optical path. The cascaded unit-magnification image relay architecture of the present disclosure extends the collimated optical path length without large beam expansion. As a result, WDM techniques well-established in the optical communication industry can be readily adapted for fluorescence light detection. In particular, multiple colored bands present in the beam of light can be separated using dichroic filters located along the optical path with the separated light being tightly focused into small spots compatible with low noise semiconductor photodetectors.

In one embodiment of a WDM, the first optical element is a lens and the second element is a concave mirror, although it is apparent to those skilled in the art that other types of refractive and/or reflective optical components may also be used to achieve the

same design goal. The optical path in the WDM of the present disclosure may be folded using dichroic filters. In one embodiment of the present disclosure, the light path may be folded into a zig-zag configuration. To facilitate the flow cytometer's reliable reconfiguration, each dichroic filter may be bonded to a mechanical holder having a reference surface that is optically parallel to the filter's reflective surface. As a result, all of the WDM's filters can be accurately positioned along the optical path by referencing the filter's holder against a common optical flat. In another embodiment of the present disclosure, the collimated beam passing through the dichroic filter is further branched out into multiple colored bands using secondary dichroic filters. It is apparent to those skilled in the art that dichroic filters may be inserted anywhere along the long, narrow and collimated beam path afforded by the present disclosure's relay imaging to thereby permit delivering a tightly focused beam to photo detectors using a variety of optical configurations, such as the star configuration discussed in United States Patent no. 6,683,314, the branched configuration discussed in United States Patent no. 4,727,020 and other types of WDM optical configuration widely practiced in the optical communication industry. Instead of concave mirrors, the WDM may be replaced by curved dichroic filters to further increase the number of colored bands selected by the WDM.

According to some exemplary embodiments, an optical system for impinging beams of light into a viewing zone in which a sample flow carrying objects and a sheath flow pass through includes a first light source for emitting a first beam of light along a first beam path to illuminate objects in the viewing zone at a first location, a second light source for emitting a second beam of light along a second beam path to illuminate objects in the viewing zone at a second location, a beam compressing optical element for reducing widths of the first and second beams of light on their major axes to a width less than the width of the sheath flow, and a first chromatic compensation element located on at least one of the first beam path and the second beam path for compensating chromatic aberration in the viewing zone such that the first location and the second locations are on a common plane parallel to the direction of the sample flow. The wavelength of the second light source is different from the wavelength of the first light source. The chromatic compensation allows compensating the properties of the different paths, resulting e.g. from the different wavelengths, the different path lengths, different locations in the flow path etc. This applies

also for multiple compensating elements in different paths, in particular when using two, three or more wavelengths for illumination.

According to some exemplary embodiments, an optical system includes a first light source for emitting a first beam of light to illuminate objects at a first location in a viewing zone, a composite microscope objective for imaging light scattered from and fluoresced by the objects at the first location in the viewing zone at an image plane external to the composite microscope, and a beam splitter for reflecting or transmitting scattered and fluoresced light, wherein the light source and the image plane are on two sides of the beam splitter. The composite microscope includes a concave mirror and an aberration corrector plate. The aberration corrector plate is an aspheric lens that has a first zone with negative optical power and a second zone with positive optical power radially inside the first zone. The viewing zone is positioned between the concave mirror and the aberration corrector plate. This allows a compact build-up, as the illumination and the detection of light scattered from and fluoresced by the objects in the viewing zone may be conducted from the same side of the microscope objective.

According to some exemplary embodiments, an axial light detection system includes a concave mirror for reflecting light that propagates from a viewing zone, and a detector for measuring axial light loss produced by an object in the viewing zone by detecting light reflected by the concave mirror. This allows an effective detection of light loss which may serve as a base for better interpretation of the measured values.

According to some exemplary embodiments, a power monitoring system for adjusting power of a light source includes a first light source for emitting a first beam of light, a second light source for emitting a second beam of light, a first dichroic filter for reflecting the first beam of light and passing the second beam of light, a second dichroic filter for reflecting the second beam of light, a first detector for measuring residual power of the first and second beams of light downstream of the first dichroic filter on a time-division multiplexing basis, and a control unit coupled with the first detector and the first and second light sources, wherein the control unit adjusts power of one or more of the first and second light sources based on measured residual power of the first and second beams of light by the first detector. This allows an effective detection of light power which may

serve as a base for better interpretation of the measured values, as well as an effective control procedure, in particular when controlling or adaption respective light sources.

According to another exemplary embodiment, an optical system includes an objective adapted for imaging light scattered from and fluoresced by an illuminated object within a viewing zone, an optical transmission member for propagating light received from the aspheric lens, a wavelength division multiplexer (WDM) for receiving light propagated by the optical transmission member. The objective includes an aspheric lens with a first zone with negative optical power and a second zone inside the first zone with positive optical power, and a concave mirror for reflecting light scattered from and fluoresced by the illuminated object through the aspheric lens, wherein the viewing zone is located between said concave mirror and the aspheric lens. The WDM includes a first optical element that produces a beam of light with an image of substantially the same size as the effective size of said first optical element, at least one dichroic filter located between said first optical element and said image, a second optical element located in one of said branches, and an image relay optical element located near the image produced by said first optical element in the other branch. The dichroic filter separates the beam of light into two branches of distinctive colors. The beam of light in said branch is focused to a spot by said second optical element. The image relay optical element produces an image of said first optical element at substantially unit magnification. This allows an adapted combined operation of the microscope objective and the WDM, as well as the optical coupling there between. In particular the microscope objective and the WDM as well as the optical coupling may be adapted to match to each other with respect to wavelength and other parameters.

According to another exemplary embodiment, an optical system includes a light source for emitting a beam of light to illuminate an object in a viewing zone, a concave mirror for receiving and reflecting light scattered from and fluoresced by the illuminated object, an aspheric lens with a first zone with negative optical power and a second zone inside the first zone with positive optical power, wherein light reflected by the concave mirror passes through the aspheric lens, and wherein the viewing zone is located between said concave mirror and the aspheric lens, an optical transmission member for receiving and propagating light from the aspheric lens, and a multiplexer for receiving light from the

optical transmission member and separating the light into at least two colors. This allows an adapted combined operation of the illumination system, the microscope objective and the WDM, as well as the optical coupling there between. In particular the illumination system, the microscope objective and the WDM as well as the optical coupling may be adapted to match to each other with respect to wavelength and other parameters.

According to another exemplary embodiment, an apparatus for imaging light scattered from and fluoresced by an illuminated object within a viewing zone includes a fluid delivery system for delivering an object to a viewing zone, a light source for illuminating the object in the viewing zone, a concave mirror located on one side of the viewing zone for reflecting light scattered from and fluoresced by the illuminated object, and an aspheric lens located on another side of the viewing zone for receiving the light reflected by the concave mirror and forming an image at an image plane, the aspheric lens having a first zone with negative optical power and a second zone radially inside the first zone with positive optical power. This allows an adapted combined operation of the fluid delivery system, the illumination system, and the microscope objective. In particular the fluid delivery system, the illumination system, and the microscope objective may be adapted to match to each other with respect to wavelength and other parameters.

According to other exemplary embodiments, an optical method for impinging beams of light into a viewing zone includes directing a first beam of light to illuminate objects in a viewing zone to produce scattered and fluoresced light, reflecting the scattered and fluoresced light using a concave mirror toward an aberration corrector plate, correcting aberrations in the reflected light with the aberration corrector plate, wherein the aberration corrector plate has a first zone with negative optical power a second zone radially inside the first zone with positive optical power, and reflecting or transmitting the corrected light using a beam splitter.

According to other exemplary embodiments, an optical method for detecting light includes reflecting light that propagates from a viewing zone using a concave mirror, and measuring axial light loss produced by an object in the viewing zone by detecting light reflected by the concave mirror.

According to other exemplary embodiments, a method of gathering and imaging light scattered from or fluoresced by objects in a viewing includes delivering an object to a viewing zone, illuminating the object in the viewing zone to produce scattered and fluoresced light, reflecting the scattered and fluoresced light using a concave mirror toward a transparent aberration corrector plate, and correcting spherical aberrations in the reflected light with the transparent aberration corrector plate, wherein the transparent aberration corrector plate has a first zone with negative optical power and a second zone radially inside the first zone with positive optical power.

According to other exemplary embodiments, a composite microscope objective adapted for imaging light scattered from and fluoresced by an object present within a viewing zone, comprises a viewing zone, a concave mirror arrangement, an exit area and an illumination beam forming arrangement, wherein the viewing zone is arranged between the concave mirror arrangement and the exit area, and wherein the concave mirror is arranged to reflect scattered and fluoresced light impinging from an object present in the viewing zone to the exit area, and wherein the illumination beam forming arrangement is arranged so that an illumination beam entering the illumination beam forming arrangement is pre-definitely formed at the viewing zone. According to other exemplary embodiments there may be provided an aberration corrector plate, in particular an aspheric lens in the exit area. This allows an effective build-up of the microscope objective. It should be noted that the aberration corrector plate is not necessary when providing a concave mirror shape allowing a sufficient imaging of the light scattered and fluoresced from an object in the viewing zone. If required an aberration corrector plate, in particular an aspheric lens may be arranged in the exit area.

According to other exemplary embodiments a wavelength division multiplexer (WDM) for separating light emitted from a light source into multiple colored bands comprises an imaging optical arrangement, a dichroic filter arrangement, a semiconductor photo detector, and a focusing optical arrangement, wherein the imaging optical arrangement forms a beam of light from the light emitted from a light source and produces an image of substantially the same size as the effective size of said imaging optical arrangement, and wherein the dichroic filter arrangement is located between said imaging optical arrangement and said image, and separates the beam of light into a first branch and

a second branch of distinctive colors, and wherein the semiconductor photo detector is located in the first branch, and wherein the focusing optical arrangement is located between the dichroic filter arrangement and the semiconductor photo detector so as to focus the beam of light onto the semiconductor photo detector. Thus, an effective detection arrangement may be provided, which may be operated with a semiconductor detector. The semiconductor detector may be a semiconductor photo detector. The semiconductor detector may be an avalanche photo diode or a carbon nanotube detector. Thus, a reduced signal to noise ratio can be achieved.

In first aspect of the disclosure, a flow cytometer includes a laser diode (LD) based optical subsystem for directing a beam of light into a viewing zone of said flow cytometer through which a sample liquid carrying particles flows, the sample liquid being hydrodynamically focused within the viewing zone by a liquid sheath flow that also flows through the viewing zone, a composite microscope objective for imaging light scattered from and fluoresced by a particle present within the viewing zone, a fluidic subsystem for supplying the liquid sheath flow to the viewing zone, the liquid sheath flow lacking pulsations, a peristaltic pump for supplying the sample liquid carrying the particles, the sample liquid being hydrodynamically focused within the viewing zone by the liquid sheath flow, a peristaltic pump for supplying the sample liquid carrying the particles, the sample liquid being hydrodynamically focused within the viewing zone by the liquid sheath flow, and a wavelength division multiplexer (WDM) for separating into multiple colored bands a beam of light emitted initially from the viewing zone and imaged by the composite microscope objective into an optical fiber for transmission to the WDM. The LD based optical subsystem may include a LD for emitting a diverging beam of light from an edge thereof, the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis, a collimating lens for converting the diverging beam of light emitted from said LD into a collimated elliptical beam of light, wherein the minor axis of said collimated elliptical beam of light is oriented parallel to a direction in which particles pass through the viewing zone, a beam compressing optical el ement for reducing the size of said elliptical beam of light at the viewing zone whereby a width of said major axis of said elliptical beam of light oriented perpendicular to the direction in which particles pass through the viewing zone is less than a width of said liquid sheath

flow, a cylindrical focusing element positioned adjacent to the viewing zone with an axis of said cylindrical focusing element being oriented perpendicular to the direction in which particles pass through the viewing zone whereby said minor axis of said beam of light becomes focused at the viewing zone, and the size of said major axis of said elliptical beam of light at the viewing zone remains essentially unchanged. The composite microscope objective may include a concave mirror upon which scattered and fluoresced light impinges and an aberration corrector plate made of optically transparent material. The aberration corrector plate is an aspheric lens that has a first zone of said aberration corrector plate having negative optical power outside a neutral zone and a second zone of said aberration corrector plate inside the neutral zone having positive optical power light. The neutral zone is the thinnest portion of the aberration corrector plate. Light reflected from the concave mirror passes through said aberration corrector plate. The viewing zone of said flow cytometer is located between said concave mirror and said aberration corrector plate. The fluidic subsystem may include a liquid pump for supplying liquid drawn from a reservoir and a T-coupling having at least one (1) inlet and two (2) outlets. The inlet of said T-coupling receives liquid from said liquid pump. A first fraction of the liquid received by the inlet flows via a first one of the outlets and via a bypass conduit back to the reservoir. A second fraction of the liquid received by the inlet flows via a second one of the outlets and via a particle filter to the viewing zone of said flow cytometer. The peristaltic pump may include a pump housing having a arcuate curved track formed therein that extends between a pump inlet and a pump outlet, a plurality of rollers that are attached to a rotor, the rollers having a substantially equal angular spacing between each pair of immediately adjacent rollers, the rotor being rotatable together with the rollers attached thereto inside said pump housing, and a compressible tube sandwiched between said rollers and the arcuate curved track of said pump housing. The arcuate curved track includes an exit section and at least one pumping section along the arcuate curved track between the pump inlet and the pump outlet. As a roller rolls through the exit section, said compressible tube adjacent to said roller progressively expands from fully closed at a beginning of said exit section to fully open at the pump outlet where said roller breaks contact with said compressible tube. Said compressible tube is compressed to fully closed by at least one of said rollers. The wavelength division multiplexer (WDM) may include a collimating optical element that

magnifies an to produce an image of substantially the same size as the effective size of said collimating optical element, at least one dichroic filter located between said collimating optical element and said image, said dichroic filter separating the collimated beam of light into two (2) branches of distinctive colors, a focusing optical element located in one of said branches, the beam of light in said branch being focused to a spot having a diameter of less than 1.0 mm by said focusing optical element, and an image relay optical element located near the image produced by said collimating optical element in the other branch, said image relay optical element producing an image of said collimating optical element at substantially unit magnification.

In second aspect of the disclosure, said cuvette may have a rectangularly-shaped cross-section, and the viewing zone of the flow cytometer is located within a channel having a rectangularly-shaped cross-section that is located within said cuvette.

In third aspect of the disclosure, said cuvette may have a tubularly-shaped cross-section, and the viewing zone of the flow cytometer is located within a channel having a circularly-shaped cross-section that is located within said cuvette.

In fourth aspect of the disclosure, the sample liquid and the liquid sheath flow form a jet stream in which the viewing zone of the flow cytometer is located.

In fifth aspect of the disclosure, said cylindrical focusing element is in optical contact with an entrance face of said rectangularly-shaped cuvette.

In sixth aspect of the disclosure, said cylindrical focusing element is separated from said rectangularly-shaped cuvette.

In seventh aspect of the disclosure, said cylindrical focusing element is separated from said tubularly-shaped cuvette.

In eighth aspect of the present disclosure, said cylindrical focusing element is separated from said jet stream.

In ninth aspect of the present disclosure, the flow cytometer further comprises a polarization conditioning element through which said collimated elliptical beam of light passes.

In tenth aspect of the present disclosure, an optical image of the viewing zone is formed outside the composite microscope objective.

In eleventh aspect of the present disclosure, the viewing zone is located within a flow channel included in a rectangularly-shaped cuvette made of optically transparent material.

In twelfth aspect of the present disclosure, said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

In thirteenth aspect of the present disclosure, the plano-surface of said plano-concave back surface mirror is optically coupled to a flat surface of said cuvette.

In fourteenth aspect of the present disclosure, an optical adhesive material accomplishes the optical coupling.

In fifteenth aspect of the present disclosure, an index matching gel accomplishes the optical coupling.

In sixteenth aspect of the present disclosure, an index matching fluid accomplishes the optical coupling.

In seventeenth aspect of the present disclosure, optical contact bonding accomplishes the optical coupling.

In eighteenth aspect of the present disclosure, the plano-concave back surface mirror formed integrally with said cuvette means.

In nineteenth aspect of the present disclosure, said aberration corrector plate is a plano-aspherical lens.

In twentieth aspect of the present disclosure, a plano-surface of said aberration corrector plate is optically coupled to a flat surface of said cuvette opposite of said plano-concave back surface mirror.

In twenty-first aspect of the present disclosure, an index matching gel accomplishes the optical coupling.

In twenty-second aspect of the present disclosure, an index matching fluid accomplishes the optical coupling.

In twenty-third aspect of the present disclosure, optical contact bonding accomplishes the optical coupling.

In twenty-fourth aspect of the present disclosure, the plano-aspherical lens is formed integrally with said cuvette.

In twenty-fifth aspect of the present disclosure, said aberration corrector plate is detached from said cuvette.

In twenty-sixth aspect of the present disclosure, the viewing zone is inside a jet stream.

In twenty-seventh aspect of the present disclosure, said concave mirror is a front surface mirror.

In twenty-eighth aspect of the present disclosure, the viewing zone is located on a surface of a flat, transparent substrate.

In twenty-ninth aspect of the present disclosure, said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

In thirtieth aspect of the present disclosure, the plano-surface of said plano-concave back surface mirror is optically coupled to said flat, transparent substrate.

In thirty-first aspect of the present disclosure, an optical adhesive material accomplishes the optical coupling.

In thirty-second aspect of the present disclosure, an index matching gel accomplishes the optical coupling.

In thirty-third aspect of the present disclosure, an index matching fluid accomplishes the optical coupling.

In thirty-fourth aspect of the present disclosure, optical contact bonding accomplishes the optical coupling.

In thirty-fifth aspect of the present disclosure, said plano-concave back surface mirror is formed integrally with said flat, transparent substrate.

In thirty-sixth aspect of the present disclosure, said aberration corrector plate is detached from said flat, transparent substrate.

In thirty-seventh aspect of the present disclosure, the particle filter has inlet thereto for receiving liquid from the T-coupling, the inlet of the particle filter being disposed so that air becomes trapped within the particle filter at the inlet thereto.

In thirty-eighth aspect of the present disclosure when said liquid pump is turned off air cannot enter into the bypass conduit.

In thirty-ninth aspect of the present disclosure, the flow cytometer further comprises a small capsule disposed between the second one of the outlets of said T-coupling and the particle filter for storing air ejected from the particle filter when the liquid pump is turned off.

In fortieth aspect of the present disclosure, the flow cytometer further comprises a length of tubing disposed between the second one of the outlets of said T-coupling and the particle filter for storing air ejected from the particle filter when the liquid pump is turned off.

In forty-first aspect of the present disclosure, the flow cytometer further comprises an adjustable valve located in the bypass conduit between the first one of the outlets of the T-coupling and the reservoir for restricting liquid flow therebetween.

In forty-second aspect of the present disclosure, the flow cytometer further comprises an adjustable valve located between the second one of the outlets of the T-coupling and the viewing zone for restricting liquid flow therebetween.

In forty-third aspect of the present disclosure, the throughput of the liquid pump is adjustable.

In forty-fourth aspect of the present disclosure, the arcuate curved track of said pump housing includes at least two (2) pumping sections, the arcuate curved track further including at least one recess section located between said pumping sections along the arcuate curved track, and said compressible tube at said recess section becoming decompressed to full expansion then compressed to fully closed when one (1) of said rollers rolls through said recess section.

In forty-fifth aspect of the present disclosure, the peristaltic pump includes a plurality of recess sections along said arcuate curved track upstream of the pump outlet, the angular spacing between the compression part of said recess section adjacent to the pump outlet and said exit section of said arcuate curved track being substantially the same as the angular spacing between each pair of immediately adjacent rollers.

In forty-sixth aspect of the present disclosure, said compression part of said recess section adjacent to the pump outlet has a shape complementing a shape of said exit section of said arcuate curved track to maintain the total fluid volume inside a section of said compressible tube extending from said recess section to the pump outlet substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

In forty-seventh aspect of the present disclosure, the peristaltic pump includes a plurality of recess sections respectively interspersed between immediately adjacent pairs of a plurality of pumping sections.

In forty-eighth aspect of the present disclosure, both angular spacing between adjacent pairs of recess sections, and angular spacing between said exit section of said arcuate curved track and an adjacent recess section to said exit section are substantially the same as the angular spacing between each pair of immediately adjacent rollers.

In forty-ninth aspect of the present disclosure, shapes of a plurality of recess sections of said arcuate curved track complement a shape of said exit section of said arcuate curved track to maintain a fluid volume in sections of said compressible tube at the plurality of recess sections and said exit section substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

In fiftieth aspect of the present disclosure, a speed of said rotor is programmably controlled to vary substantially in inverse proportion to the fluid volume change rate in said compressible tube due to its changing compression near the exit section of said arcuate curved track.

In fifty-first aspect of the present disclosure, at least one additional dichroic filter is located between said image relay optical element and the image produced by said image

relay optical element, said dichroic filter producing two (2) branches of the beam of light having distinctive colors.

In fifty-second aspect of the present disclosure, another focusing optical element is located in one of said branches and focuses the beam of light in the branch into a spot having a diameter of less than 1.0 mm.

In fifty-third aspect of the present disclosure, wherein successive combinations of said image relay optical element, dichroic filter, and focusing optical element are cascaded to produce additional focused spots having a diameter of less than 1.0 mm for multiple colored bands of said beam of light.

In fifty-fourth aspect of the present disclosure, the dichroic filter is assembled using a template that include two (2) optically flat glass plates bonded together in optical contact, and the dichroic filter is bonded to a filter holder using the template such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder.

In fifty-fifth aspect of the present disclosure, the reference surface of the filter holder rests against an optically flat surface of an reference block included in the WDM thereby providing consistent optical alignment when installing the dichroic filter into the WDM.

In fifty-sixth aspect of the present disclosure, the LD based optical subsystem includes a LD for emitting a diverging beam of light from an edge thereof, the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis, a collimating lens for converting the diverging beam of light emitted from said LD into a collimated elliptical beam of light, wherein the minor axis of said collimated elliptical beam of light is oriented parallel to a direction in which particles pass through the viewing zone, a beam compressing optical element for reducing the size of said elliptical beam of light at the viewing zone whereby a width of said major axis of said elliptical beam of light oriented perpendicular to the direction in which particles pass through the viewing zone is less than a width of said liquid sheath flow, a cylindrical focusing element positioned adjacent to the viewing zone with an axis of said cylindrical focusing element being oriented perpendicular to the direction in which particles pass through the viewing

zone whereby said minor axis of said beam of light becomes focused at the viewing zone, and the size of said major axis of said elliptical beam of light at the viewing zone remains essentially unchanged.

In fifty-seventh aspect of the present disclosure, the optical subsystem may further comprise a cuvette having a rectangularly-shaped cross-section, and the viewing zone may be located within a channel having a rectangularly-shaped cross-section that is located within said cuvette.

In fifty-eighth aspect of the present disclosure, the optical subsystem further comprises a cuvette having a tubularly-shaped cross-section, and the viewing zone is located within a channel having a circularly-shaped cross-section that is located within said cuvette.

In fifty-ninth aspect of the present disclosure, the sample liquid and the liquid sheath flow form a jet stream in which the viewing zone is located.

In sixtieth aspect of the present disclosure, cylindrical focusing element is in optical contact with an entrance face of said rectangularly-shaped cuvette.

In sixty-first aspect of the present disclosure, said cylindrical focusing element is separated from said rectangularly-shaped cuvette.

In sixty-second aspect of the present disclosure, said cylindrical focusing element is separated from said tubularly-shaped cuvette.

In sixty-third aspect of the present disclosure, said cylindrical focusing element is separated from said jet stream.

In sixty-fourth aspect of the present disclosure, the optical subsystem (50) further comprises a polarization conditioning element through which said collimated elliptical beam of light passes.

In sixty-fifth aspect of the present disclosure, a method for delivering an elliptically shaped beam of light using a LD based optical subsystem (50), the beam of light having a smooth profile at a focus of a minor axis thereof that is located at a viewing zone through which a sample liquid flows, the sample liquid being hydrodynamically focused within the viewing zone by a liquid sheath flow that also flows through the viewing zone, the method

includes the steps of:

providing a LD that emits a diverging beam of light from an edge thereof, the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis, impinging the diverging beam of light emitted by the LD upon a collimating lens for converting the diverging beam of light emitted therefrom into a collimated elliptical beam of light wherein the minor axis of said collimated elliptical beam of light is oriented parallel to a direction in which sample liquid passes through the viewing zone, after passing through said collimating lens, impinging the collimated elliptical beam of light upon an beam compressing optical element for reducing the size of said elliptical beam of light at the viewing zone whereby a width of said major axis of said elliptical beam of light oriented perpendicular to the direction in which sample liquid passes through the viewing zone becomes less than a width of said liquid sheath flow, and after passing through said beam compressing optical element, impinging the beam of light upon a cylindrical focusing element positioned adjacent to the viewing zone with an axis of said cylindrical focusing element being oriented perpendicular to the direction in which sample liquid passes through the viewing zone whereby said minor axis of said beam of light becomes focused at the viewing zone, and the size of said major axis of said elliptical beam of light at the viewing zone remains essentially unchanged.

In sixty-sixth aspect of the present disclosure, the viewing zone is located within a channel having a rectangularly-shaped cross-section that is located within a cuvette.

In sixty-seventh aspect of the present disclosure, the viewing zone is located within a channel having a circularly-shaped cross-section that is located within a cuvette.

In sixty-eighth aspect of the present disclosure, the viewing zone is located within a jet stream.

In sixty-ninth aspect of the present disclosure, the method further comprises a step of establishing an optical contact between said cylindrical focusing element and an entrance face of said cuvette.

In seventieth aspect of the present disclosure, the method further comprises a step of establishing a spacing between said cylindrical focusing element and said cuvette.

In seventy-first aspect of the present disclosure, the method further comprises a step of establishing a spacing between said cylindrical focusing element and said cuvette.

In seventy-second aspect of the present disclosure, the method further comprise a step of establishing a spacing between said cylindrical focusing element and said jet stream.

In seventy-third aspect of the present disclosure, the method further comprises a step of inserting a polarization conditioning element between the collimating lens and the beam compressing optical element whereby the collimated elliptical beam of light passes through the polarization conditioning element.

In seventy-fourth aspect of the present disclosure, The composite microscope objective includes a concave mirror upon which scattered and fluoresced light impinges and an aberration corrector plate made of optically transparent material. The aberration corrector plate is an aspheric lens that has a first zone of said aberration corrector plate having negative optical power outside a neutral zone and a second zone of said aberration corrector plate inside the neutral zone having positive optical power light. The neutral zone is the thinnest portion of the aberration corrector plate. Light reflected from the concave mirror passes through said aberration corrector plate. The viewing zone of said flow cytometer is located between said concave mirror and said aberration corrector plate.

In seventy-fifth aspect of the present disclosure, an optical image of the viewing zone is formed outside the composite microscope objective.

In seventy-sixth aspect of the present disclosure, the viewing zone is located within a flow channel included in a rectangularly-shaped cuvette made of optically transparent material.

In seventy-seventh aspect of the present disclosure, said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

In seventy-eighth aspect of the present disclosure, a plano-surface of said plano-concave back surface mirror is optically coupled to a flat surface of said cuvette.

In seventy-ninth aspect of the present disclosure, an optical adhesive material accomplishes the optical coupling.

In eightieth aspect of the present disclosure an index matching gel accomplishes the optical coupling.

In eighty-first aspect of the present disclosure, an index matching fluid accomplishes the optical coupling.

In eighty-second aspect of the present disclosure, optical contact bonding accomplishes the optical coupling.

In eighty-third aspect of the present disclosure, the plano-concave back surface mirror formed integrally with said cuvette means.

In eighty-fourth aspect of the present disclosure, said aberration corrector plate is a plano-aspherical lens.

In eighty-fifth aspect of the present disclosure, a plano-surface of said aberration corrector plate is optically coupled to a flat surface of said cuvette opposite of said plano-concave back surface mirror.

In eighty-sixth aspect of the present disclosure, an index matching gel accomplishes the optical coupling.

In eighty-seventh aspect of the present disclosure, an index matching fluid accomplishes the optical coupling.

In eighty-eighth aspect of the present disclosure, optical contact bonding accomplishes the optical coupling.

In eighty-ninth aspect of the present disclosure, the plano-aspherical lens is formed integrally with said cuvette.

In ninetieth aspect of the present disclosure said aberration corrector plate is detached from said cuvette.

In ninety-first aspect of the present disclosure, the viewing zone is inside a jet stream.

In ninety-second aspect of the present disclosure, said concave mirror is a front surface mirror.

In ninety-third aspect of the present disclosure, the viewing zone is located on a surface of a flat, transparent substrate.

In ninety-fourth aspect of the present disclosure, said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

In ninety-fifth aspect of the present disclosure, a plano-surface of said plano-concave back surface mirror is optically coupled to said flat, transparent substrate.

In ninety-sixth aspect of the present disclosure, an optical adhesive material accomplishes the optical coupling.

In ninety-seventh aspect of the present disclosure, an index matching gel accomplishes the optical coupling.

In ninety-eighth aspect of the present disclosure, an index matching fluid accomplishes the optical coupling.

In ninety-ninth aspect of the present disclosure, optical contact bonding accomplishes the optical coupling.

In one hundredth aspect of the present disclosure, said plano-concave back surface mirror is formed integrally with said flat, transparent substrate.

In one hundred first aspect of the present disclosure, said aberration corrector plate is detached from said flat, transparent substrate.

In one hundred second aspect of the present disclosure, a method for characterizing microscopic species using a microscope objective device includes a concave mirror, an aberration corrector plate made of optically transparent material, and a viewing zone located in between said concave mirror and said aberration corrector plate. The aberration corrector plate is an aspheric lens that has a first zone of said aberration corrector plate having negative optical power outside a neutral zone and a second zone of said aberration corrector plate inside the neutral zone having positive optical power light. The neutral zone is the thinnest portion of the aberration corrector plate.

In one hundred third aspect of the present disclosure, an optical image of the viewing zone is formed outside the device.

In one hundred fourth aspect of the present disclosure, the viewing zone is located within a flow channel contained in a rectangularly-shaped cuvette means made of optically transparent material.

In one hundred fifth aspect of the present disclosure, said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

In one hundred sixth aspect of the present disclosure, a plano-surface of said plano-concave back surface mirror means is optically coupled to a flat surface of said cuvette means.

In one hundred seventh aspect of the present disclosure, an optical adhesive material accomplishes the optical coupling.

In one hundred eighth aspect of the present disclosure, an index matching gel accomplishes the optical coupling.

In one hundred ninth aspect of the present disclosure, an index matching fluid accomplishes the optical coupling.

In one hundred tenth aspect of the present disclosure, optical contact bonding accomplishes the optical coupling.

In one hundred eleventh aspect of the present disclosure the plano-concave back surface mirror is formed integrally with said cuvette.

In one hundred twelfth aspect of the present disclosure, said aberration corrector plate is a plano-aspherical lens.

In one hundred thirteenth aspect of the present disclosure, a plano-surface of said aberration corrector plate is optically coupled to a flat surface of said cuvette means opposite of said concave mirror.

In one hundred fourteenth aspect of the present disclosure an index matching gel accomplishes the optical coupling.

In one hundred fifteenth aspect of the present disclosure, an index matching fluid accomplishes the optical coupling.

In one hundred sixteenth aspect of the present disclosure, optical contact bonding accomplishes the optical coupling.

In one hundred seventeenth aspect of the present disclosure, the plano-aspherical lens is formed integrally with said cuvette.

In one hundred eighteenth aspect of the present disclosure, said aberration corrector plate is detached from said cuvette.

In one hundred nineteenth aspect of the present disclosure, the viewing zone is inside a jet stream.

In one hundred twentieth aspect of the present disclosure, said concave mirror is a front surface mirror.

In one hundred twenty-first aspect of the present disclosure, the viewing zone is located on a surface of a flat, transparent substrate.

In one hundred twenty-second aspect of the present disclosure said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

In one hundred twenty-third aspect of the present disclosure, a plano-surface of said plano-concave back surface mirror means is optically coupled to said flat, transparent substrate.

In one hundred twenty-fourth aspect of the present disclosure, an optical adhesive material accomplishes the optical coupling.

In one hundred twenty-fifth aspect of the present disclosure, an index matching gel accomplishes the optical coupling.

In one hundred twenty-sixth aspect of the present disclosure, an index matching fluid accomplishes the optical coupling.

In one hundred twenty-seventh aspect of the present disclosure, optical contact bonding accomplishes the optical coupling.

In one hundred twenty-eighth aspect of the present disclosure, said plano-concave back surface mirror is formed integrally with said flat, transparent substrate.

In one hundred twenty-ninth aspect of the present disclosure, said aberration corrector plate is detached from said flat, transparent substrate.

In one hundred thirtieth aspect of the present disclosure, a fluidic subsystem for supplying a liquid flow pulsation free to an outlet of the fluidic subsystem includes a liquid pump for supplying liquid drawn from a reservoir and a T-coupling having at least one inlet and two outlets. The inlet of said T-coupling receives liquid from said liquid pump. A first fraction of the liquid received by the inlet flows via a first one of the outlets and via a bypass conduit back to the reservoir. A second fraction of the liquid received by the inlet flows via a second one of the outlets and via a particle filter to the viewing zone of said flow cytometer.

In one hundred thirty-first aspect of the present disclosure, the particle filter has an inlet thereto for receiving liquid from the T-coupling, the inlet of the particle filter being disposed so that air becomes trapped within the particle filter at the inlet thereto.

In one hundred thirty-second aspect of the present disclosure, when said liquid pump is turned off air cannot enter into the bypass conduit.

In one hundred thirty-third aspect of the present disclosure, the fluidic subsystem further comprises a small capsule disposed between the second one of the outlets of said T-coupling and the particle filter for storing air ejected from the particle filter when the liquid pump is turned off.

In one hundred thirty-fourth aspect of the present disclosure, the fluidic subsystem further comprises a length of tubing disposed between the second one of the outlets of said T-coupling and the particle filter for storing air ejected from the particle filter when the liquid pump is turned off.

In one hundred thirty-fifth aspect of the present disclosure, the fluidic subsystem further comprises an adjustable valve located in the bypass conduit between the first one of the outlets of the T-coupling and the reservoir for restricting liquid flow therebetween.

In one hundred thirty-sixth aspect of the present disclosure, the fluidic subsystem further comprises an adjustable valve located between the second one of the outlets of the T-coupling and the outlet of the fluidic subsystem for restricting liquid flow therebetween.

In one hundred thirty-seventh aspect of the present disclosure, the throughput of the liquid pump is adjustable.

In one hundred thirty-eighth aspect of the present disclosure, a method for supplying a liquid flow pulsation free to an outlet of the fluidic subsystem includes a liquid pump for supplying liquid drawn from a reservoir and a T-coupling having at least one (1) inlet and two (2) outlets. The inlet of said T-coupling receives liquid from said liquid pump. A first fraction of the liquid received by the inlet flows via a first one of the outlets and via a bypass conduit back to the reservoir. A second fraction of the liquid received by the inlet flows via a second one of the outlets and via a particle filter to the viewing zone of said flow cytometer.

In one hundred thirty-ninth aspect of the present disclosure, during normal operation certain amount of air is trapped near the inlet portion of said filter cartridge means.

In one hundred fortieth aspect of the present disclosure said reservoir means holds sufficient amount of liquid such that when said pump means is turned off, portion of the tubing between said T-coupling means and said reservoir means is still filled with liquid, preventing said trapped air from leaking into said bypass means.

In one hundred forty-first aspect of the present disclosure, said reservoir means is a capsule.

In one hundred forty-second aspect of the present disclosure, said reservoir means is a piece of tubing.

In one hundred forty-third aspect of the present disclosure an adjustable flow restrictor means is placed in the bypass route.

In one hundred forty-fourth aspect of the present disclosure an adjustable flow restrictor means is placed in the sheath route.

In one hundred forty-fifth aspect of the present disclosure, the throughput of the sheath pump is adjustable.

In one hundred forty-sixth aspect of the present disclosure, a peristaltic pump includes a pump housing having an arcuate curved track formed therein that extends between a pump inlet and a pump outlet, a plurality of rollers that are attached to a rotor,

the rollers having a substantially equal angular spacing between each pair of immediately adjacent rollers, the rotor being rotatable together with the rollers attached thereto inside said pump housing, and a compressible tube sandwiched between said rollers and the arcuate curved track of said pump housing. The arcuate curved track includes an exit section and at least one pumping section along the arcuate curved track between the pump inlet and the pump outlet. As a roller rolls through the exit section, said compressible tube adjacent to said roller progressively expands from fully closed at a beginning of said exit section to fully open at the pump outlet where said roller breaks contact with said compressible tube. Said compressible tube is compressed to fully closed by at least one of said rollers.

In one hundred forty-seventh aspect of the present disclosure, the arcuate curved track of said pump housing includes at least two (2) pumping sections, the arcuate curved track further including at least one recess section located between said pumping sections along the arcuate curved track, and wherein said compressible tube at said recess section becomes decompressed to full expansion then compressed to fully closed when one (1) of said rollers rolls through said recess section.

In one hundred forty-eighth aspect of the present disclosure, the peristaltic pump includes a plurality of recess sections along said arcuate curved track upstream of the pump outlet, the angular spacing between the compression part of said recess section adjacent to the pump outlet and said exit section of said arcuate curved track being substantially the same as the angular spacing between each pair of immediately adjacent rollers.

In one hundred forty-ninth aspect of the present disclosure, said compression part of said recess section adjacent to the pump outlet has a shape complementing a shape of said exit section of said arcuate curved track to maintain the total fluid volume inside a section of said compressible tube extending from said recess section to the pump outlet substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

In one hundred fiftieth aspect of the present disclosure, the peristaltic pump has a plurality of recess sections respectively interspersed between immediately adjacent pairs of a plurality of pumping sections.

In one hundred fifty-first aspect of the present disclosure, both angular spacing between adjacent pairs of recess sections, and angular spacing between said exit section of said arcuate curved track and an adjacent recess section to said exit section are substantially the same as the angular spacing between each pair of immediately adjacent roller.

In one hundred fifty-second aspect of the present disclosure, shapes of a plurality of recess sections of said arcuate curved track complement a shape of said exit section of said arcuate curved track to maintain a fluid volume in sections of said compressible tube at the plurality of recess sections and said exit section substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

In one hundred fifty-third aspect of the present disclosure, a speed of said rotor is programmably controlled to vary substantially in inverse proportion to the fluid volume change rate in said compressible tube due to its changing compression near the exit section of said arcuate curved track.

In one hundred fifty-fourth aspect of the present disclosure, a method for delivering liquid using a peristaltic pump includes a pump housing having a arcuate curved track formed therein that extends between a pump inlet and a pump outlet, a plurality of rollers that are attached to a rotor, the rollers having a substantially equal angular spacing between each pair of immediately adjacent rollers, the rotor being rotatable together with the rollers attached thereto inside said pump housing, and a compressible tube sandwiched between said rollers and the arcuate curved track of said pump housing. The arcuate curved track includes an exit section and at least one pumping section along the arcuate curved track between the pump inlet and the pump outlet. As a roller rolls through the exit section, said compressible tube adjacent to said roller progressively expands from fully closed at a beginning of said exit section to fully open at the pump outlet where said roller breaks contact with said compressible tube. Said compressible tube is compressed to fully closed by at least one of said rollers.

In one hundred fifty-fifth aspect of the present disclosure the arcuate curved track of said pump housing includes at least two (2) pumping sections, the arcuate curved track further including at least one recess section located between said pumping sections along the arcuate curved track, and wherein said compressible tube at said recess section becomes

decompressed to full expansion then compressed to fully closed when one (1) of said rollers rolls through said recess section.

In one hundred fifty-sixth aspect of the present disclosure, the peristaltic pump includes a plurality of recess sections along said arcuate curved track upstream of the pump outlet; The angular spacing between the compression part of said recess section adjacent to the pump outlet and said exit section of said arcuate curved track being substantially the same as the angular spacing between each pair of immediately adjacent rollers.

In one hundred fifty-seventh aspect of the present disclosure, said compression part of said recess section adjacent to the pump outlet has a shape complementing a shape of said exit section of said arcuate curved track to maintain the total fluid volume inside a section of said compressible tube extending from said recess section to the pump outlet substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

In one hundred fifty-eighth aspect of the present disclosure, the pump has a plurality of recess sections respectively interspersed between immediately adjacent pairs of a plurality of pumping sections.

In one hundred fifty-ninth aspect of the present disclosure, both angular spacing between adjacent pairs of recess sections, and angular spacing between said exit section of said arcuate curved track and an adjacent recess section to said exit section are substantially the same as the angular spacing between each pair of immediately adjacent roller.

In one hundred sixtieth aspect of the present disclosure, shapes of a plurality of recess sections of said arcuate curved track complement a shape of said exit section of said arcuate curved track to maintain a fluid volume in sections of said compressible tube at the plurality of recess sections and said exit section substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

In one hundred sixty-first aspect of the present disclosure, a speed of said rotor of the peristaltic pump is programmably controlled to vary substantially in inverse proportion to the fluid volume change rate in said compressible tube due to its changing compression near the exit section of said arcuate curved track.

In one hundred sixty-second aspect of the present disclosure, the wavelength division multiplexer (WDM) includes a collimating optical element that magnifies an to produce an image of substantially the same size as the effective size of said collimating optical element, at least one dichroic filter located between said collimating optical element and said image, said dichroic filter separating the collimated beam of light into two (2) branches of distinctive colors, a focusing optical element located in one of said branches, the beam of light in said branch being focused to a spot having a diameter of less than 1.0 mm by said focusing optical element, and an image relay optical element located near the image produced by said collimating optical element in the other branch, said image relay optical element producing an image of said collimating optical element at substantially unit magnification.

In one hundred sixty-third aspect of the present disclosure, at least one additional dichroic filter is located between said image relay optical element and the image produced by said image relay optical element, wherein said dichroic filter produces two (2) branches of the beam of light having distinctive colors.

In one hundred sixty-fourth aspect of the present disclosure, another focusing optical element is located in one of said branches and focuses the beam of light in the branch into a spot having a diameter of less than 1.0 mm.

In one hundred sixty-fifth aspect of the present disclosure, successive combinations of said image relay optical element, dichroic filter and focusing optical element are cascaded to produce additional focused spots having a diameter of less than 1.0 mm for multiple colored bands of said beam of light.

In one hundred sixty-sixth aspect of the present disclosure, the dichroic filter is assembled using a template that include two (2) optically flat glass plates bonded together in optical contact, and wherein the dichroic filter is bonded to a filter holder using the template such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder.

In one hundred sixty-seventh aspect of the present disclosure, the reference surface of the filter holder rests against an optically flat surface of an reference block included in

the WDM thereby providing consistent optical alignment when installing the dichroic filter into the WDM.

In one hundred sixty-eighth aspect of the present disclosure, a method for separating beam of light into colored bands using a WDM includes a collimating optical element that magnifies an to produce an image of substantially the same size as the effective size of said collimating optical element, at least one dichroic filter located between said collimating optical element and said image, said dichroic filter separating the collimated beam of light into two (2) branches of distinctive colors, a focusing optical element located in one of said branches, the beam of light in said branch being focused to a spot having a diameter of less than 1.0 mm by said focusing optical element, and an image relay optical element located near the image produced by said collimating optical element in the other branch, said image relay optical element producing an image of said collimating optical element at substantially unit magnification.

In one hundred sixty-ninth aspect of the present disclosure, at least one additional dichroic filter may be located between said image relay optical element and the image produced by said image relay optical element, wherein said dichroic filter produces two (2) branches of beam of light having distinctive colors.

In one hundred seventieth aspect of the present disclosure, another focusing optical element is located in one of said branches and focuses the beam of light in the branch into a spot having a diameter of less than 1.0 mm.

In one hundred seventy-first aspect of the present disclosure, successive combinations of said image relay optical element, dichroic filter and focusing optical element are cascaded to produce additional focused spots having a diameter of less than 1.0 mm for multiple colored bands of said beam of light.

In one hundred seventy-second aspect of the present disclosure, the dichroic filter is assembled using a template that include two (2) optically flat glass plates bonded together in optical contact, and wherein the dichroic filter is bonded to a filter holder using the template such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder.

In one hundred seventy-third aspect of the present disclosure, the reference surface of the filter holder rests against an optically flat surface of an reference block included in the WDM thereby providing consistent optical alignment when installing the dichroic filter into the WDM.

In one aspect of the disclosure, a flow cytometer having a wavelength division multiplexer (WDM), which includes an extended light source providing light that forms an object, a collimating optical element that captures light from the extended light source and projects a magnified image of the object as a first light beam, and a first focusing optical element configured to focus the first light beam to a size smaller than the object of the extended light source to a first semiconductor detector.

In an additional aspect of the disclosure, a flow cytometer includes a viewing zone where a particle in a flow stream is illuminated by light, and a composite microscope objective. The composite microscope objective further includes a concave mirror configured to gather light scattered from or fluoresced by the illuminated particle and to reflect the light back towards the viewing zone, and an aberration corrector plate configured to reduce optical aberrations in the reflected light caused by the concave mirror.

In an additional aspect of the disclosure, a flow cytometer having a fluidic system, which includes a liquid pump for supplying liquid drawn from a reservoir, and a T-coupling having at least one inlet and two outlets. The inlet of the T-coupling receives the liquid from the liquid pump. The first fraction of the liquid received by the inlet flows via a first one of the outlets and via a bypass conduit back to the reservoir. The second fraction of the liquid received by the inlet flows via a second one of the outlets and via a particle filter to the outlet of the fluidic system.

In an additional aspect of the disclosure, a flow cytometer having a peristaltic pump, which includes a pump housing having an arcuate curved track formed therein that extends between a pump inlet and a pump outlet, a plurality of rollers that are attached to a rotor, the rollers having a substantially equal angular spacing between each pair of immediately adjacent rollers, the rotor being rotatable together with the rollers attached thereto inside the pump housing, a compressible tube sandwiched between the rollers and the arcuate curved track of the pump housing, and a recess section located between the at least two pumping

sections. The compressible tube at the recess section is not fully closed. The arcuate curved track further includes an exit section and at least two pumping sections along the arcuate curved track between the pump inlet and the pump outlet. As one of the plurality of rollers rolls through the exit section, the compressible tube adjacent to the roller progressively expands from fully closed at a beginning of the exit section to fully open at the pump outlet where the roller breaks contact with the compressible tube. The compressible tube is compressed to fully closed by at least one of the plurality of rollers at the at least two pumping sections.

In an additional aspect of the disclosure, a flow cytometer having a laser diode (LD) system, which includes a LD for emitting a diverging beam of light from an edge thereof, the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis, a collimating lens for converting the diverging beam of light emitted from the LD into a collimated elliptical beam of light, the minor axis of the collimated elliptical beam of light being oriented parallel to a direction in which particles pass through a viewing zone, a beam compressing optical element for reducing the size of the elliptical beam of light at the viewing zone whereby a width of the elliptical beam of light oriented perpendicular to the direction in which the particles pass through the viewing zone is less than a width of a liquid sheath flow, and a cylindrical focusing element positioned adjacent to the viewing zone with an axis of the cylindrical focusing element being oriented perpendicular to the direction in which the particles pass through the viewing zone whereby the minor axis of the elliptical beam of light becomes focused at the viewing zone; and the size of the major axis of the elliptical beam of light at the viewing zone remains essentially unchanged.

The foregoing has outlined rather broadly the features and technical advantages of the present application in order that the detailed description that follows may be better understood. Additional features and advantages will be described hereinafter which form the subject of the claims. It should be appreciated by those skilled in the art that the conception and specific aspect disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present application. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the present application and the appended claims. The

novel features which are believed to be characteristic of aspects, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagram schematically illustrating an embodiment of a flow cytometer in accordance with the present disclosure that includes:

a) a LD based optical illumination subsystem;

b) a composite microscope objective upon which light emitted from the LD based optical illumination subsystem impinges, the composite microscope objective having a fluid-passing channel formed therethrough with a particle illumination viewing zone located inside a cuvette thereof;

c) a fluidic system for supplying a pulsation free flow of sheath liquid to the fluid-passing channel formed through the composite microscope objective;

d) a peristaltic pump for introducing a pulsation free flow of sample liquid that carries cells or particles to be analyzed into the sheath flow of liquid supplied by the fluidic system; and

e) a wavelength division multiplexer ("WDM") having a zig-zag configuration for separating a beam of light into several different colored bands, the WDM receiving light via an optical fiber that is scattered from cells or particles as they pass through the composite microscope objective's fluid passing channel and are illuminated therein by light emitted from the LD based optical illumination subsystem.

FIG. 2 is a schematic view depicting a typical high power edge emitting LD that illustrates the fast and slow axes of light emitted therefrom.

FIG. 2A shows a typical far field profile for a laser beam emitted from the LD chip depicted in FIG. 2.

FIG. 3A depicts a 3-dimensional view of a conventional LD based optical illumination subsystem for flow cytometric instruments together with the system's flow cell.

FIG. 3B depicts a typical time dependent profile of light scattering from a cell or particle passing through the laser beam depicted in FIG. 3A at the focus thereof within the system's flow cell.

FIG. 4A is an elevational view of an alternative prior art LD based optical illumination subsystem configuration across the liquid flowing through the fluid-passing channel with the beam profile at the focus thereof in the flow cytometer system's viewing zone.

FIG. 4B is a plan view along the liquid flowing through the fluid-passing channel of the alternative prior art LD based optical illumination subsystem depicted in FIG. 4A.

FIG. 5A is an elevational view across liquid flowing through the fluid-passing channel of the composite microscope objective depicted in FIG. 1, wherein the LD's slow axis oriented transversely to the liquid flow in accordance with one aspect of the present disclosure.

FIG. 5B is a plan view along liquid flowing through the fluid-passing channel of the composite microscope objective depicted in FIG. 1, wherein the LD's slow axis oriented transversely to the liquid flow in accordance with one aspect of the present disclosure.

FIG. 5C depicts a typical time dependent profile of light scattering from a cell or particle passing through the fluid passing channel of the composite microscope objective depicted in FIG. 1.

FIG. 5D depicts a perspective view of the cylindrical lens of the LD based optical subsystem coupled with the composite microscope objective in accordance with one aspect of the present disclosure.

FIG. 5E depicts an enlarged view of the beam profile shown in FIG. 5D.

FIG. 6 is a perspective view of an alternative embodiment of a LD based optical illumination subsystem in accordance with one aspect of the present disclosure adapted for

use in a flow cytometer system in which a jet stream of liquid passes through the viewing zone.

FIG. 6A is an enlarged perspective view of the alternative embodiment of a LD based optical illumination subsystem of Fig. 6 depicting in greater detail the jet stream of liquid passes through the viewing zone.

FIG. 7 is a perspective view of an alternative embodiment of a LD based optical illumination subsystem in accordance with one aspect of the present disclosure adapted for use in a flow cytometer system which orients the LD's slow axis parallel to the direction of liquid flowing through the fluid-passing channel of the composite microscope objective depicted in FIG. 1.

FIG. 8 is a perspective view of a composite microscope objective in accordance with one aspect of the present disclosure adapted for use in the flow cytometer system depicted in FIG. 1, the composite microscope objective having a fluid-passing channel formed therethrough with the particle illumination viewing zone located inside a cuvette therein.

FIG. 8A is a perspective view of a combined microscope objective in accordance with one aspect of the present disclosure.

FIG. 8B illustrates the combined microscope objective depicted in FIG. 8A coupled with a flow cell in accordance with one aspect of the present disclosure.

FIG. 9A is a cross-sectional elevational view of the composite microscope objective taken along the line 9A-9A in FIG. 8 that includes ray traces from three (3) spatially separated locations in the viewing zone to an image plane for the objective illustrating scatter and fluorescence emission propagation.

FIGs. 9B1-9B3 are spot diagrams near the image plane depicted in FIG. 9A for the three (3) spatially separated light emission locations depicted in FIG. 9A.

FIG. 10 is a cross-sectional elevational view similar to that of FIG. 9A for an alternative embodiment composite microscope objective in accordance with one aspect of the present disclosure that includes ray traces from three (3) spatially separated locations in

a viewing zone therein to an image plane for the objective illustrating scatter and fluorescence emission propagation.

FIG. 11 is a perspective view of yet another alternative embodiment a composite microscope objective in accordance with one aspect of the present disclosure adapted for use in the flow cytometer system depicted in FIG. 1, the alternative embodiment composite microscope objective having a fluid-passing channel formed therethrough with the particle illumination viewing zone located inside a cuvette therein.

FIG. 12 is a perspective view of an embodiment of a composite microscope objective in accordance with one aspect of the present disclosure adapted for use in the flow cytometer system depicted in FIG. 1, adapted for use wherein a viewing zone is located inside the jet stream depicted in FIGs. 6 and 6A.

FIG. 13 is a perspective view of an embodiment of a composite microscope objective in accordance with one aspect of the present disclosure adapted for use wherein a viewing zone is located on the surface of a microscope slide.

FIG. 14 is a schematic diagram depicting a fluidic system in accordance with one aspect of the present disclosure for supplying stable liquid sheath flow to a flow cytometer flow cell that includes:

1. a small capsule located between a sheath liquid pump and the flow cell; and

2. a particle filter located between the small capsule and the flow cell, both the particle filter and the small capsule providing air reservoirs for dampening pump pulsations.

FIG. 15 is a schematic diagram depicting an embodiment of a fluidic subsystem similar to that illustrated in FIG 14 that replaces the small capsule with a length of tubing for providing an air reservoir.

FIGs. 16A and 16B are histograms that compare measured particle flight times at the flow cell when the inlet portion of the particle filter has air trapped therein (FIG. 16A), and when there is no air within the fluidic subsystem between the sheath liquid pump and the flow cell (FIG. 16B).

FIG. 17 is a perspective view of a 3-roller peristaltic pump in accordance with one aspect of the present disclosure depicting the pump's rollers, tube and surrounding pump housing.

FIGs. 18A through 18D depict simplified views for several states of the 3-roller peristaltic pump depicted in FIG. 17 with the rollers in different locations.

FIGs. 19 is a detailed longitudinal cross-sectional view of the peristaltic pump's tube being partially compressed by the pump's roller.

FIG. 19A and 19B are detailed cross-sectional views orthogonal to the peristaltic pump's tube's length taken along the lines 19A and 19B in FIG. 19 illustrating the tube's partial compression by the roller.

FIGs. 20A and 20B are schematic diagrams illustrating the pump's rollers and tube viewed along the pump's circular coordinates to depicting the pulseless flow provided by the peristaltic pump.

FIG. 21 is a graph depicting the functional relationship with respect to the roller position when it rolls off the exit section of the compressible tube of:

1. the total volume of liquid in the exit half of the pump; as well as
2. the liquid volumes in the pump's:
   a. recess section; and
   b. exit section.

FIG. 22 is a simplified plan view of a 4-roller peristaltic pump in accordance with one aspect of the present disclosure.

FIG. 23 is a simplified plan view of a 6-roller peristaltic pump in accordance with one aspect of the present disclosure.

FIG. 24A is a longitudinal cross-sectional illustration of rollers and a compressible tube for a pulsation minimizing 3-roller peristaltic pump in accordance with one aspect of the present disclosure having a rotor with programmable speed.

FIG. 24B is simplified plan view illustrating the pulsation minimizing 3-roller peristaltic pump in accordance with one aspect of the present disclosure having a rotor with programmable speed.

FIG. 24C is a graph depicting for the pulsation minimizing peristaltic pump depicted in FIG. 24B having a programmable speed rotor:

1. negative volume change rate with respect to the roller position;

2. rotor speed; and

3. pump flow rate.

FIG. 25 is a diagram illustrating optical ray tracing for an exemplary 6 port wavelength division multiplexer ("WDM") using a zig-zag configuration in accordance with one aspect of the present disclosure.

FIG. 25A illustrates a top view of a light detection assembly of the WDM illustrated in FIG. 25 in accordance with one aspect of the present disclosure.

FIG. 25B illustrates a front perspective view of the light detection assembly of the WDM illustrated in FIGs. 25 and 25A.

FIG. 26 is a diagram illustrating ray tracing of prior art collimating devices showing the device's limitation in collimating an extended light source.

FIG. 27 is a perspective illustration of an embodiment of a 6 port WDM using a combination of zig-zag and branched configurations in accordance with one aspect of the present disclosure.

FIG. 28 is a perspective illustration of another embodiment of a WDM having concave dichroic filters in accordance with one aspect of the present disclosure.

FIGs. 29A and 29B are perspective illustrations depicting an assembly process for constructing a replaceable dichroic filter assembly for a reconfigurable WDM in accordance with the present disclosure.

FIG. 29C is a perspective illustration of a replaceable dichroic filter assembly build in accordance with the illustrations of FIGs. 29A and 29B.

FIGs. 30A and 30B are perspective illustrations of a WDM in accordance with one aspect of the present disclosure depicting installing into the WDM the replaceable dichroic filter assembly depicted in FIG. 29C and its removal therefrom.

FIG. 31 is a diagram schematically illustrating an optical system with a single light source in accordance with one aspect of the present disclosure.

FIG. 32 is a diagram schematically illustrating an optical system with multiple light sources in accordance with one aspect of the present disclosure.

FIG. 33 illustrates an enlarged view of beams of light shown in FIG. 32.

FIG. 34 is a diagram schematically illustrating an optical system with chromatic compensation elements in accordance with one aspect of the present disclosure.

FIG. 35 is a diagram schematically illustrating a power monitoring system in accordance with one aspect of the present disclosure.

FIG. 36 is a diagram schematically illustrating an optical system in accordance with one aspect of the present disclosure.

FIG. 37 is a diagram schematically illustrating an axial light loss detection system in accordance with one aspect of the present disclosure.

FIG. 38 is a diagram schematically illustrating an axial light loss detection system coupled with a second light detection system in accordance with one aspect of the present disclosure.

DETAILED DESCRIPTION

Flow Cytometer

A flow cytometer system may include one or more following components.

1. A flow cell through which a liquid stream, usually called a sheath flow, carries and hydrodynamically aligns cells or particles so that they pass single file through the flow cell.

2. A measuring subsystem system coupled to the flow cell that detects cells or particles passing through the flow cell and is usually either:

a. an impedance or conductivity measuring subsystem; or

b. an optical illumination subsystem together with an optical sensing subsystem.

3. A conversion subsystem for converting the output signal from the measuring subsystem into computer processable data.

4. A computer for analyzing the data produced by the conversion subsystem.

The optical illumination subsystem provides a collimated and then focused beam of light, usually laser light of a single wavelength, that impinges upon the hydrodynamically-focused stream of liquid passing through the flow cell. Accordingly, the flow cytometer system may have one or more light sources that may include:

1. one or more lamps, e.g., mercury or xenon;

2. one or more high-power water-cooled lasers, e.g., argon, krypton or dye laser;

3. one or more low-power air-cooled lasers, e.g., argon (488 nm), HeNe (red-633 nm), HeNe (green) and HeCd (UV); and/or

4. one or more diode lasers (blue, green, red and violet).

The optical sensing subsystem includes one or more detectors aimed where the focused liquid stream passes through the light beam. Such detectors may include:

1. detectors in line with the light beam (Forward Scatter or FSC);

2. detectors perpendicular to it (Side Scatter or SSC); and

3. fluorescence detectors.

Each suspended particle passing through the beam scatters the light, and fluorescent material present in the particle or attached to the particle excited by the impinging light emit light at a longer wavelength than that of the impinging light.

Detecting and analyzing brightness changes in a combination of scattered and fluorescent light at each detector (one for each fluorescent emission peak) permits deriving various types of information about the physical and chemical structure of each individual particle. FSC correlates with cell volume. Due to light being scattered off of internal components within a cell, SSC depends on the inner complexity of the particle (i.e., shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness). Some flow cytometers omit a fluorescence detector and detect only scattered light. Other flow cytometers form images of each cell's fluorescence, scattered light, and transmitted light. The flow cytometer system's conversion subsystem, which may include one or more amplifiers which may be either linear or logarithmic, generally includes one or

more Analogue-to-Digital Converters ("ADCs") for converting the measuring subsystem's output signal into data that is then processed by the computer.

Modern flow cytometers usually include up to four (4) lasers and numerous fluorescence detectors. Increasing the number of lasers and detectors permits labeling cells with several different antibodies, and can more precisely identify a target population by their phenotypic markers. Some instruments can even capture digital images of individual cells, allowing for the analysis of a fluorescent signal location within or on the surface of cells.

FIG. 1 depicts a flow cytometer in accordance with one aspect of the present disclosure identified by the general reference number 40. The flow cytometer 40 may include:

1. a LD based optical subsystem 50;

2. a composite microscope objective 60;

3. a fluidic subsystem 70 for supplying a liquid sheath flow;

4. a peristaltic pump 80 for injecting a liquid sample flow that contains particles to be analyzed into the liquid sheath flow supplied by the fluidic subsystem 70, the liquid sample flow becoming hydrodynamically focused by the liquid sheath flow passes through a viewing zone with the composite microscope objective 60 gathering and imaging light scattered and/or fluoresced by particles in the viewing zone;

5. an optical fiber 852 that receives light scattered and/or fluoresced by particles in the viewing zone that the composite microscope objective 60 gathers and images;

6. a wavelength division multiplexer 90 ("WDM 90") for optically processing scattered and/or fluoresced light received from the optical fiber 852; and

7. a photodetector system 938 to detect the light processed by the WDM 90.

Optical Subsystem 50

In most of the instruments, particles of interest, such as blood cells or microspheres, are carried by the sheath flow using hydrodynamic focusing into a viewing zone inside a

cuvette or jet stream and illuminated there by a focused laser beam. The technique provides the means to accurately identify and count particles of interest without being overwhelmed by background noise occurring outside a registration time window (Practical Flow Cytometry, Howard M. Shapiro, Wiley (2003) ISBN 0471411256). To increase detection sensitivity, the cross section of the focused laser beam is usually elliptical, with the minor axis along the direction of flow. In order to maintain the threshold integrity, the laser profile must have a smooth or bell shaped profile along the flow direction. One common method for producing such an beam is to elongate a 5 nearly collimated circular Gaussian beam along the direction of flow with a beam expander made of either prism or cylindrical lens pair, then focus the beam down with a spherical lens. Since the shape of the beam at the focus is the spatial Fourier transform of the beam at far field, this produces a Gaussian shaped elliptical spot with minor axis along the flow.

Conventional lasers are expensive, bulky and power hungry. More recently, laser diodes ("LD") have become available. Differing from conventional lasers, the new generation of LDs is cost effective, compact and power efficient, and shows great promise for new generation of compact biomedical instruments. A LD emits light having an elliptical cross-section with the ellipse's major axis, frequently called the fast axis, perpendicular to the LD's junction, and the ellipse's minor axis, frequently called the slow axis, parallel to the LD's junction. Unfortunately, the beam quality of a typical LD, particularly along its fast axis, leaves much to be desired, preventing its wide acceptance in flow cytometric applications.

In principle, the quality of the LD beam can be significantly improved by spatial filtering. If a small pinhole or a single mode optical fiber is positioned at the focal point of a lens, such that it only accepts the lowest order spatial mode, the beam passing through the pinhole or single mode optical fiber will be of nearly perfect Gaussian shape. United States Patent no. 5,788,927 discloses that such a beam can then be collimated and expanded in the direction of flow through the cytometer, and finally focused down to an elliptical shaped Gaussian beam with minor axis along the flow direction. Unfortunately, the size of desktop instrumentation limits the diameter of a pinhole to less than 5 micron. The core size of a visible wavelength single mode optical fiber also has a similar dimension. The challenge to

manufacture such a precision spatial filter and maintain its long-term stability not only increases the cost of LD based laser system, but also reduces its reliability.

More recently, in an effort to reduce the possible side lobes due to the edge effect of limited numerical aperture of collimating lens, United States Patent no. 6,713,019 ("the '019 patent") discloses rotating the LD by ninety degrees (90°) such that its slow axis is parallel to the direction of flow. A beam diffusing section, such as a concave cylindrical lens, is then introduced to diffuse the collimated beam in the direction perpendicular to the flow, followed by a beam spot forming section, such as a spherical focusing lens, to form an elliptical spot within the cytometer's particle viewing zone. As described in detail in the '019 patent, the laser beam after the spot forming section is extremely astigmatic. In particular, the width of the beam at the viewing zone in the direction perpendicular to the flow is comparable or even wider than the width of the flow channel. This not only reduces the amount of laser energy impinging upon the particle and consequently the signal intensity, but also increases undesired background scattering from the liquid-flow cell interface. Instead of rotating the LD, United States Patent nos. 7,385,682 and 7,561,267 disclose using a large numerical aperture aspheric lens for LD collimation. Such a design, however, cannot correct the fringe effect inherent in the LD's beam profile. Consequently, there presently exists a need for a simple LD based optical system for use in flow cytometers that can reliably produce a focused elliptical beam with near Gaussian shape along its minor axis and a width along major axis.

In accordance with one aspect of the present disclosure, the optical subsystem 50 may include a LD 501 that, as depicted in greater detail in FIG. 2, emits a diverging beam of light from an edge thereof. As more graphically depicted in FIGs 2 and 2A, the diverging beam of light has an elliptically shaped cross-sectional profile with both a major axis, a.k.a. the fast axis, and a minor axis, a.k.a. the slow axis. The diverging beam of light emitted from the LD 501 may impinge upon a collimating lens 502 which converts the diverging beam of light emitted by LD 501 into a collimated beam of light having an elliptical cross-section. Although not essential, the optical subsystem 50 may also include an optional mirror 503 positioned to direct the collimated elliptical beam of light towards the composite microscope objective 60. A plano-convex lens 504, positioned near the composite microscope objective 60, may reduce the major axis of the elliptically shaped beam of light

that is oriented perpendicular to the direction in which the liquid sample and the surrounding liquid sheath flow through the viewing zone within the composite microscope objective 60. At the viewing zone, the width of the elliptically shaped beam of light:

1. perpendicular to the direction in which the liquid sample flow passes through the viewing zone may be slightly less than the width of the liquid sheath flow; while

2. still sufficiently wide so particles in the sample flow pass through a nearly flat portion of the elliptically shaped beam of light at the beam's maximum intensity.

In accordance with one aspect of the present disclosure, it is apparent to those skilled in the art that the plano-convex lens 504 may be replaced by other types of optical elements such as an achromatic doublet lens or combination of spherical lenses, cylindrical lenses, and/or prism pairs. Alternatively, the mirror 503 and the lens 504 may also be replaced by a concave mirror. For polarization sensitive applications of the flow cytometer 40, an optional polarization conditioning element, such as a half-wave plate, may also be placed in the collimated section of the beam of light extending from the collimating lens 502 to the lens 504. Finally, before passing through the viewing zone the beam of light may pass through a high power cylindrical lens 505, positioned adjacent to the viewing zone. As depicted in FIG. 1, the axis of the cylindrical lens 505 is oriented perpendicular to the direction in which the liquid sample flow passes through the viewing zone, and the focal length of the cylindrical lens 505 produces a tight focusing of the beam of light's minor axis at the viewing zone.

An advantage of the optical subsystem 50 in comparison with conventional LD based optical subsystem may be discerned more clearly in FIGs. 2 and 2A. Most commercially available laser diodes suitable for use in a flow cytometer emit a beam of light from an edge thereof. As depicted in FIG. 2, a gain section 509 of such a LD chip 510 is highly confined in the transverse direction indicated by an arrow 511. Consequently, to achieve high output power LD manufacturers often sacrifice beam quality, particularly along the transverse or fast axis direction that is oriented parallel to the arrow 511. FIG. 2A shows this characteristic of light emitted from a LD wherein multiple fringes 512 due to gain confinement are clearly visible at the far field in the minor axis direction of the emitted beam of light. It should be noted that the fringes 512 appearing in the illustration of FIG.

2A contain only a minor amount of total energy in the beam of light, and therefore have little impact on the conventional M-square characterization of the corresponding beam profile. However, as discussed in greater detail below, the fringes 512 do have a detrimental effect on the performance of conventional flow cytometers. Alternatively, gain confinement along the slow axis direction of an edge emitting LD that is oriented perpendicular to the arrow 511 is much more relaxed. Consequently, as shown in FIG. 2A, the far field beam profile is much smoother along the slow axis of the LD's beam of light.

FIG. 3A depicts a conventional LD based optical subsystem for a flow cytometer. Those elements depicted in FIG. 3A that are common to the optical subsystem 50 illustrated in FIG. 1 carry the same reference numeral distinguished by a prime (') designation. As depicted in FIG. 3A, the conventional optical subsystem orients the fast axis of the LD 501 parallel to the direction in which the liquid sample flow passes through the viewing zone. In its most simplified configuration, the elliptical beam profile of the LD 501' is directly transposed by the spherical focusing lens 504' into the viewing zone. In an attempt to achieve optimal aspect ratio for the focused beam of light, various different conventional LD based optical subsystems have also included beam shaping optical elements in addition to those depicted in FIG. 3A.

The detrimental effect of fringes 512 along the fast axis of the LD 501' for conventional optical subsystem configurations clearly appears in the light scattering time profile depicted in FIG. 3B. Since scattering or fluorescence intensity is directly proportional to the local laser power impinging upon a particle, any fine structure in the beam of light's profile along the direction in which the liquid sample flow passes through the viewing zone will appear in the time profile of the signal produced by the flow cytometer. Such structures in the time profile are indistinguishable from signals generated by small particles, and will therefore cause the flow cytometer to trigger falsely and misidentify particles. In addition, the fringes 512 will also lead to uncertainty in the measurement of other cytometric parameters, such as in the area and width of the pulse depicted in FIG. 3B.

FIGs. 4A and 4B depict a yet another prior art optical subsystem for LD based flow cytometric applications disclosed in the '019 patent identified previously. Those elements

depicted in FIGs. 4A and 4B that are common to the optical subsystem 50 illustrated either in FIG. 1 or 3A carry the same reference numeral distinguished by a double prime (") designation. As depicted in FIGs. 4A and 4B, by orienting the slow axis of the LD 501" parallel to the direction in which the liquid sample flow passes through the viewing zone the optical subsystem depicted in FIGs. 4A & 4B effectively overcomes the problem caused by the fringes 512 as described above. Unfortunately, the beam-diffusing element 513" placed before the spherical focusing lens 504" in FIGs. 4A and 4B to diffuse the beam of light perpendicular to the direction in which the liquid sample flow passes through the viewing zone produces a highly astigmatic beam of light near the viewing zone. Specifically, focusing this astigmatic beam of light at the viewing zone in the direction in which the liquid sample flow passes through the viewing zone increases the width of the beam of light perpendicular to the direction in which the liquid sample flow passes through the viewing zone so the beam's width become similar to or even wider than the sheath flow. Consequently, the optical subsystem depicted in FIGs. 4A and 4B not only diminishes the amount of light energy impinging upon particles flowing through the viewing zone, the optical subsystem also increases undesirable scattering of light from the interface between the liquid sheath flow and adjacent parts of the composite microscope objective 60.

FIG. 5 highlights the main differences between the optical subsystem disclosed in the '019 patent and the optical subsystem 50 depicted in FIG. 1. Instead of placing an out-of-plane beam-diffusing element 513" before the spherical beam focusing lens 504 as shown in FIG. 4, the high power cylindrical lens 505, depicted in FIGs. 5A and 5B as a cylindrical plano-convex lens, may be placed along the beam of light after the spherical beam focusing lens 504 and may be juxtaposed with the composite microscope objective 60. As shown in FIGs. 5A and 5B, the cylindrical lens 505 may focus the minor axis of the beam of light in the viewing zone while leaving the major axis of the beam of light essentially unchanged. Consequently, the optical subsystem 50 depicted in FIGs. 1, 5A and 5B may establish a beam of light profile at the viewing zone which is elliptical with:

1. a tightly focused minor axis that spans across the combined liquid sample and sheath flows; and

2. a smooth minor axis profile in the direction of the combined liquid sample and sheath flows that is the Fourier conjugate of the far field beam profile along the slow axis of LD 501.

Meanwhile, as shown in FIG. 5B, the out-of-plane beam width may be unaffected by the cylindrical lens 505. FIG. 5C shows a measured time profile of light scattered from a micro particle using the optical subsystem 50 depicted in FIGs. 1, 5A and 5B. The LD 501 used in making the measurement presented in FIG. 5C is the same as that used in generating the measured time profile of light scattered from a micro particle appearing in FIG. 3B. As shown in FIG. 5C, the side lobes caused by the fringes 512 along the fast axis of the LD 501 no longer have any material effect on performance of the flow cytometer 40.

FIG. 5D illustrates a perspective view of the cylindrical lens 505 of the LD based optical subsystem 50 coupled with the composite microscope objective 60 in accordance with some embodiments of the present disclosure. A beam of light may pass through the cylindrical lens 505 and a cuvette 603 of the microscope objective 60 substantially along the z axis and establish a beam profile 524 on a x-y plane at the viewing zone inside a flow channel 604 of the composite microscope objective 60.

FIG. 5E illustrates an enlarged view of the beam profile 524 shown in FIG. 5D in accordance with some embodiments of the present disclosure. FIG. 5E shows that the minor axis of the beam of light may be along the y axis and substantially parallel to the direction of liquid sample and sheath flows and the major axis of the beam of light may be along the x axis and substantially perpendicular to the direction of liquid sample and sheath flows.

FIG. 6 depicts yet another alternative diode laser based optical subsystem in accordance with some embodiments of the present disclosure adapted for use in a flow cytometer. Those elements depicted in FIGs. 6 and 6A that are common to the optical subsystem 50 illustrated in FIGs. 1, 5A and 5B carry the same reference numeral distinguished by a triple prime (‴) designation. The optical subsystem 50‴ depicted in FIGs. 6A and 6B is almost identical to that shown in FIGs. 1, 5A and 5B except that the viewing zone occurs without a composite microscope objective 60 because it occurs in a free-flowing jet stream 519 that includes both the sample and sheath flows that is emitted

from a nozzle 518. Consequently, for the configuration of the optical subsystem 50''' depicted in FIGs. 6A and 6B, the high power cylindrical lens 505 is detached from the viewing zone that is located within the jet stream 519.

In the exemplary embodiments of the present disclosure depicted in FIGs. 1, 5A, 5B, 6A and 6B, the minor axis, i.e. the slow axis, of the LD 501 is substantially oriented perpendicular to the direction in which the liquid sample flow passes through the viewing zone. However, it will be apparent to those skilled in the art that using an alternative optical configuration the major axis, i.e. the fast axis, of the LD 501 may be reoriented to be perpendicular to the direction in which the liquid sample flow passes through the viewing zone. FIG. 7 depicts one example of such an alternative configuration of optical elements. Those elements depicted in FIG.7 that are common to the optical subsystem 50 illustrated in FIGs. 1, 5A, 5B, 6A and 6B carry the same reference numeral distinguished by a quadruple prime ("") designation. As shown, the slow axis of the LD 501"" is oriented in the z-direction. The beam of light emitted from the LD 501"" is then rotated to the in-plane y-direction by a pair of ninety degrees (90°) reflection mirrors 523a and 523b. In the illustration of FIG. 7, a normal to the first elliptically-shaped light beam reorienting mirror 523a is oriented in the x-y plane at forty-five degrees (45°) to the x-axis, and a normal to the second elliptically-shaped light beam reorienting mirror 523b is oriented in the y-z plane at forty-five degrees (45°) to the z-axis.

Composite Microscope Objective 60

Modern flow cytometers include a spatial filter, usually either a mechanical pinhole or a large core optical fiber, located at an image location of an objective lens to prevent undesired background light from entering the cytometer's detector(s). Because particles remain in the cytometer's viewing zone for a few microseconds, microscope objectives with large numerical aperture must be used to maximize light collection efficiency. To support multiple spatially separated excitation laser beams in flow cytometers, as disclosed in United States Patent No. 4,727,020, it is also desirable to use an objective with large field of view. In order to achieve these goals, United States Patent Nos. 6,5100,07 and 7,110,192 disclose an objective design using a modified apochromat with a gel-coupled or epoxy bonded near hemisphere lens as the optical element closest to the sample that is followed

by multiple meniscus lenses. While such microscope objectives provide both a satisfactory numerical aperture and field of view, they significantly sacrificed image quality thereby:

1. limiting effective use of the spatial filter; and

2. exhibiting poor background light discrimination.

Further, such refractive microscope objectives are bulky, expensive to manufacture and often exhibit severe chromatic aberration. To overcome these limitations, Published Patent Cooperation Treaty ("PCT") Patent Application No. WO 01/27590 discloses an alternative objective design based on a spherical concave mirror. The design offers large numerical aperture and good image quality along the optical axis. However, due to its poor off-axis characteristics, such a design is unsuitable for flow cytometers having multiple, spatially separated laser beams.

FIG. 8 depicts one embodiment in accordance with the present disclosure for the composite microscope objective 60 depicted in FIGs. 1, 5A, 5B and 7. As depicted in FIG. 8, the composite microscope objective 60 may image a viewing zone that is located inside a prismatically-shaped glass cuvette 603 within a small flow channel 604, that may have a rectangular cross-sectional shape, through which passes the particle carried by combined liquid sample and sheath flows. A plano-concave back-surface mirror 601 included in the composite microscope objective 60 may be made of an optically transparent material that may have a refractive index similar to that of the glass cuvette 603, such as glass or optical quality plastics. To minimize optical loss, the back-surface mirror 601 may include a flat front surface that is optically coupled to an abutting flat surface of the prismatically-shaped cuvette 603. Optical coupling of the back-surface mirror 601 to the cuvette 603 may employ an index-matching gel, optical adhesive or direct optical bonding. Alternatively, the back-surface mirror 601 may also be formed integrally with the cuvette 603.

The composite microscope objective 60 may also include a plano-aspheric corrector plate 602 that is also made of an optically transparent material that may have a refractive index similar to that of the glass cuvette 603, such as glass or optical quality plastics. To reduce optical loss, a flat surface of the corrector plate 602 may be optically coupled to an abutting flat surface of the prismatically-shaped cuvette 603 on a face thereof that is diametrically opposite to the back-surface mirror 601. Optical coupling of the corrector

plate 602 to the cuvette 603 may employ an index-matching gel, optical adhesive or direct optical bonding. The aspheric surface of the corrector plate 602 furthest from the corrector plate 602 may carry an anti-reflective coating to reduce optical transmission loss, although such a coating is not a mandatory requirement for a composite microscope objective 60 in accordance with some embodiments of the present disclosure. The shape of the aspheric surface of the corrector plate 602 is similar to that in a classical Schmidt camera, (Schmidt, B., *Mitt. Hamburg Sternwart* 7 (36) 1932). As known by those skilled in the art, the corrector plate of a Schmidt camera includes a circularly shaped neutral zone where the corrector plate does not deviate rays of light passing through the plate. For use in the composite microscope objective 60, outside of the neutral zone of the corrector plate 602, where the plate thickness is thinnest, the corrector plate 602 may have negative optical power while inside the neutral zone the corrector plate 602 may have positive optical power. The exact shape of the aspheric corrector plate 602 may be readily obtained using any commercially available optical ray tracing tool by any person having ordinary skill in the art. Note that in the flow cytometer 40, the beam of light generated by the optical subsystem 50 depicted in FIGs. 1, 5A, 5B and 7 enters the cuvette 603 perpendicularly to the flow channel 604 through one (1) of the two (2) faces of the cuvette 603 that do not abut the back-surface mirror 601 or corrector plate 602.

Combined Microscope Objective 65

FIG. 8A illustrates a perspective view of a combined microscope objective 65 in accordance with some embodiments of the present disclosure. The combined microscope objective 65 may include the composite microscope objective 60, as illustrated in FIG. 8 and the cylindrical lens 505. The cylindrical lens 505 may direct a beam of light to the viewing zone in the flow channel 604 to illuminate particles in the sample flow. After particles are illuminated in the viewing zone, the composite microscope objective 60 then may collect imaging light scattered from and fluoresced by particles within the view zone.

FIG. 8B illustrates a perspective view of the combined microscope objective 65 coupled with a flow cell 619 in according with some embodiments of the present disclosure. Liquid sample 623 may be pumped up from a sample tube 621 into a flow section 620 of the flow cell 619 by a pump 624. The pump 624 may be the peristaltic pump

80, as illustrated in FIG. 17. Liquid sheath 622 may be also pumped into the flow section 620 of the flow cell 619. The pump for pumping the liquid sheath 622 into the flow cell 619 may be a part of the fluidic system 70, as illustrated in FIG. 14 or 15. The liquid sample 623 may be combined with the liquid sheath 622 in the flow section 620 of the flow cell 619 and then hydro-dynamically focused within the viewing zone inside the flow channel 604 of the combined microscope objective 65. The combined microscope objective 65 or the composite microscope objective 60 may be positioned on the flow cell 619. Persons skilled in the art may also refer to a combination of the microscope objective 65 or the composite microscope objective 60 and the flow cell 619 as a flow cell. The cross-sectional area of the flow section 620 at the top of the flow cell 619 may be smaller than the cross-sectional area of the flow section 620 at the bottom of the flow cell 619 to facilitate hydrodynamic focusing of the liquid sample 623 in the viewing zone. It should be noted that the various aspects of the present disclosure are not limited to specific direction of liquid sheath or sample flow and specific shape of the flow cell or the microscope objective.

FIG. 9A depicts the result of ray tracing for the embodiment of composite microscope objective 60 illustrated in FIG. 8. As depicted in FIG. 9A, scatter and fluorescence emissions from three (3) spatially separated locations in the flow channel 604 near the center of the cuvette 603 may:

1. initially propagate toward back-surface mirror 601 and pass first through the cuvette 603 to be internally reflected by the back-surface mirror 601;

2. then pass through the cuvette 603;

3. subsequently pass through the aspheric corrector plate 602; and

4. finally forms three (3) distinct images near an image plane 605.

Note that rays traversing the composite microscope objective 60 depicted in FIG. 9A are nearly optically-uniform and that light emitted near the center of the cuvette 603 traverses the corrector plate 602 at near normal incidence. Consequently, the composite microscope objective 60 introduces very little chromatic dispersion in light emitted near the center of the cuvette 603.

Further, it is well known in the astrophysics community that Schmidt camera offers the unparalleled combination of a fast focal ratio and a large field of view with near diffraction limited optical performance. The principal drawback in a conventional Schmidt camera is that the image surface lies inside the instrument. For the composite microscope objective 60, light near the center of the cuvette 603 propagates opposite to that of a conventional Schmidt camera and therefore the image surface lies outside the composite microscope objective 60. Consequently, the present disclosure takes full advantage of the optical performance of the Schmidt camera without experiencing its limitation. FIGs. 9B1 through 9B3 depict spot diagrams near the image plane 605 for three (3) emission locations, 606, 607, 608 in viewing zone within the flow channel 604 that may be separated 150 micron from each other. The diameters of all images depicted in FIGs. 9B1 through 9B3 may be less than 35 microns.

Light emitted from the viewing zone within the flow channel 604 of the composite microscope objective 60 depicted in FIGs. 8 and 9A that traverses the aspheric corrector plate 602 may suffer from a small amount of chromatic aberration. FIG. 10 depicts an alternative embodiment for the composite microscope objective 60 depicted in FIGs. 1, 5A, 5B and 7 in accordance with some embodiments of the present disclosure. Those elements depicted in FIG. 10 that are common to the composite microscope objective 60 illustrated in FIGs. 8 and 9A carry the same reference numeral distinguished by a prime (') designation. The shapes of the back-surface mirror 601' and the aberration corrector plate 602' depicted in FIG. 10 are modified slightly to produce collimated afocal images of the emission locations near the viewing zone within the flow channel 604'. In FIG. 10 the composite microscope objective 60' may include a chromatic compensating doublet lens 609 inserted between the corrector plate 602' and the image plane 605'. In addition to focusing the light emitted from the corrector plate 602' onto the image plane 605', the doublet lens 609 may also serve to further reduce the residual chromatic aberration introduced by the aspheric corrector plate 602'.

It is not essential that the flat surface of the corrector plate 602 to be optically coupled to the cuvette 603. FIG. 11 depicts an alternative embodiment of the composite microscope objective 60 in accordance with some embodiments of the present disclosure. Those elements depicted in FIG. 11 that are common to the composite microscope

objective 60 illustrated in FIGs. 8 and 9A carry the same reference numeral distinguished by a double prime (") designation. FIG. 11 depicts the aberration corrector plate 602" optically decoupled from the cuvette 603". Although not essential for operation of the composite microscope objective 60", to improve the light transmission efficiency both surfaces of the corrector plate 602" and the exposed flat surface of the cuvette 603" may carry an anti-reflectively coating. It is understood that the corrector plate 602" shown in FIG. 11 may be held in fixed relationship to the combined back-surface mirror 601 and cuvette 603 by a mechanical support not depicted in FIG. 11. Similar to the composite microscope objective 60 and 60' depicted respectively in FIGs. 9A and 10, the composite microscope objective 60" with detached corrector plate 602" may be configured to provide either finite focal length image, or an afocal system which in turn is focused to a finite distance image plane by an additional chromatic compensating doublet lens 609.

FIG. 12 depicts yet another alternative embodiment of the composite microscope objective 60. Those elements depicted in FIG. 12 that are common to the composite microscope objective 60 illustrated in FIGs. 8, 9A and 11 carry the same reference numeral distinguished by a triple prime ("') designation. The composite microscope objective 60"' depicted in FIG. 12 is adapted for collecting scatter and fluorescence emissions from cells or other microscopic particles carried in the jet stream 519 emitted by the nozzle 518. The composite microscope objective 60"' may include a concave, spherically shaped, front surface mirror 610 and an aberration corrector plate 612. The front surface mirror 610 may be made of glass or other types of hard material with a highly reflective coating on the concave surface 611 or made of metal with polished concave surface 611. Similar to the corrector plate 602, the plano-aspheric corrector plate 612 may be made of a thin piece of transparent material, such as glass or optical quality plastics. The aspheric surface may be formed on either side of the corrector plate 612. Both surfaces of the corrector plate 612 may be coated with an anti-reflective coating to reduce optical transmission loss, although such a coating is not a mandatory requirement for a corrector plate 612 in accordance with some embodiments of the present disclosure. It is understood that the front surface mirror 610 and the corrector plate 612 may be held in fixed relationship to each other by a mechanical support not depicted in FIG. 12. Scatter and fluorescence light emitted from cells or other types of microscopic particles in the viewing zone inside the jet stream 519

may be reflected by the concave surface 611 of the front surface mirror 610. The aberration due to reflection from the concave surface 611 may be corrected by the corrector plate 612 after light traverses through the corrector plate 612. It is understood by those having skill in the art that the composite microscope objective 60''' may be configured to provide either a finite focused image similar to that depicted in FIG. 9A, or a collimated afocal image which is focused at finite distance from the composite microscope objective 60''' by a chromatic aberration correction doublet similar to the doublet lens 609 depicted in FIG. 10.

FIG. 13 depicts an adaptation of the composite microscope objective 60 for imaging specimens fixed to the surface of a transparent substrate such as a glass slide. Those elements depicted in FIG. 13 that are common to the composite microscope objective 60 illustrated in FIGs. 8, 9A and 11 carry the same reference numeral distinguished by a quadruple prime ("") designation. The composite microscope objective 60"" depicted in FIG. 13 may include two (2) optical elements, one a plano-concave back surface mirror 617 made of a transparent material, such as glass or optical quality plastics, and an aberration corrector plate 618. As depicted in FIG. 13, the specimen to be imaged may be fixed to a front surface 615 of a transparent, usually glass slide 616. The slide 616 may be optically coupled, for example, using a thin layer of index matching fluid, to the flat surface of the back surface mirror 617. Scatter and fluorescence light emitted by the specimen may:

1. initially propagate through the slide 616 and the back surface mirror 617;

2. be internally reflected by the back surface mirror 617 back through the slide 616;

3. then pass through the corrector plate 618; and

4. finally form an image at an image plane that is located beyond the corrector plate 618.

Fluidic Subsystem 70

The performance of a flow cytometer depends critically on a stable liquid sheath flow. In particular, flow cytometers that have multiple spatially separated excitation laser beams or perform droplet sorting rely on a constant velocity of the liquid sheath flow for timing synchronization. As disclosed in United States Patent No. 5,245,318, conventional

flow cytometers provide a stable liquid sheath flow by using an airtight fluidic system that either:

1. applies constant air pressure in a sheath liquid reservoir to push the fluid through the flow cell; or

2. by sucking the fluid from the sheath liquid reservoir through the flow cell using a vacuum pump.

These systems are bulky, expensive to manufacture, and prone to failure. More recently, United States Patent No. 8,187,888 discloses including a sheath liquid subsystem that pumps the liquid sheath flow from the sheath liquid reservoir into the viewing zone and a waste sheath liquid pump that pumps waste sheath liquid from the viewing zone into the waste tank. Although it appears that the disclosed sheath liquid subsystem has never been used in velocity critical flow cytometers, this patent reports that the disclosed sheath liquid subsystem overcomes most of the drawbacks of conventional sheath liquid flow stabilization by:

1. damping pump pulsations by locating:

   a. one fluidic capacitor between the sheath liquid pump and the flow cell; and
   b. another fluidic capacitor between the flow cell and the waste pump; and

2. a pump controller whose operation is responsive to a pressure sensor that measures the pressure difference between the inlet and outlet of the flow cell.

However, the disclosed sheath liquid subsystem has other limitations. For example, the pressure sensor located near the outlet of the flow cell could be a potential source of contamination.

FIG. 14 depicts a fluidic subsystem 70 in accordance with some embodiments of the present disclosure that includes a sheath liquid reservoir 702 and a liquid pump 701 that draws sheath liquid from the sheath liquid reservoir 702. The liquid pump 701 may be a diaphragm pump, a peristaltic pump, a piston pump, or any types of continuous fluid pump. An outlet of the liquid pump 701 may connect to an inlet of a T-coupling 703 that receives sheath liquid from the liquid pump 701. The T-coupling 703 may have two (2) outlets. The first outlet may connect to a bypass conduit 710 for returning a fraction of the sheath liquid

received by the T-coupling 703 from the liquid pump 701 back to the sheath liquid reservoir 702. Returning a fraction of the sheath liquid received by the T-coupling 703 from the liquid pump 701 back to the sheath liquid reservoir 702 is advantageous for two (2) reasons.

1. As depicted in FIG. 14, the bypass conduit 710 is left open to the surrounding atmosphere which effectively dampens pulsation to thereby significantly reducing the pulsation inherent in the operation of the liquid pump 701.

2. Returning a fraction of the sheath liquid received by the T-coupling 703 from the liquid pump 701 back to the sheath liquid reservoir 702 also effectively reduces the throughput of the liquid pump 701 thereby allowing the use of comparatively high flow rate, low cost pumps in the flow cytometer 40.

Denote the flow resistance of the bypass conduit 710 as *"r"* and the flow resistance of path from the T-coupling 703 to the flow channel 604 of the cuvette 603 as *"R."* The output resistance to the sheath pump R_p is then equal to:

$$R_p \frac{rR}{r+R} \quad (1)$$

Since $R >> r$, the behavior of the liquid pump 701 is therefore dominated by the resistance of the bypass conduit 710 whose fluid dynamic properties may be temperature insensitive. Thus, the configuration of the fluidic subsystem 70 depicted in FIG. 14 may also provide a simple mechanism for achieving a temperature insensitive sheath liquid flow to the flow channel 604. As depicted in FIG. 14, the second outlet of the T-coupling 703 connects to the flow channel 604 that extends through the cuvette 603 first via a small reservoir capsule 704 and then via a filter cartridge 705. As depicted in FIG. 15, a piece of tubing 704', which may be, for example, about 4 ft. long may be substituted for the small reservoir capsule 704. During initialization of the fluidic subsystem 70, some air becomes trapped in the filter cartridge 705 near its inlet which as depicted in FIG. 15 is located above an outlet of the filter cartridge 705. The air trapped in the filter cartridge 705 may act as an additional fluidic capacitor effectively reducing to negligible level the pulsation in sheath liquid emitted into the flow channel 604. Due to the large fluidic resistance at the flow channel 604, the air trapped inside the filter cartridge 705 becomes compressed. When

the liquid pump 701 is turned off, a trapped in the filter cartridge 705 is pushed back towards the T-coupling 703 analogous to a discharging capacitor. Without the small reservoir capsule 704, some air ejected from the filter cartridge 705 reaches the bypass conduit 710 due to its low fluidic resistance, and will be pushed out of the fluidic subsystem 70 once the liquid pump 701
is turned on again. Without additional air supply, such a scenario will repeat until most of the air becomes purged from the fluidic subsystem 70 causing the filter cartridge 705 to lose its effectiveness as a pulsation damper. The purpose of the small reservoir capsule 704 or the piece of tubing 704' is therefore to provide a reservoir for isolating the filter cartridge 705 from the bypass conduit 710 ensuring that air trapped inside the filter cartridge 705 remains within the fluidic subsystem 70 despite repeated on-off operations of the liquid pump 701.

The pulsation damping effect of the trapped air near the inlet of the filter cartridge 705 is clearly evident in the histograms depicted in FIGs. 16A and 16B. FIG. 16A depicts measured particle flight times at the flow channel 604 when a pocket of air is trapped near the inlet of the filter cartridge 705. FIG. 16B depicts measured particle flight times at the flow channel 604 when the trapped air is purged from the fluidic subsystem 70. The result depicted in the histograms of FIGs. 16A and 16B is made using two (2) knife edge shaped laser beams focused near the center of the flow channel 604 that are spaced approximately 200 micrometers apart. The horizontal axis of the FIGs. 16A and 16B is the flight time a particle takes from one laser beam to the other measured by recording the peak arrival time of light scattered from the particle at ninety degrees (90°) from the excitation beams. In both cases, the average flight time for particles to cross the two laser beams is the same. As shown in FIG. 16A, when the filter cartridge 705 retains some air, all particles take about the same amount of time to cross the two laser beams. If the filter cartridge 705 retains no air, as shown in FIG. 16B, the distribution of flight times not only broadens, but also becomes bimodal. In other words, some particles take less time while others take longer than average amount of time to cross the two laser beams, a phenomenon that can be easily attributed to sheath liquid velocity pulsation at the flow channel 604.

In the embodiments of the present disclosure discussed so far, the fluidic resistance along bypass conduit 710 as well as between the T-coupling 703 and the flow channel 604

may not be adjustable. As should be apparent to those ordinary skilled in the art, flow restrictors such a fixed restrictor or adjustable valves 712, 712' and 711, 711' and may be advantageously inserted in the bypass conduit 710 and between the T-coupling 703 and the flow channel 604 to permit adjusting the flow rate through the flow channel 604. Alternatively, the velocity of sheath liquid flowing through the flow channel 604 may also be adjusted using a liquid pump 701 that is driven by a variable speed brushless DC motor.

Peristaltic Pump 80

Peristaltic pumps are volumetric pumps in which a set of linearly or circularly moving rollers progressively compress a compressible tube to propel the fluid through the tube. Peristaltic pumps are widely used particularly to pump clean/sterile or aggressive fluids to avoid cross contamination with exposed pump components. Conventional peristaltic pump exhibits a pulsation. Each time a roller rolls off the tube near the pump outlet, caused by the temporary increase of tube volume when the compressed tube expands back to its original shape. The pulsation is undesirable in applications that require smooth flow. Many attempts have been made in the past to reduce the pulsation. For example, United States Patent Nos. 3,726,613 and 3,826,593 introduced a cam operated pusher which synchronously exerts an external pressure on the tube to compensate for the tube expansion. In United States Patent No. 4,834,630, a plurality of tubes mounted on segmented rollers are joined together at the pump inlet and outlet by T-shaped couplers such that pulsations from individual tubes would be reduced by averaging. United States Patent No. 7,645,127 proposed a pump tube with slightly larger inner diameter near the inlet so that the tube decompression near the pump outlet is compensated by the compression of a larger volume tube near the inlet. The various methods either significantly increased the complexity of the peristaltic pump or had limited success in reducing the pulsation effect.

A peristaltic pump 80 in accordance with some embodiments of the present disclosure is illustrated in FIG. 17. The pump may include a housing 809 with arcuate curved track 808, three rollers 810, 811 and 812 attached to a rotor 816 rotatable within the housing 809, and a compressible tube 807 sandwiched between the arcuate curved track 808 of the housing 809 and the rollers 810, 811 and 812, in particular at the surface 814 of

rollers 810, 811 and 812. As depicted schematically in FIGs. 18A through 18D, the rollers 810, 811 and 812 of the peristaltic pump 80 are spaced at substantially equal angular distances, separations or spacings from each other around the perimeter of the rotor 816. The rollers 810, 811, 812 may rotate about a longitudinal axis thereof, so that limited friction occurs between the rollers and the compressible tube. This may also apply for the subsequently described rollers. For simplicity, it is assumed in the following discussions that the rotor 816 rotates counterclockwise, although it is to be understood that the discussions apply equally well to a peristaltic pump with clockwise rotating rotor. The compressible tube 807 of the housing 809 may be divided into several sections:

1. an open section between point 801 and point 806 where the compressible tube 807 experiences no compression;

2. a pump inlet section between point 801 and point 802 where the compressible tube 807 is progressively compressed until fully closed when a roller rolls over the section;

3. two pumping sections between point 802 and point 803, as well as between point 804 and point 805 wherein the compressible tube 807 is fully closed by the roller;

4. a recess section between point 803 and point 804 in which the compressible tube 807 progressively expands from fully closed to fully open as a roller rolls through the expansion part of the recess section from point 803 to point 813;

5. then the compressible tube 807 is progressively compressed to fully closed as a roller rolls through a compression part of the recess section from point 813 to point 804; and

6. the exit section between point 805 and point 806 where the compressible tube 807 progressively expands from fully closed to fully open as a roller rolls through the section.

In other words, when a roller rolls anticlockwise over the compressible tube 807 from inlet point 801 to outlet point 806, the inner gap of the compressible tube 807 may:

1. progressively decrease from fully open at point 801, to fully closed at point 802 and remain closed until point 803;

2. then progressively expand back to fully open at point 813;

3. then progressively decrease to fully closed at point 804, and remain closed until the roller reaches point 805; and

4. finally progressively expand back to fully open at point 806.

The size of the gap inside the compressible tube 807 is schematically illustrated in FIGs. 18A through 18D as the spacing between dashed circle and the solid compressible tube 807. As illustrated in FIGs. 18A through 18D, in this embodiment of the peristaltic pump 80, the angular distances, separations or spacings between points 801 and 803, points 802 and 813, point 813 and 805, as well as between point 804 and 806 may be identical to the angle between adjacent rollers. As a result, when the roller 810 rolls through the pumping section from point 804 to point 805, as depicted in FIGs. 18A through 18B, its interaction with the compressible tube 807 may completely determine the fluid flow rate of the peristaltic pump 80. Once the roller 810 reaches the exit section between point 805 and 806, as shown in FIG. 18C, the compressible tube 807 underneath the roller 810 may start to progressively expand and a gap may start to grow. Meanwhile, the roller 811 may arrive at the compression part of the recess section and start to progressively compress the compressible tube 807. In the peristaltic pump 80, the shape of the compression part of the recess section between point 813 and point 804 along the compressible tube 807 is such that the volume of liquid pushed out by the compression of the underneath roller 811 in the compression part of the recess section between point 813 and point 804 may substantially fill the volume created by the compressible tube 807 expansion underneath roller 810 in the exit section between point 805 and point 806. During this period, the compressible tube 807 is partially open underneath both rollers 810 and 811 and completely closed underneath roller 812. Consequently, the pumping action may be mainly delivered by roller 812. In particular, since by design the total volume of liquid in the section of the compressible tube 807 between point 813 and point 816 remains substantially constant during this period, the flow rate of the peristaltic pump 80 in the state shown in FIG. 18C may remain substantially the same as that in the state shown in FIGs. 18A and 18B. Once the roller 810 passes point 806, roller 811 reaches the pumping section between point 804 and point 805. Note there is no physical difference between the rollers 810, 811 and 812, the flow rate of the peristaltic pump 80 may therefore remain substantially constant throughout the entire process.

The mechanism of the pulseless peristaltic pump in accordance with some embodiments of the present disclosure may be understood more clearly if it is viewed along a circular coordinate following the movement of the rollers. Referring to FIG. 19, denote as V the volume of the fluid inside a compressible tube 819 from the outlet to a nearest roller 820 that closes off the compressible tube 819, i.e., the amount of fluid represented by the hatched area 818 shown in FIG. 19. Clearly, V depends on the angular position, θ, of the roller 820, as well as δ, the amount of tube compression exerted by all other downstream rollers.

$$V = V(\theta, \delta_1, \delta_2, ...) \quad (2)$$

Consequently, the flow rate, F, of a peristaltic pump is related to the time derivative of Vc by:

$$-F = \frac{dv}{dt} = \frac{\partial v}{\partial \theta} R + \sum_i \frac{\partial v}{\partial \delta_i} \frac{d\delta_i}{dt} \quad (3)$$

Here R is the rotational speed of the rotor and the subscripts are used to identify multiple downstream rollers. The first term on the right hand side of Eqn. (3) represents the contribution from the roller that closes off the tube. The partial derivative $\frac{\partial v}{\partial \theta}$ is therefore independent of θ. The summation term represents contributions from all other downstream rollers partially compressing the compressible tube 819. Now let ΔS be the cross sectional area change due to the compression of the compressible tube 819 by the roller 817, and L be the length of tube where its cross sectional shape is affected by the tube compression. Then, it is obvious to a person skilled in the art that L is proportional to the tube compression δ, and ΔS proportional to its square, δ^2. Consequently, ΔV, the volume of fluid lost due to the compression of the compressible tube 807 by the roller, follows Eqn. (4):

$$\Delta V \alpha L \cdot \Delta S \alpha \delta^3 = (D - G)^3 \quad (4)$$

where D is the inner diameter of the compressible tube and G is the minimum gap indicated in FIGs. 19, 19A and 19B which is also represented in FIGs. 18A through 18D by the spacing between the dashed circle and the solid track 808 of the housing 809. FIG. 19A and

19B are detailed cross-sectional views orthogonal to the peristaltic pump's tube's length taken along the lines 19A and 19B in FIG. 19 illustrating the tube's partial compression by the roller. FIG. 19A shows the cross-sectional area 818a taken along the line 19A in FIG. 19. FIG. 19B shows the cross-sectional area 818b taken along the line 19B in FIG. 19. Now referring to FIGS. 20A and 20B, in the circular coordinate system, FIG. 20A corresponds to the state of pump shown in FIGs. 18A and 18B. During this period, there is no roller downstream of the roller 810' and the summation term in Eqn. (3) vanishes. FIG. 20B corresponds to the state of the pump shown in FIG. 18C. The compressible tube 807 is closed off by roller 812' and partially compressed by the rollers 810' and 811'. However, volumetric changes introduced by the two rollers 810' and 811' substantially cancel each other. Consequently, the summation term is Eqn.(3) vanishes as well. As a result, the flow rate of the peristaltic pump 80 may remain substantially constant regardless of roller positions.

The shape of the compressible tube 807 satisfying the above requirement can be readily derived from Eqn. (4). Referring to FIG. 18C, if the gaps of the arcuate curved track 808 in the compression part of the recess section between point 813 and point 804, $G_{13,4}$, and in the exit section between point 805 and point 806, $G_{5,6}$, follow the equation:

$$(D - G_{13,4})^3 + (D - G_{5,6})^3 = D^3 \quad (5)$$

then the total fluid volume in the two sections may remain substantially constant, as shown in FIG. 21. In the peristaltic pump 80, the shape of the pump housing 809 may be symmetrical with respect to its center line, such that the entrance half of the pump housing 809 is the mirror image of the exit half of the housing 809, as shown in FIG. 17. The peristaltic pump 80 can therefore be operated both in counterclockwise and clockwise rotation with very little pulsation, although it is understood that the symmetry is not required to realize a pulseless peristaltic pump in accordance with some embodiments of the present disclosure. For example, as long as the gaps of the arcuate curved track 808 in the section between point 813 and point 803, $G_{13,3}$, and in the section between point 802 and point 801, $G_{2,1}$, follow Eqn. (6)

$$(D - G_{13,3})^3 + (D - G_{2,1})^3 = D^3 \quad (6)$$

a peristaltic pump in accordance with some embodiments of the present disclosure will exhibit little pulsation when the rotor 816 rotates clockwise.

FIG. 22 depicts an alternative embodiment of a peristaltic pump in accordance with some embodiments of the present disclosure. Those elements depicted in FIG. 22 that are common to the peristaltic pump 80 illustrated in FIG. 17 carry the same reference numeral distinguished by a prime (') designation. The peristaltic pump 80' may include an arcuate curved track 808' having two (2) recesses 818 and 819, and four (4) rollers 820, 821, 822 and 823. In the embodiment depicted in FIG. 22, the fluid volume loss due to the tube expansion near the outlet of the pump is compensated by the combined effect of the compression of the compressible tube by rollers 820 and 823 near the two recesses 818 and 819.

FIG. 23 depicts yet another alternative embodiment of a peristaltic pump in accordance with the present disclosure. Those elements depicted in FIG. 23 that are common to the peristaltic pump 80 illustrated in FIG. 17 and the peristaltic pump 80' illustrated in FIG. 22 carry the same reference numeral distinguished by a double prime (") designation. The peristaltic pump 80" may include six (6) rollers 820, 821, 822, 823, 824, 825 and an arcuate curved track 808" having two recesses 818" and 819". In the peristaltic pump 80", the fluid volume loss due to the tube expansion near the outlet of the pump is compensated by the action of the roller immediately upstream of the one recess 818" or 819" near the pump outlet.

Pulsation due to the expansion of a compressed compressible tube near the outlet of a peristaltic pump may also be overcome by a peristaltic pump having a programmable rotor speed. FIGs. 24A through 24C illustrate pertinent aspects of an alternative embodiment mechanism for minimizing peristaltic pump pulsation in accordance with the present disclosure for a 3-roller peristaltic pump. As depicted in FIG. 24B, the track 828 is substantially circular between the pump inlet and pump exit section. Consequently, as indicated by the spacing between the dashed circle 829 and the solid curve of the track 828, the compressible tube is completely closed by various ones of the pump's three (3) rollers 825, 826, 827 between the pump inlet and pump outlet. FIG. 24A illustrates schematically in a circular coordinate system the roller positions for the peristaltic pump depicted in FIG.

24B. Since there is only one roller downstream of the one that closes off the tube, Eqn. (3) is much simplified:

$$-F = R(\frac{\partial V_c}{\partial \theta} + \frac{\partial V_c}{\partial \delta}\frac{d\delta}{d\theta}) \quad (7)$$

Here the tube compression $\delta(\theta)$ is explicitly expressed as a function of roller position θ. The terms inside the parentheses represent the change rate of fluid volume with respect to roller position. The first term is the contribution from the roller that closes off the tube, i.e., roller 827 in FIG. 24A, and the second term the contribution from the roller in the exit section. Note that by definition, the volume change rate is negative and the second term inside the parentheses vanishes when there is no roller in the exit section. The dotted curve in FIG. 24C is a representative plot of the negative volume change rate with respect to the position of the roller. The bump along the curve, due to the tube expansion when a roller rolls off the tube near the pump outlet, is the cause for pulsation in conventional peristaltic pumps having a constant rotor speed. However, for the peristaltic pump depicted in FIGs. 24A through 24C, the rotor speed, R, shown as dashed curve in FIG. 24C, may be set to vary in synchronism with the rotor position and inversely proportional to the change rate of the fluid volume. Consequently, the flow rate of the pump, which is the product of the rotor speed and the change rate of the fluid volume, may remain constant, as indicated by the solid line at the top of FIG. 24C. Note the terms inside the parentheses of Eqn. (7) may be uniquely determined by the mechanical structure of the pump. The rotor speed profile can therefore be readily generated from the shape of the track 828 in accordance with Eqn. (4). To those skilled

in the art, there are many ways to realize a programmable rotor, for example, with stepping motor or DC servo motor.

WDM Device 90

In many multicolor fluorescence detection instrumentations, such as flow cytometers, (Practical Flow Cytometry, Howard M. Shapiro, Wiley (2003) ISBN 0471411256), the fluorescence light emitted from the object of interest is:

1. collected by a microscope objective;

2. reimaged through a small pinhole or a multimode optical fiber;

3. then collimated and separated into multiple colored bands; and

4. finally detected by photo detector, such as photomultiplier tube (PMT), PIN photodiode or avalanche photodiode (APD).

A PMT is essentially a special type of electron tube. This "pre-semiconductor age" device is bulky and expensive. In addition, it has poorer quantum efficiency and less reproducible spectral response than silicon based semiconductor detectors, particularly in the biologically important red to near infrared spectral region. Despite the disadvantages, PMT has excellent noise characteristics. For example, the dark current of a typical 13mm PMT (e.g., the R9305 from Hamamatsu Corporation of Japan) is only about 1nA. In contrast, an APD's dark current would be 10 times greater even if its active area were reduced to 1/20th of that of the PMT. As a result, PMT has been the de-facto low-level light detector in many commercial fluorescence detection flow cytometers. Only in certain scientific applications where event rate is low and dark current may be discriminated against by expensive photon- counting techniques that the PMT has been replaced by APD detectors. (c.f., High-Throughput Flow Cytometric DNA Fragment Sizing, A.V. Orden, R.A. Keller, and W. P.Ambrose, *Anal. Chem.*, 2000, 72 (1), p 37–41). More recently, a Geiger mode APD array was also promoted as PMT replacement. (For example, the multi pixel photon counter of Hamamatsu Photonics of Japan and the solid-state photomultiplier of SensL Inc. of Ireland.) These detectors, however, also have high dark current and are nonlinear at high event rate.

The only industry where APD has found wide acceptance is in optical communication. It is known that if the APD's active area is reduced to less than $1mm^2$, the corresponding dark current will be reduced to the same level as a PMT. In optical communication, the light is a laser beam out of single mode optical fiber. Such a beam can be easily collimated then focused down to an area much smaller than $1mm^2$. It should be noted that the color separation devices used in the fluorescence light detection instruments, as described in United States Patent Vo. 6,683,314 and references therein, are almost identical in function and architecture to the wavelength division multiplexers (WDM) widely used in optical communication, as described in United States Patent Nos. 4,482,994,

and 5,786,915. A fundamental reasons preventing the use of small area APD in fluorescence detection instrumentation is the well-known theorem of etendue conservation: the fluorescence light coming through a pinhole or multimode optical fiber is an extended light source with an etendue hundreds of times greater than that of a laser beam out of a single mode optical fiber. Consequently, as illustrated in FIG. 26, it cannot be collimated over an extended distance unless the diameter of the beam is significantly expanded. Unfortunately, the larger the beam diameter, the greater the technical challenge to focus it down to a small spot. Since efficient color separation can only be accomplished economically with collimated light beam, small area APD has not been considered viable for multicolor fluorescence light detection applications. Clearly, a technology capable of collimating a large etendue light beam over an extended distance without significantly expanding the beam diameter would be highly desirable. Such a technology would enable a WDM like device for fluorescence light detection with characteristics comparable to low noise semiconductor detectors.

FIG. 25 shows the optical ray trace for an exemplary 6 port wavelength division multiplexer of the present disclosure using zig-zag configuration. As shown in FIG. 25, fluorescence light going through a pinhole or emitted from the facet of a multimode optical fiber, such as the optical fiber 852 depicted in FIG. 1, forms an extended object or light source at location 901, i.e. the optical input of the WDM 90. The size of the object is defined by the diameter of the pinhole or the core diameter of the multimode optical fiber. Note that the practical size of the pinhole or the core diameter of the multimode optical fiber is measured in millimeters, in contrast to the diameter of single mode optical fibers that are measured in micrometers. Consequently, the etendue of the fluorescence light source, defined as the product of beam size and its divergence angle, is hundreds times greater than its counterpart in optical communication. According to the theorem of the conservation of etendue (Julio Chaves, *Introduction to Nonimaging Optics*, CRC Press, 2008 [ISBN 978-1420054293]), light from such an extended source, similar to that from a flash light, can only be kept collimated for a very limited distance, particularly when the diameter of the collimated portion needs to be small.

As depicted in FIG. 25, a collimating optical element, in this case an achromatic doublet lens 902, may capture the light from source 901, and project a magnified image of

the object near a final focusing lens 905. The size of the image near 905 may be kept approximately the same as the effective size of the collimating optical element 902. Consequently, beam of light propagating between the collimating optical element 902 and the focusing lens 905 may be effectively collimated. As shown in FIG. 25, so long as the magnification factor is kept small, for example, less than around 10, using a simple singlet lens as the focusing lens 905, the collimated beam of light can readily focused down to a spot smaller than that of the beam of light received by the WDM 90 at location 901. The ability to focus the beam of light down to such a small size permits placing a small area semiconductor detector at a focal point 906 of the focusing lens 905 for efficient photo detection.

A dichroic filter 903, oriented at a slanted angle, may be inserted into the optical path in between the collimating optical element 902 and the focusing lens 905. The dichroic filter 903 may pass the color band of interest and reflects the remaining colors in the beam of light for further processing within the WDM 90. An optional band pass filter 904 may be inserted following the dichroic filter 903 to further improve the color isolation capability of the WDM 90.

Light reflected from the dichroic filter 903 may impinge upon a second optical element 907, such as a concave mirror. The concave mirror 907 may a radius of curvature approximately equal to the distance between the collimating optical element 902 and the image near focusing lens 905. The concave mirror 907 therefore creates a second image of the collimating lens 902 near a second focusing lens 908. The light beam between the concave mirror 907 and the second image at the lens 908 may have substantially the same diameter as the beam of light between the collimating lens 902 and the first image near the focusing lens 905. The relay imaging concave mirror 907 therefore effectively doubles the collimated beam path without expanding the beam's diameter. Again, the extended yet collimated beam can be easily focused down to a spot smaller than that of the light source at 901. The diameter of the spot may be smaller than 1mm, for example, around $600\,\mu m$, A second dichroic filter 909 may then be inserted in between the relay imaging concave mirror 907 and the second image near focusing lens 908. The second dichroic filter 909 may pass another band of color in the beam of light received by the WDM 90 at location

901 and reflect the remainder of the impinging beam of light for further processing. The first and second dichroic filters 903 and 909 may be inserted approximately midway between the collimating optical element 902 and the focusing lens 905 and between the relay imaging concave mirror 907 and the second image near focusing lens 908, respectively.

As shown in FIG. 25, additional relay collimating optical elements 910, 911, 912, 913 and dichroic filters 914, 915, 916, 917 can be cascaded in the same way to produce multiple images near focusing lenses 918, 919, 920 and 921, each of these images corresponding to a specific color band of light received by the WDM 90 at location 901. As shown in FIG. 25, due to the present disclosure's 1:1 imaging relay architecture, the spots of light produced by focusing lenses 905, 908, 918, 919, 920 and 921 are all smaller than the source of the beam of light, and therefore can be easily captured by small area APD's.

Although FIG 25 illustrates a 6-port wavelength division multiplexer for a beam of light from the extended light source, it is readily apparent to those skilled in the art that WDM's having different numbers of ports can be easily built in accordance with some embodiments of the present disclosure. It is also apparent to those skilled in the art that although the WDM 90 may use achromatic doublets as the first collimating optical element, singlet lens can also be used since the images created before the focusing lenses 905, 908, 918, 919, 920 and 921 are all nearly monochromatic. Instead of using concave mirrors for relaying the beam of light reflected from the dichroic filters, one may also use refractive optics, such as a convex lens, as a relay element to extend the path of the collimated beam of light. One of the advantages of the zig-zag architecture used in the WDM 90, however, is the possibility for using array detectors, which would lead to a more compact WDM suitable for portable instrumentation.

FIG. 25A illustrates a top view of a light detection assembly 937 with the WDM 90 illustrated in FIG. 25 in accordance with some embodiments of the present disclosure. The light detection assembly 937 may include the WDM 90 and a photodetector system 938. A beam of light which emits from the facet of the optical fiber 852 may be processed by the WDM 90 and detected by the photodetector system 938. The concave mirrors 907, 910, 911, 912, and 913 and the dichroic filters 903, 909, 914, 915, 916, and 917 may be formed

on two sides of a reference block 935. The reference block 935 may be made of glass or any material which allows light to pass through it. Accordingly, a zig-zag optical pattern, as illustrated in FIG. 25 may be formed among the collimating optical element 902, the dichroic filters 903, 909, 914, 915, 916, and 917, the reference block 935, the concave mirrors 907, 910, 911, 912, and 913, and the focusing lens 905, 908, 918, 919, 920, and 921. After being processed by the WDM 90, the beam of light emitting from the facet of the optical fiber 852 may split into multiple colored bands with different wavelengths to be detected by photodetectors 940, 941, 942, 943, 944, and 945, respectively. The photodetector can be, but is not limited to, a semiconductor detector, an avalanche photodetector (APD), and a carbon nanotube detector.

In some embodiments, the concave mirrors 907, 910, 911, 912, and 913 may be structurally formed on a relaying assembly 939. It should be understood by those having skill in the art that the concave mirror can be replaced with a convex lens, which is also able to converge and relay the beam of light.

In some embodiments, the dichroic filter can be replaced with a mirror to prevent the beam of light from entering a photodetector when a user wants to decrease the number of light signal channels to be detected. It should be understood by those having skill in the art that the dichroic filter can also be replaced by a dichroic mirror, a beam splitter, or any optical element which is able to split or filter a beam of light.

FIG. 25B illustrates a front view of the light detection assembly 937 with the WDM 90 illustrated in FIGs. 25 and 25A in accordance with some embodiments of the present disclosure. The light detection assembly 937 may have a top cover 940 which is openable. Therefore, the user can open the top cover 940 to change the dichroic mirrors 903, 909, 914, 915, 916, and 917 and modify the light detection system 938 or the WDM 90 inside.

FIG. 26 illustrates an optical ray trace for a prior art collimating device. The technique depicted in FIG. 26 is extensively used in conventional multicolor fluorescence instruments, for example, in United States Patent No. 6,683,314. As shown in FIG. 26, the beam of light diverges rapidly beyond the image 924 created by the collimating optical element 923. Consequently, the only option for constructing a multi-color device is to insert dichroic filters in between the collimating element 923 and its image 924.

Due to the constraint of etendue conservation, the diameter of the collimated beam must be significantly expanded to accept multiple dichroic filters in the section. The expanded beam creates serious challenge to refocusing the collimated beam down to small spots suitable for small area semiconductor detectors. To overcome these difficulties, some instrument manufacturers have chosen to use PMT exclusively for fluorescence detection such as in the main stream flow cytometers manufactured by Becton-Dickinson, Beckman Coulter and Partec 's and the MegaBACE series of DNA sequencers by GE Amersham. Other instruments, such as the Luminex multiplexed bead analyzers, have selected certain color bands with known bright fluorescence, and uses large area APD for detecting light in the selected color bands.

FIG. 27 illustrates a perspective view of an alternative embodiment for a 6-port WDM 90 using a combination of zig-zag and branched configurations. The design is a modification of zig-zag configuration depicted in FIG. 25. In the alternative embodiment depicted in FIG. 27, band pass filter 904 of FIG. 25 may be replaced by a dichroic filter 904'. The filter 904' is positioned to let one color pass through and reflects other colors at ninety degrees (90°). The optical path length of the beam of light passing through the dichroic filter 904' and that being reflected from the 904' are substantially the same, such that one arm is focused by lenses 905 and the other by lens
905' to small spots compatible with small area semiconductor detectors placed at focal location 906 and 906'. As shown in FIG. 25, the remaining color of the light reflected by dichroic filter 903 is relay imaged by a concave mirror 907 and the configuration including optical elements 903, 904', 905 and 905' is cascaded two (2) more times to form a 6 port-WDM.

FIG. 28 illustrates a perspective view of an alternative embodiment for a 8-port WDM 90. By replacing the concave mirrors 907 and 910 in FIG. 27 with concave shaped dichroic filters 907' and 910', the WDM depicted in FIG. 28 may provide 2 more color bands in comparison with the WDMs depicted in FIGs. 25 and 27.

Numerous fluorescence probes for use in flow cytometry have been developed over the years. More recently, multiple fluorescence proteins have also become an important tool in biomedical studies. To accommodate different types of fluorescence probe, various

techniques have been developed to enable user selection of dichroic filters suitable for their particular needs. A significant challenge for replaceable dichroic filters is avoiding direct contact of the coated filter surface with any hard flow cytometer reference frame. Repeated direct contact between the coated filter surface and any hard reference frame may damage a replaceable dichroic filter. Presently, most conventional solutions addressing this problem use precision-machined mechanical spacers for holding replaceable dichroic filters in place. One example of such a solution appears in United States Patent No. 6,683,314. However, such a solution becomes unreliable if the detector's active area is smaller than 1.0 mm^2.

FIGs. 29A and 29B depict fabricating a replaceable dichroic filter assembly 934 illustrated in FIG. 29C suitable for small area detectors. Assembly of the replaceable dichroic filter assembly 934 begins in FIG. 29A which depicts constructing a reference template for its fabrication. The reference template may be a staircase made of two (2) optically parallel glass plates 925 and 926. Bonding the two (2) glass plates 925 and 926 together in optical contact can ensure that a surface 929 of the glass plate 925 becomes optically parallel to a surface 930 of the glass plate 926. A front surface 932 of a replaceable dichroic filter 927 may then be pressed against the surface 929 of the template. A filter holder 928, which loosely fits the dichroic filter 927, may include a reference surface 931 and a filter slot 933. During assembly of the replaceable dichroic filter, the filter slot 933 may be partially filled with epoxy adhesive and the reference surface 931 of the filter holder 928 may be pressed against the surface 930 of the template while filter holder 928 slides toward the dichroic filter 927. While the epoxy adhesive sets, part of the dichroic filter 927 remains seated within the filter slot 933 while pressure is applied against the dichroic filter 927 and filter holder 928. It should be apparent to those skilled in the art that the epoxy adhesive may be either UV or thermally curable, or made by blending together components of an A/B mixture. FIG. 29C depicts a dichroic filter fabricated as depicted in FIGs. 29A and 29B and described above. The assembly process depicted in FIGs. 29A and 29B and described above can ensure that the front surface 932 of the replaceable dichroic filter assembly 934 can be optically parallel to the reference surface 931, and indented with respect to the latter at a spacing accurately determined by the thickness of the glass plate 925. The dichroic filter 927 depicted in FIGs. 29A, 29B, and

29C may be the dichroic filters 903, 909, 914, 915, 916, or 917 used with the WDM illustrated in FIG. 25 or the dichroic filter 903 or the filter 904' used with the WDM illustrated in FIG. 27.

FIGs. 30A and 30B depict an embodiment of the present disclosure where the fore-mentioned replaceable dichroic filter assembly 934 is used in the WDM 90 for optically processing a beam of light from an extended light source. A notable feature of the WDM 90 is a glass reference block 935 having an optically flat surface. As will be apparent to those skilled in the art, the glass reference block 935 may be made of other materials. As shown in FIG 30B, when installing a dichroic filter 927 the reference surface 931 of the replaceable dichroic filter assembly 934 may slide against the flat surface of the glass reference block 935 and be kept in contact therewith by a spring loaded screw 936. Consequently, the coated front surface 932 of the replaceable dichroic filter assembly 934 can remain optically parallel to the optical flat and accurately located. In the meantime, the indentation of front surface 932 with respect to the reference surface 931 may protect it from in physical contact with any object during filter replacement. It is apparent to those skilled in the art that many modifications and variations of the described embodiments of the replaceable dichroic filter assembly 934 are possible. For example, an alternative embodiment of the present disclosure may be a pedestal assembled using a first and a second round optical flat. When assembling the replaceable dichroic filter assembly 934, the reference surface of a filter holder may rest against a surface of the first optical flat and the coated surface of the dichroic filter may rest against the flat surface of the second optical flat. Epoxy bonding then may hold the coated surface of the dichroic filter optically parallel to the reference surface of a filter holder, yet indented at a distance accurately determined by the thickness of the second optical flat.

Optical System with Single Light Source 41

FIG. 31 is a diagram schematically illustrating an optical system with a single light source 41 in accordance with some embodiments of the present disclosure. The optical system with a single light source 41 may include a LD based optical subsystem 50, a composite microscope objective 60, a WDM 90, and a light detection system 938. The beam of light may propagate substantially along the z axis and enter the composite microscope objective 60 from the LD based optical subsystem 50 to illuminate particles

present within the viewing zone inside the composite microscope objective 60. The light scattered from and fluoresced by particles may then be reflected by the concave mirror 601, corrected by the corrector plate 602, and collected by the optical fiber 852 substantially along the x axis. The optical fiber 852 may be fixed by a fiber holder 940.

Common wavelengths of light sources may include, but not limited to, 375nm, 405nm, 440nm, 488nm, 502nm, 534nm, 561nm, 591nm, 637nm, and 637nm. The light detection system 938 may be coupled with circuits for processing light signals. The more ports the WDM 90 has, the more light signal channels the user can use.

Optical System with Multiple Light sources 42

FIG. 32 is a diagram schematically illustrating an optical system with multiple light sources 42 in accordance with some embodiments of the present disclosure. The optical system with multiple light sources 42 may include multiple LD based optical subsystems 50, multiple WDMs 90, multiple light detection systems 938, and a composite microscope objective 60 with a viewing zone. The number of WDMs 90 may correspond to the number of LD based optical subsystems 50. In FIG. 32, the optical system with multiple light sources 42 includes three laser diodes 501 for emitting multiple beams of light with different wavelengths, three collimating lenses 502 in front of the three LDs for collimating the beams of light respectively, three dichroic filters 506, 507, and 508 for passing beams of light with certain wavelength range or reflecting beams of light with certain wavelength range, a plano-convex lens 504 for shaping the beams of light on the major axis, a cylindrical lens 505 for focusing the beams of light onto three spatially separated locations in the flow channel 604, a composite microscope objective 60 for directing the light scattered from and fluoresced by the illuminated particles at three spatially separated locations to be collected by three optical fibers 852, respectively, three optical fibers 852 for collecting scatter and fluorescence emissions and transmitting the emissions to three WDMs 90, respectively, and three WDMs 90 and light detection systems 938 for processing and detecting the scatter and fluorescence light, respectively. The direction of beams of light entering the composite microscope objective 60 may be perpendicular to the direction of scatter and fluoresce emissions to be collected by the optical fiber 852. It should be noted that the plano-convex lens 504 and the cylindrical lens 505 can be replaced

with any conventional beam shaper and any focusing lens. It should also be noted that the various aspects of the present disclosure are not limited to specific numbers of laser diodes, collimating lenses, dichroic filters, plano-convex lenses, composite microscope objectives, optical fibers, WDMs, and light detection systems and specific wavelength and direction of each beam of light.

FIG. 33 illustrates an enlarged view of beams of light 509 and 510 shown in FIG. 32. The beams of light 509 and 510 are emitted from different laser diodes 501 with different wavelengths and then are focused onto spatially divided locations in the flow channel 604 inside the composite microscope objective 60.

Optical System with Chromatic Compensation Elements 51

FIG. 34 is a diagram schematically illustrating an optical system with chromatic compensation elements 51 in accordance with one aspect of the present disclosure in accordance with some embodiments of the present disclosure. The optical system with chromatic compensation elements 51 may include the optical system with multiple light sources 42, as shown in FIG. 32, and multiple chromatic compensation elements 514, 515, and 516. Each of the chromatic compensation elements 514, 515, and 516 may be positioned on the beam paths of beams of light emitting from light sources 511, 512, and 513, respectively, and compensate chromatic aberration in the viewing zone. As such, the beams of light emitting from the light sources 511, 512, and 513 with different wavelengths may be focused onto three spatially divided locations on a common plane which is in the viewing zone and substantially parallel to the direction of a sample flow. The optical properties of chromatic compensation elements 514, 515, and 516 may be different from each other. For example, their thicknesses and shapes may be different to accommodate various beams of light with different wavelengths.

In some embodiments, the optical system shown in FIG. 34 may only need one or two chromatic compensation elements to compensate chromatic aberration in the viewing zone. It should be noted that the various aspects of the present disclosure are not limited to specific numbers or optical properties of chromatic compensation elements.

Power Monitoring System 43

FIG. 35 is a diagram schematically illustrating a power monitoring system 43 in accordance with some embodiments of the present disclosure. The power monitoring system 43 may include a first light source 513 for emitting a first beam of light, a second light source 512 for emitting a second beam of light, a first dichroic filter 519 for reflecting the first beam of light and passing the second beam of light, a second dichroic filter 518 for reflecting the second beam of light, a first detector 401 for measuring residual power of the first and second beams of light downstream of the first dichroic filter 519 on a time-division multiplexing basis, and a control unit 522 coupled with the first detector 401, the first light source 513, and the second light source 512. The first detector 401 may be positioned near or coupled to the first dichroic filter 519. The first and second light sources 513 and 512 may emit beams of light with different wavelengths.

In order to reduce interference between the residual power of the first and second beams of light, the first detector 401 may measure the residual power of the first beam of light when the second light source 512 is off or measure the residual power of the second beam of light when the first light source 513 is off. The residual power of the first and second beams of light may include power of the first beam of light passing through the first dichroic filter 519 and power of the second beam of light reflected by the first dichroic filter 519.

In some embodiments, the control unit 522 may include a feedback circuit to increase the power of the light source when residual power of the light source drops below a certain level or to lower the power of the light source when the residual power of the light source increases above a certain level.

In some embodiments, a second detector 400 may be applied with the power monitoring system 43 and positioned near or coupled to the second dichroic filter 518 to measure the residual power of the second beam of light downstream of the second dichroic filter 518. The residual power of the second beam of light downstream of the second dichroic filter 518 may include power of the second beam of light passing through the second dichroic filter 518. The second detector 400 may also be coupled to the control

circuit 522. When the second detector 400 is applied to the power monitoring system 43, the first detector 401 may only need to monitor the residual power of the first beam of light.

In some embodiments, a third light source 511 for emitting a third beam of light and a third dichroic filter 517 for reflecting the third light may be also applied with the power monitoring system 43. The third light source 511 may be also coupled to the control circuit 522. As such, the first detector 401 may measure residual power of the first, second, and third beams of light downstream of the first dichroic filter 519 on a time-division multiplexing basis.

In some embodiments, the second detector 400 may measure residual power of the second and third beams of light downstream of the second dichroic filter 518 on a time-division multiplexing basis. The residual power of the second and third beams of light downstream of the second dichroic filter 518 may include power of the second beam of light passing through the second dichroic filter 518 and power of the third beam of light reflected by the second dichroic filter 518.

In some embodiments, a third detector (not shown in FIG. 35) may also be applied with the power monitoring system 43 and positioned near or coupled to the third dichroic filter 517 to measure residual power of the third beam of light downstream of the third dichroic filter 517. The third detector may be also coupled to the control circuit 522. The residual power of the third beam of light at the downstream of the third dichroic filter 517 may include power of the third beam of light passing through the third dichroic filter 517.

The second beam of light can either be detected by the first detector 401 or the second detector 400. The third beam of light can be detected by the first detector 401, the second detector 400, or the third detector which is positioned near or coupled to the third dichroic filter 517. The control circuit 522 may control the operation of the detectors and light sources.

It should be understood by those having skill in the art that the dichroic filter can also be replaced by a dichroic mirror or a beam splitter. It should also be noted that the various aspects of the present disclosure are not limited to specific numbers of light sources, dichroic filters, and detectors.

Optical System 44

FIG. 36 is a diagram schematically illustrating an optical system 44 in accordance with some embodiments of the present disclosure. The optical system 44 may include a composite microscope objective 60, as shown in FIG. 8, a light source 403, and a beam splitter 402. The light source 403 may emit beams of light to illuminate objects in a viewing zone, which is located in a flow channel 604 inside a cuvette 603. The composite microscope objective 60 may image light scattered from and fluoresced by the objects in the viewing zone at an image plane 404 external to the composite microscope objective 60. The light source 403 and the image plane 404 may be located on two sides of the beam splitter 402.

The composite microscope objective 60 may include a concave mirror 601 and an aberration corrector plate 602 coupled to the two sides of the cuvette 603. The aberration corrector plate 602 may be an aspheric lens that has a first zone with negative optical power and a second zone with positive optical power radially inside the first zone. A neutral zone may be the thinnest portion of the aberration corrector plate 602 and located between the first zone and the second zone. The aspheric lens may be a plano-aspherical lens. The concave mirror may be a plano-concave back surface mirror or a front surface mirror. The concave mirror 604 and the aberration corrector plate 602 may be made of an optically transparent material.

In FIG. 36, the beam of light emitting from the light source 403 may be reflected by the beam splitter 402 and enter into the composite microscope objective 60 to illuminate objects in the viewing zone. The light scattered from and fluoresced by objects may be reflected by the concave mirror 604, transmit through the aberration corrector plate 602 and the beam splitter 402, and form an image at the image plane 404 external to the composite microscope objective 60. The light source 403 may include multiple laser diodes 403a, 403b, and 403c emitting multiple beams of light with different wavelengths to illuminate objects at multiple locations in the flow channel 604. Accordingly, multiple images 404a, 404b, 404c may be formed at the image plane 404.

In some embodiments, the locations of the light source 403 and the image plane 404 may be swapped. Accordingly, the beam of light emitting from the light source 403 may

transmit through the beam splitter 402 and enter into the composite microscope objective 60 to illuminate objects in the viewing zone. The light scattered from and fluoresced by objects may be reflected by the concave mirror 604, transmit through the aberration corrector plate 602, be reflected by the beam splitter 402, and form an image at the image plane 404 external to the composite microscope objective 60.

In some embodiments, the viewing zone may be located in a jet stream or a surface of a substrate containing objects (not shown in FIG. 36). The objects may be delivered into the viewing zone by a fluidic system, such as a fluidic system shown in FIG. 14 or 15.

In some embodiments, the scattered and fluoresced light imaged at the image plane 404 may be received by a fiber (not shown in FIG. 36) which transmits the light to a photodetector. The scattered and fluoresced light may be processed by a wavelength division multiplexer (WDM) (not shown in FIG. 36) before being detected by the photodetector. The WDM may be configured as a WDM illustrated in FIGs. 25, 25A, and 25B. The photodetector can be, but not limited to, a semiconductor photodetector, a multi-pixel photon counter, and a carbon nanotube detector.

In some embodiments, the light source 403 may emit coherent light or incoherent light. The light source 403 can be single or multiple laser diodes, light emitting diodes, illumination devices emitting beam of light, or any combination of them.

In some embodiments, a chromatic compensating lens (not shown in the figure) may be inserted between the aberration corrector place 602 and the image plane 404 to serve to reduce the residual chromatic aberration.

Axial Light Loss Detection System 45

FIG. 37 is a diagram schematically illustrating an axial light loss detection system 45 in accordance with some embodiments of the present disclosure. The axial light loss detection system 45 may include a concave mirror 406 for reflecting light that propagates from a viewing zone and a detector 408 for measuring axial light loss produced by the object in the viewing zone by detecting light reflected by the concave mirror 406. The light reflected by the concave mirror 406 may include forward scattered light (FSC) and remaining light of beam of light entering into the viewing zone from a light source 412 to

irradiate the object therein, which is so called axial light loss (ALL). The axial light loss of the beam of light along its propagation direction may result from the object passing through the beam of light. The beam of light may be blocked or absorbed by the object.

The axial light loss detection system 45 may utilize the concave mirror 406 to direct both FSC and remaining light into the detector 408 in order to determine the size of object. The FSC and remaining light may have the same wavelength, and therefore the signals of FSC and remaining light detected by the detector 408 may be proportional to square of the sum of their electric fields as follows:

$$(E_{FSC} + E_{ALL})^2 \quad (8)$$

E_{FSC} represents the electric field of FSC. E_{ALL} represents the electric field of remaining light.

On the contrary, a conventional ALL detection system disclosed in prior art usually requires a pinhole positioned along a laser beam path to block FSC in order to detect remaining light of the laser beam. Accordingly, the signals of remaining light detected by an ALL detector is proportional to square of its electric field as follows:

$$(E_{ALL})^2 \quad (9)$$

Further, a conventional FSC detection system disclosed in prior art usually requires a mask positioned along a laser beam path to block remaining light of the laser beam in order to detect FSC. Accordingly, the light signals of FSC detected by a FSC detector is proportional to square of its electric field as follows:

$$(E_{FSC})^2 \quad (10)$$

Apparently, neither of the conventional ALL detection system nor conventional FSC detection system could operate without using a pinhole or a mask.

In some embodiments, the concave mirror 406 may be an ellipsoidal mirror or a combination of a flat mirror and a lens. The detector 408 may be an axial light loss detector to determine the size of the object.

In some embodiments, the detector 408 may be in a heterodyne mode detecting the coherent interference of FSC and the remaining light. The wavelengths of FSC and remaining light may be the same.

In some embodiments, a light source 412 emitting beam of light may be used to illuminate the object in the viewing zone. The optical axis of the beam of light is substantially perpendicular to the flow direction of the object.

In some embodiments, multiple light sources 412 emitting beams of light with different wavelengths may be used to illuminate the objects in the viewing zone. When multiple light sources 412 are applied to the axial light loss detection system 45, a filter 407 may be positioned upstream of the detector 408 to separate the light irradiated by the first light source and reflected by the concave mirror 406 and the light irradiated by the second light source and reflected by the concave mirror 406. As such, the detector 408 may measure them separately, for example, on a time-division multiplexing basis.

In some embodiments, the viewing zone may be located within a microscope objective 410. The viewing zone may be located in a flow channel 409, a jet stream, or a substrate. In some embodiments, a cylindrical lens 411 may be coupled to the microscope objective 410 to focus beams of light emitting from the light source 412 to the viewing zone. The optical axis of the cylindrical lens 411 is substantially perpendicular to the optical axis of the light reflected by the concave mirror 406.

FIG. 38 is a diagram schematically illustrating an axial light loss detection system 45 coupled with a second light detection system 413 in accordance with some embodiments of the present disclosure. The axial light loss detection system 45 may utilize a composite microscope objective 60, as illustrated in FIG. 8 The composite microscope objective 60 may include a second concave mirror 415 and an aberration corrector plate 414 located on two sides of the viewing zone of the composite microscope objective 60. The optical axes of the second concave mirror 415 and the aberration corrector plate 414 are substantially parallel to the optical axis of the light reflected by the concave mirror 406. The FSC and remaining light propagating from the viewing zone may be reflected by the concave mirror 406 and detected by the detector 408 while the side-scattered fluoresced light may be reflected by the second concave mirror 415, propagate out of the composite microscope

objective 60 via the aberration corrector plate 414, and be detected by the second light detection system 413.

In some embodiments, one or more control circuits may be coupled with one or more of the detector 408, the second light detection system 413, and the light source 412 to process detected light signals. As known by one skilled in the art, the control circuit may include an amplifier to amplify detected light signals, a noise filter to reduce noise interference, and a processor to process detected light signals and generate corresponding information regarding the properties of the object.

Alternative Combined Microscope Objective

FIGs. 11, 12 and 13 illustrate the build-up of a composite microscope objective 60 adapted for imaging light scattered from and fluoresced by an object present within a viewing zone. The illustrated composite microscope objective comprises a viewing zone, a concave mirror arrangement 601, 610, 617, an exit area and an illumination beam forming arrangement 505, as illustrated in FIG. 8A. It should be noted that in Fig. 11, 12 and 13 the beam forming arrangement is not illustrated. The viewing zone in Fig. 11 may be located in e.g. a channel 604 of a cuvette 603. In FIG. 12, the viewing zone may be located e.g. along the droplets of the jet stream 519 leaving the nozzle 518. In FIG. 13, the viewing zone maybe located e.g. in the plane of the substrate. The exit area of the microscope objective is an area through which scattered light and fluoresced light impinging from an object present in the viewing zone passes, which scattered and fluorescent light is reflected by the concave mirror of the microscope objective. The aberration corrector plate 602 in FIG. 11, 612 in FIG. 12 and 618 in FIG. 13 may be located in the exit area. It should be noted that the corrector plate may be used, in particular in combination with a spherical mirror 601, 610, 617. However the corrector plate may be omitted when using a concave mirror having already implemented a correcting shape. Thus, the exit area does not have to include a corrector, if a concave mirror has an appropriate shape and the gain of the corrector plate is not required. As can be seen in FIGs. 11, 12 and 13, the viewing zone is arranged between the concave mirror arrangement and the exit area. The concave mirror 601, 610, 617 is arranged to reflect scattered and fluoresced light impinging from an object present in the

viewing zone to the exit area. The illumination beam forming arrangement 505 is illustrated e.g. in FIG. 8A. Fig. 8A illustrates an arrangement of FIG. 11 having attached illumination beam arrangement 505 to the cuvette 603. The illumination beam arrangement 505 is arranged so that an illumination beam entering the illumination beam forming arrangement is pre-definitely formed at the viewing zone. A path of an illumination beam from an illumination system can be seen e.g. in FIG. 1 or 3A. FIGs 11, 12 and 13 as well as FIG. 8A illustrate that the concave mirror arrangement, the viewing zone and the exit area are arranged along a first axis, also referred to as x-axis. FIG. 9A illustrated that an optical image of the viewing zone, e.g. within channel 604 in FIG. 9A is formed outside the composite microscope objective in the image plane 605 with image locations 606, 607 and 608. The illumination beam forming arrangement 505 is arranged so that an illumination beam impinges the viewing zone along a second axis, also referred to as z-axis, which is substantially perpendicular to said x-axis. The above described cuvette 603 may be manufactured of an optical transparent material, wherein the viewing zone is formed in the cuvette, in particular in the channel 604 of the cuvette 603. The channel extends along a third axis, also referred to as y-axis being substantially perpendicular to the x-axis and the z-axis, so that a liquid flow in the channel flows along the y-axis, as illustrated e.g. in FIG. 5D, wherein the viewing zone is located within the channel. FIG. 8 and 8A illustrated that the cuvette 603 may be of rectangular cross section in a plane of the first axis/x-axis and second axis/z-axis. It should be noted that the cross section of the cuvette 603 may also be of a form, that a sheath flow covering the sample flow forces the sample flow into a rectangular cross section. The cross section of the channel 604 may be constant along the y-axis, but may also vary along the y-axis. In particular the channel may have a focused cross section in the area of the viewing zone. The viewing area may include a plurality of predefined viewing points distributed along the y-axis for different illumination wavelengths, as can be seen in FIG. 33, or along the z-axis, which may be a varying focal point varying along the z-axis, when adjusting the objective 60 with respect to the illumination system 50, as will described later. Although element 505 may be allocated to the illumination system 50, element 505 may also be part of the objective 60, in particular it may be attached to the cuvette 603. The illumination beam forming arrangement 505 is adapted to compress an illumination beam, so that the illumination beam in the viewing

zone has a compressed dimension along the y-axis. The illumination beam forming arrangement 505 may be a cylindrical lens, in particular having a cylindrical axis parallel to the x-axis, as can be seen in FIGs. 5D and 5E. It should be noted that the illumination beam forming arrangement can be assembled by a plurality of optical elements, so that the arrangement 505 may not have a defined axis. The aberration corrector arrangement 602, 612, 618 may be arranged in the exit area, as can be seen in FIGs. 11, 12 and 13. The aberration corrector arrangement may be an aspheric lens made of optically transparent material. As can be seen in FIG. 9A, said aberration corrector arrangement may have a first zone with negative optical power, a second zone radially inside the first zone with positive optical power, and a neutral zone between the first zone and the second zone. The neutral zone in FIG. 9A is thinner than each of the first zone and the second zone, so that light reflected from the concave mirror arrangement passing through said aberration corrector arrangement forms a focal area. Although FIG. 9A illustrates a corrector plate with convex and concave portions, it should be noted that the positive and negative optical power may be achieved by using different optical materials at different locations of the corrector plate. The concave mirror arrangement 601, the viewing zone and the aberration corrector arrangement 602 form a reversed Schmidt camera. The concave mirror arrangement may be formed by plane-convex lens, as can be seen in FIG. 11. The concave mirror may be a plano-concave back surface mirror. The plano-concave back surface mirror may be made from an optically transparent material. As can be seen in FIG. 8A a plano-surface of said plano-concave back surface mirror is optically coupled to a flat surface of said cuvette. Plano-concave back surface mirror means that although the optical lens body is a plano-convex lens body, the surface when seeing into the mirror, which is the inside of the optic body, is concave. The plano-surface of said plano-concave back surface mirror may also be optically coupled to said flat, transparent substrate, as can be seen in FIG. 13. Said concave mirror may also be a front surface mirror, as can be seen from FIG. 12. It should be noted that the mirror of FIG. 12 can also be used in combination with a cuvette, and the mirror of FIG. 11 can also be used for a jet stream. The concave mirror arrangement, the aberration corrector arrangement and the illumination beam forming arrangement may be attached to the cuvette by at least one of index matching gel, index matching fluid, optical adhesive

material and optical contact bonding. It should be noted that also a combination may be used for attaching.

As can be seen in FIGs. 1, 3A, 31, 32 or 34-38, the composite microscope objective 60 may be combined with or comprise an illumination system. Although not mandatory, the illumination may be an illumination system as described above, in particular with a laser source 501, an collimating optical arrangement 502 to form a collimated laser beam and a beam shaping arrangement 504, 505 being adapted to shape a collimated beam, wherein the beam shaping arrangement includes the illumination beam forming arrangement 505. The laser source may be a laser diode and the collimating arrangement may be arranged with respect to the laser diode so as to form a collimated beam. As an alternative, the laser source may be a conventional laser with an optic arrangement to form a collimated beam of a desired cross section. The laser diode and the collimation optical arrangement, or alternatively the conventional laser with the optics are adapted to form a beam having an elliptical cross section having a major axis and a minor axis, wherein the minor axis is oriented substantially along the y-axis and the major axis is oriented substantially along the x-axis, as can be seen in FIG. 5E. The beam shaping arrangement may include a major axis optical beam compressing arrangement 504 being adapted to compress at least the major axis of the collimated elliptical beam. The illumination beam forming arrangement 505 is adapted to compress at least the minor axis of the collimated elliptical beam. This can be seen for example in FIGs 1, 3A or 6.

The viewing zone may be movable along the z-axis with respect to the illumination system so as to vary a focus of the compressed elliptical beam within the viewing zone along the z-axis. This allows a scanning along the z-axis. In particular this allows to sense or scan properties of a cell in the viewing zone at different locations. It should be understood, that either the objective 60 may be controllably moved or the illumination system 50 or both. It should also be understood that the variation of the focus may also be achieved by moving single components of the illumination system, e.g. one of the mirrors 523b, 523a or the element 504, as illustrated in FIG. 7. Also single components of the objective may be moved to vary the focus along the z-axis. The actuation can be conducted by e.g. piezo actuators or acoustic actuators. In particular the varying focus can be achieved by a modulation or an sinusoidal oscillation of the respective component. Thus the cuvette

is movable with respect to the laser source so as to spatially vary a focus of the laser source in the channel. For this purpose, a control unit can be provided being adapted to control the movement of components of the composite microscope objective along the z-axis so as to spatially vary a focus of the laser source in the channel. It should be noted that likewise also a variation of the focus can be achieved along the y-axis or even the x-axis.

Combined Wavelength Division Multiplexer (WDM) with Semiconductor Photo Detector

FIGs. 25, 27, and 28 illustrate a wavelength division multiplexer (WDM) for separating light emitted from a light source into multiple colored bands. The wavelength division multiplexer may comprise an imaging optical arrangement 902, a dichroic filter arrangement 903, 904, a semiconductor photo detector 906 and a focusing optical arrangement 905. The imaging optical arrangement 902 forms a beam of light from the light emitted from a light source 901 and produces an image of substantially the same size as the effective size of said imaging optical arrangement. The light source may be an outlet of an optical fiber, which fiber may transfer detected light from the microscope objective 60 to the WDM. The dichroic filter arrangement 903, 904 may be located between said imaging optical arrangement 902 and said image, and separates the beam of light into a first branch and a second branch of distinctive colors. As can be seen in FIG. 25, the first branch travels toward element 905, whereas the second branch travels toward element 907. The semiconductor photo detector 906 is located in the first branch behind the focusing optical arrangement 905, which is located between the dichroic filter arrangement 903, 904 and the semiconductor photo detector 906 so as to focus the beam of light onto the semiconductor photo detector. Light means an electromagnetic wave, coherent or non-coherent, particularly having a wavelength which transits the used optical elements. In particular, the term light is not limited to the visible part of light, e.g., light between 380nm and 780nm. It should be noted that also infrared light and ultraviolet light may be used, if the used optical components are capable of being operated with such wavelengths. The focusing optical element arrangement 905 is located in or in proximity to an image plane of said image. It should be noted that the imaging arrangement 902 as well as the focusing arrangement 905

may be composed of more than one optical element. In particular, a plurality of lenses may be combined so as to form the imaging arrangement 902 or the focusing arrangement 905. Likewise the dichotic filters may be composed of more than one filter or optic element. In particular to compose particular properties of the respective arrangements. The focusing optical arrangement and the semiconductor photo sensor may be arranged to each other that the beam of light is focused to a spot having a diameter of less than 1.0 mm, particularly of less than 0.6 mm. In particular when using semiconductor sensors the signal to noise ration SNR can be significantly reduced. As can be seen in FIG. 25, the wavelength division multiplexer as described above may further comprise an image relay optical arrangement 907. This image relay optical arrangement may be located in or in proximity to an image plane produced by said imaging optical arrangement in the second branch, wherein said image relay optical arrangement is adapted to produce an image of said imaging optical arrangement in a third branch having substantially the same size as the image in the second branch. The third branch in FIG. 25 is the beam traveling from the element 907 toward element 909. The effective size of the optical element is the area where beams from the object transit the optical element. Consequently, producing an image of substantially the same size as the effective size of said optical element means that between the optical element and the image the beam is within a virtual parallel tube. For illustration purposes, in the hypothetical case the object is a pinhole the optical element produces a collimated beam. The image relay optical arrangement 907 may be a concave mirror. Alternatively the image relay optical arrangement 907 may be a combination of a lens and a mirror, in particular a plane mirror. The wavelength division multiplexer as described above may further comprise an additional dichroic filter arrangement 909, wherein the additional dichroic filter arrangement is located between said image relay optical arrangement 907 and an image produced by said image relay optical arrangement 907. Said additional dichroic filter arrangement 909 from the third branch produces a fourth branch and a fifth branch of the beam of light having distinctive colors. The fourth branch in FIG. 25 is the beam traveling from the dichroic filter 909 toward the focusing element 908, whereas the fifth branch is the beam traveling from the dichroic filter toward the element 910. The above described wavelength division multiplexer further may comprise an additional focusing optical arrangement 908 and an additional semiconductor photo detector, wherein the

additional focusing optical arrangement 908 is located in the fourth branch and focuses the beam of light in the fourth branch so as to focus the beam of light onto the additional semiconductor photo detector. The wavelength division multiplexer may further comprise a plurality of image relay optical arrangements 910, 911, 912, 913, a plurality of dichroic filter arrangements 914, 915, 916, 917, a plurality of semiconductor photo detectors and a plurality of focusing optical arrangements 918, 919, 920, 921, wherein each of the plurality of focusing optical arrangements is arranged between a respective one of the plurality of dichroic filter arrangements and a respective one of the plurality of semiconductor photo detectors so as to form a cascaded arrangement, as can be seen in FIG. 25. The additional focused spots may have a diameter of less than 1.0 mm, particularly of less than 0.6 mm for multiple colored bands of said beam of light. The plurality of dichroic filter arrangements are arranged in a common plane, as can be seen in FIG. 25A. The wavelength division multiplexer may further comprise a plan-parallel optical basis having a first surface and second surface parallel thereto, wherein the plurality of dichroic filter arrangements are arranged parallel, preferably in abutment to the first surface of the plan-parallel optical basis, as described with respect to the WDM 90. The dichroic filter arrangements are assembled using a template that includes two optically flat glass plates bonded together in optical contact, wherein the dichroic filter arrangements are bonded to a filter holder using the template such that a coated filter surface of the dichroic filter arrangements are indented and optically parallel to a reference surface of the filter holder, as can be seen in FIGs 29A, B and C. The reference surface of the filter holder rests against an optically flat surface of an reference block included in the wavelength division multiplexer thereby providing consistent optical alignment when installing the dichroic filter arrangements into the wavelength division multiplexer. The respective image relay optical arrangements may be formed into the second surface of the plan-parallel optical basis, as can be seen in FIG. 25A. At least one of the semiconductor photo detectors is an avalanche photo diode detector. As an alternative or in addition, at least one of the semiconductor photo detectors is a carbon nanotube detector.

Industrial Applicability

Although an embodiment of the present disclosure of an LD based optical system for flow cytometric application has been described in some detail, and equally advantageous embodiments have also been described for a stream based flow cytometric instrument, it will be apparent to those of ordinary skill in the art that many modifications and variations of the described embodiment are possible in the light of the above teachings without departing from the principles and concepts of the disclosure as set forth in the claims.

Although an embodiment of the present disclosure of wavelength division multiplexing device for separating light beam from an extended light source into multiple color bands has been described in some detail, and several other equally advantageous embodiments have also been described, it will be apparent to those ordinary skilled in the art that many modifications and variations of the described embodiments are possible in the light of the above teachings without departing from the principles and concepts of the disclosure as set forth in the claims.

Although the present disclosure describes certain exemplary embodiments, it is to be understood that such disclosure is purely illustrative and is not to be interpreted as limiting. Consequently, without departing from the spirit and scope of the disclosure, various alterations, modifications, and/or alternative applications of the disclosure will, no doubt, be suggested to those skilled in the art after having read the preceding disclosure. Accordingly, it is intended that the following claims be interpreted as encompassing all alterations, modifications, or alternative applications as fall within the true spirit and scope of the disclosure.

WHAT IS CLAIMED IS:

1. A flow cytometer (40) comprising:

a) a laser diode ("LD") based optical subsystem (50) for directing a beam of light into a viewing zone of said flow cytometer (40) through which a sample liquid carrying particles flows, the sample liquid being hydrodynamically focused within the viewing zone by a liquid sheath flow that also flows through the viewing zone, the optical subsystem (50) including:

i. a LD for emitting a diverging beam of light from an edge thereof, the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis;

ii. a collimating lens for converting the diverging beam of light emitted from said LD into a collimated elliptical beam of light, wherein the minor axis of said collimated elliptical beam of light is oriented parallel to a direction in which particles pass through the viewing zone;

iii. a beam compressing optical element for reducing the size of said elliptical beam of light at the viewing zone whereby a width of said major axis of said elliptical beam of light oriented perpendicular to the direction in which particles pass through the viewing zone is less than a width of said liquid sheath flow; and

v. a cylindrical focusing element positioned adjacent to the viewing zone with an axis of said cylindrical focusing element being oriented perpendicular to the direction in which particles pass through the viewing zone whereby:

1) said minor axis of said beam of light becomes focused at the viewing zone; and

2) the size of said major axis of said elliptical beam of light at the viewing zone remains essentially unchanged;

b) a composite microscope objective (60) for imaging light scattered from and fluoresced by a particle present within the viewing zone, the composite microscope objective (60) including:

i. a concave mirror upon which scattered and fluoresced light impinges;

ii. an aberration corrector plate made of optically transparent material wherein said aberration corrector plate is an ashperic lens that has:

1) a first zone of said aberration corrector plate outside a neutral zone thereof where said aberration corrector plate:

A. is thinnest; and

B. has negative optical power; and

2) a second zone of said aberration corrector plate inside the neutral zone has positive optical power

light reflected from the composite microscope objective (60) passing through said aberration corrector plate,

the viewing zone of said flow cytometer (40) being located between said concave mirror and said aberration corrector plate;

c) a fluidic subsystem (70) for supplying the liquid sheath flow to the viewing zone, the liquid sheath flow lacking pulsations, the fluidic subsystem (70) including:

l. a liquid pump for supplying liquid drawn from a reservoir;

ii. a T coupling having at least one (1) inlet and two (2) outlets,

1) the inlet of said T coupling receiving liquid from said liquid pump;

2) a first fraction of the liquid received by the inlet flowing via a first one of the outlets and via a bypass conduit back to the reservoir; and

3) a second fraction of the liquid received by the inlet flowing via a second one of the outlets and via a particle filter to the viewing zone of said flow cytometer (40);

d) a peristaltic pump 80 for supplying the sample liquid carrying the particles, the sample liquid being hydrodynamically focused within the viewing zone by the liquid sheath flow, the peristaltic pump 80 comprising:

i. a pump housing having an arcuate curved track formed therein that extends between a pump inlet and a pump outlet;

ii. a plurality of rollers that are attached to a rotor, the rollers having a substantially equal angular spacing between each pair of immediately adjacent rollers, the

rotor being rotatable together with the rollers attached thereto inside said pump housing; and

ii a compressible tube sandwiched between said rollers and the arcuate curved track of said pump housing, the arcuate curved track including:

1) an exit section wherein, as a roller rolls through the exit section, said compressible tube adjacent to said roller progressively expands from fully closed at a beginning of said exit section to fully open at the pump outlet where said roller breaks contact with said compressible tube; and

2) at least one pumping section along the arcuate curved track between the pump inlet and the pump outlet wherein said compressible tube is compressed to fully closed by at least one of said rollers; and

e) a wavelength division multiplexer 90 ("WDM (90)") for separating into multiple colored bands a beam of light emitted initially from the viewing zone and imaged by the composite microscope objective (60) into an optical fiber for transmission to the WDM (90), the WDM (90) comprising:

i. a collimating optical element that magnifies an to produce an image of substantially the same size as the effective size of said collimating optical element;

ii. at least one dichroic filter located between said collimating optical element and said image, wherein said dichroic filter separates the collimated beam of light into two (2) branches of distinctive colors;

iii. a focusing optical element located in one of said branches, wherein the beam of light in said branch is focused to a spot having a diameter of less than 1.0 mm by said focusing optical element; and

iv. an image relay optical element located near the image produced by said collimating optical element in the other branch, wherein said image relay optical element produces an image of said collimating optical element at substantially unit magnification.

2. The flow cytometer (40) of claim 1 wherein said cuvette has a rectangularly-shaped , and wherein the viewing zone of the flow cytometer (40) is located within a channel having a rectangularly-shaped cross-section that is located within said cuvette.

3. The flow cytometer (40) of claim 1 wherein said cuvette has a tubularly-shaped cross-section, and wherein the viewing zone of the flow cytometer (40) is located within a channel having a circularly-shaped cross section that is located within said cuvette.

4. The flow cytometer (40) of claim 1 wherein the sample liquid and the liquid sheath flow form a jet stream in which the viewing zone of the flow cytometer (40) is located.

5. The flow cytometer (40) of claim 2 wherein said cylindrical focusing element is in optical contact with an entrance face of said rectangularly-shaped cuvette.

6. The flow cytometer (40) of claim 2 wherein said cylindrical focusing element is separated from said rectangularly-shaped cuvette.

7. The flow cytometer (40) of claim 3 wherein said cylindrical focusing element is separated from said tubularly-shaped cuvette.

8. The flow cytometer (40) of claim 4 wherein said cylindrical focusing element is separated from said jet stream.

9. The flow cytometer (40) of claim 1 further comprising a polarization conditioning element through which said collimated elliptical beam of light passes.

10. The flow cytometer (40) of claim 1 wherein an optical image of the viewing zone is formed outside the composite microscope objective (60).

11. The flow cytometer (40) of claim 10 wherein the viewing zone is located within a flow channel included in a rectangularly-shapeed cuvette made of optically transparent material.

12. The flow cytometer (40) of claim 11 wherein said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

13. The flow cytometer (40) of claim 12 wherein the plano-surface of said plano-concave back surface mirror is optically coupled to a flat surface of said cuvette.

14. The flow cytometer (40) of claim 13 wherein an optical adhesive material accomplishes the optical coupling.

15. The flow cytometer (40) of claim 13 wherein an index matching gel accomplishes the optical coupling.

16. The flow cytometer (40) of claim 13 wherein an index matching fluid accomplishes the optical coupling.

17. The flow cytometer (40) of claim 13 wherein optical contact bonding accomplishes the optical coupling.

18. The flow cytometer (40) of claim 13 wherein the plano-concave back surface mirror formed integrally with said cuvette means.

19. The flow cytometer (40) of claim 11 wherein said aberration corrector plate is a plano-aspherical lens.

20. The flow cytometer (40) of claim 19 wherein a plano-surface of said aberration corrector plate is optically coupled to a flat surface of said cuvette opposite of said plano-concave back surface mirror.

21. The flow cytometer (40) of claim 20 wherein an index matching gel accomplishes the optical coupling.

22. The flow cytometer (40) of claim 20 wherein an index matching fluid accomplishes the optical coupling.

23. The flow cytometer (40) of claim 20 wherein optical contact bonding accomplishes the optical coupling.

24. The flow cytometer (40) of claim 20 wherein the plano-aspherical lens is formed integrally with said cuvette.

25. The flow cytometer (40) of claim 19 wherein said aberration corrector plate is detached from said cuvette.

26. The flow cytometer (40) of claim 10 wherein the viewing zone is inside a jet stream.

27. The flow cytometer (40) of claim 25 wherein said concave mirror is a front surface mirror.

28. The flow cytometer (40) of claim 10 wherein the viewing zone is located on a surface of a flat, transparent substrate.

29. The flow cytometer (40) of claim 28 wherein said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

30. The flow cytometer (40) of claim 29 wherein the plano-surface of said plano-concave back surface mirror is optically coupled to said flat, transparent substrate.

31. The flow cytometer (40) of claim 30 wherein an optical adhesive material accomplishes the optical coupling.

32. The flow cytometer (40) of claim 30 wherein an index matching gel accomplishes the optical coupling.

33. The flow cytometer (40) of claim 30 wherein an index matching fluid accomplishes the optical coupling.

34. The flow cytometer (40) of claim 30 wherein optical contact bonding accomplishes the optical coupling.

35. The flow cytometer (40) of claim 29 wherein said plano-concave back surface mirror is formed integrally with said flat, transparent substrate.

36. The flow cytometer (40) of claim 28 wherein said aberration corrector plate is detached from said flat, transparent substrate.

37. The flow cytometer (40) of claim 1 wherein the particle filter has inlet thereto for receiving liquid from the T-coupling, the inlet of the particle filter being disposed so that air becomes trapped within the particle filter at the inlet thereto.

38. The flow cytometer (40) of claim 37 wherein when said liquid pump is turned off air cannot enter into the bypass conduit.

39. The flow cytometer (40) of claim 38 further comprising a small capsule disposed between the second one of the outlets of said T-coupling and the particle filter for storing air ejected from the particle filter when the liquid pump is turned off.

40. The flow cytometer (40) of claim 38 further comprising a length of tubing disposed between the second one of the outlets of said T-coupling and the particle filter for storing air ejected from the particle filter when the liquid pump is turned off.

41. The flow cytometer (40) of claim 38 further comprising an adjustable valve located in the bypass conduit between the first one of the outlets of the T-coupling and the reservoir for restricting liquid flow therebetween.

42. The flow cytometer (40) of claim 38 further comprising an adjustable valve located between the second one of the outlets of the T coupling and the viewing zone for restricting liquid flow therebetween.

43. The flow cytometer (40) of claim 38 wherein the throughput of the liquid pump is adjustable.

44. The flow cytometer (40) of claim 1 wherein the arcuate curved track of said pump housing includes at least two (2) pumping sections, the arcuate curved track further

including at least one recess section located between said pumping sections along the arcuate curved track, and wherein said compressible tube at said recess section becomes decompressed to full expansion then compressed to fully closed when one (1) of said rollers rolls through said recess section.

45. The flow cytometer (40) of claim 44 includes a plurality of recess sections along said arcuate curved track upstream of the pump outlet, the angular spacing between the compression part of said recess section adjacent to the pump outlet and said exit section of said arcuate curved track being substantially the same as the angular spacing between each pair of immediately adjacent rollers.

46. The flow cytometer (40) of claim 44 wherein said compression part of said recess section adjacent to the pump outlet has a shape complementing a shape of said exit section of said arcuate curved track to maintain the total fluid volume inside a section of said compressible tube extending from said recess section to the pump outlet substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

47. The flow cytometer (40) of claim 44 having a plurality of recess sections respectively interspersed between immediately adjacent pairs of a plurality of pumping sections.

48. The flow cytometer (40) of claim 46 wherein both:

a) angular spacing between adjacent pairs of recess sections; and

b) angular spacing between said exit section of said arcuate curved track and an adjacent recess section to said exit section

are substantially the same as the angular spacing between each pair of immediately adjacent rollers.

49. The flow cytometer (40) of claim 47 wherein shapes of a plurality of recess sections of said arcuate curved track complement a shape of said exit section of said arcuate curved track to maintain a fluid volume in sections of said compressible tube at the plurality of recess sections and said exit section substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

50. The flow cytometer (40) of claim 1 wherein a speed of said rotor is programmably controlled to vary substantially in inverse proportion to the fluid volume

change rate in said compressible tube due to its changing compression near the exit section of said arcuate curved track.

51. The flow cytometer (40) of claim 1 wherein at least one additional dichroic filter is located between said image relay optical element and the image produced by said image relay optical element, wherein said dichroic filter produces two (2) branches of the beam of light having distinctive colors.

52. The flow cytometer (40) of claim 51 wherein another focusing optical element is located in one of said branches and focuses the beam of light in the branch into a spot having a diameter of less than 1.0 mm.

53. The flow cytometer (40) of claim 52 wherein successive combinations of said image relay optical element, dichroic filter and focusing optical element are cascaded to produce additional focused spots having a diameter of less than 1.0 mm for multiple colored bands of said beam of light.

54. The flow cytometer (40) of claim 52 wherein the dichroic filter is assembled using a template that include two (2) optically flat glass plates bonded together in optical contact, and wherein the dichroic filter is bonded to a filter holder using the template such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder.

55. The flow cytometer (40) of claim 54 wherein the reference surface of the filter holder rests against an optically flat surface of an reference block included in the WDM (90) thereby providing consistent optical alignment when installing the dichroic filter into the WDM (90).

56. A LD based optical subsystem (50) for impinging a beam of light into a viewing zone in which a particle is present, the optical subsystem (50) comprising:

a) a LD for emitting a diverging beam of light from an edge thereof, the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis;

b) a collimating lens for converting the diverging beam of light emitted from said LD into a collimated elliptical beam of light, wherein the minor axis of said collimated elliptical beam of light is oriented parallel to a direction in which particles pass through the viewing zone;

c) a beam compressing optical element for reducing the size of said elliptical beam of light at the viewing zone whereby a width of said major axis of said elliptical beam of light oriented perpendicular to the direction in which particles pass through the viewing zone is less than a width of said liquid sheath flow; and

d) a cylindrical focusing element positioned adjacent to the viewing zone with an axis of said cylindrical focusing element being oriented perpendicular to the direction in which particles pass through the viewing zone whereby:

i. said minor axis of said beam of light becomes focused at the viewing zone; and

ii. the size of said major axis of said elliptical beam of light at the viewing zone remains essentially unchanged.

57. The optical subsystem (50) of claim 56 further comprising a cuvette having a rectangularly-shaped cross-section, and wherein the viewing zone is located within a channel having a rectangularly-shaped cross section that is located within said cuvette.

58. The optical subsystem (50) of claim 56 further comprising a cuvette having a tubularly-shaped cross-section, and wherein the viewing zone is located within a channel having a circularly-shaped cross-section that is located within said cuvette.

59. The optical subsystem (50) of claim 56 wherein the sample liquid and the liquid sheath flow form a jet stream in which the viewing zone is located.

60. The optical subsystem (50) of claim 57 wherein said cylindrical focusing element is in optical contact with an entrance face of said rectangularly-shaped cuvette.

61. The optical subsystem (50) of claim 57 wherein said cylindrical focusing element is separated from said rectangularly-shaped cuvette.

62. The optical subsystem (50) of claim 58 wherein said cylindrical focusing element is separated from said tubularly-shaped cuvette.

63. The optical subsystem (50) of claim 59 wherein said cylindrical focusing element is separated from said jet stream.

64. The optical subsystem (50) of claim 56 further comprising a polarization conditioning element through which said collimated elliptical beam of light passes.

65. A method for delivering an elliptically shaped beam of light using a LD based optical subsystem (50), the beam of light having a smooth profile at a focus of a

minor axis thereof that is located at a viewing zone through which a sample liquid flows, the sample liquid being hydrodynamically focused within the viewing zone by a liquid sheath flow that also flows through the viewing zone, the method comprising the steps of:

a) providing a LD that emits a diverging beam of light from an edge thereof, the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis;

b) impinging the diverging beam of light emitted by the LD upon a collimating lens for converting the diverging beam of light emitted therefrom into a collimated elliptical beam of light wherein the minor axis of said collimated elliptical beam of light is oriented parallel to a direction in which sample liquid passes through the viewing zone;

c) after passing through said collimating lens, impinging the collimated elliptical beam of light upon an beam compressing optical element for reducing the size of said elliptical beam of light at the viewing zone whereby a width of said major axis of said elliptical beam of light oriented perpendicular to the direction in which sample liquid passes through the viewing zone becomes less than a width of said liquid sheath flow; and

d) after passing through said beam compressing optical element, impinging the beam of light upon a cylindrical focusing element positioned adjacent to the viewing zone with an axis of said cylindrical focusing element being oriented perpendicular to the direction in which sample liquid passes through the viewing zone whereby:

i. said minor axis of said beam of light becomes focused at the viewing zone; and

ii. the size of said major axis of said elliptical beam of light at the viewing zone remains essentially unchanged.

66. The method of claim 65 wherein the viewing zone is located within a channel having a rectangularly-shaped cross-section that is located within a cuvette.

67. The method of claim 65 wherein the viewing zone is located within a channel having a circularly-shaped cross-section that is located within a cuvette.

68. The method of claim 65 wherein the viewing zone is located within a jet stream.

69. The method of claim 66 further comprising a step of establishing an optical contact between said cylindrical focusing element and an entrance face of said cuvette.

70. The method of claim 66 further comprising a step of establishing a spacing between said cylindrical focusing element and said cuvette.

71. The method of claim 67 further comprising a step of establishing a spacing between said cylindrical focusing element and said cuvette.

72. The method of claim 68 further comprising a step of establishing a spacing between said cylindrical focusing element and said jet stream.

73. The method of claim 65 further comprising a step of inserting a polarization conditioning element between the collimating lens and the beam compressing optical element whereby the collimated elliptical beam of light passes through the polarization conditioning element.

74. A composite microscope objective (60) adapted for imaging light scattered from and fluoresced by a particle present within a viewing zone comprising:

a) a concave mirror upon which scattered and fluoresced light impinges;

b) an aberration corrector plate made of optically transparent material wherein said aberration corrector plate is an aspheric lens that has:

i. a first zone of said aberration corrector plate outside a neutral zone thereof where said aberration corrector plate:

1) is thinnest; and

2) has negative optical power; and

ii. a second zone of said aberration corrector plate inside the neutral zone has positive optical power,

light reflected from the composite microscope objective (60) passing through said aberration corrector plate,

the viewing zone being located between said concave mirror and said aberration corrector plate.

75. The composite microscope objective (60) of claim 74 wherein an optical image of the viewing zone is formed outside the composite microscope objective (60).

76. The composite microscope objective (60) of claim 75 wherein the viewing zone is located within a flow channel included in a rectangularly-shaped cuvette made of optically transparent material.

77. The composite microscope objective (60) of claim 76 wherein said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

78. The composite microscope objective (60) of claim 77 wherein a plano-surface of said plano-concave back surface mirror is optically coupled to a flat surface of said cuvette.

79. The composite microscope objective (60) of claim 78 wherein an optical adhesive material accomplishes the optical coupling.

80. The composite microscope objective (60) of claim 78 wherein an index matching gel accomplishes the optical coupling.

81. The composite microscope objective (60) of claim 78 wherein an index matching fluid accomplishes the optical coupling.

82. The composite microscope objective (60) of claim 78 wherein optical contact bonding accomplishes the optical coupling.

83. The composite microscope objective (60) of claim 78 wherein the plano-concave back surface mirror formed integrally with said cuvette means.

84. The composite microscope objective (60) of claim 76 wherein said aberration corrector plate is a plano-aspherical lens.

85. The composite microscope objective (60) of claim 84 wherein a plano-surface of said aberration corrector plate is optically coupled to a flat surface of said cuvette opposite of said plano-concave back surface mirror.

86. The composite microscope objective (60) of claim 85 wherein an index matching gel accomplishes the optical coupling.

87. The composite microscope objective (60) of claim 85 wherein an index matching fluid accomplishes the optical coupling.

88. The composite microscope objective (60) of claim 85 wherein optical contact bonding accomplishes the optical coupling.

89. The composite microscope objective (60) of claim 85 wherein the plano-aspherical lens is formed integrally with said cuvette.

90. The composite microscope objective (60) of claim 84 wherein said aberration corrector plate is detached from said cuvette.

91. The composite microscope objective (60) of claim 75 wherein the viewing zone is inside a jet stream.

92. The composite microscope objective (60) of claim 90 wherein said concave mirror is a front surface mirror.

93. The composite microscope objective (60) of claim 75 wherein the viewing zone is located on a surface of a flat, transparent substrate.

94. The composite microscope objective (60) of claim 93 wherein said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

95. The composite microscope objective (60) of claim 94 wherein a plano-surface of said plano-concave back surface mirror is optically coupled to said flat, transparent substrate.

96. The composite microscope objective (60) of claim 95 wherein an optical adhesive material accomplishes the optical coupling.

97. The composite microscope objective (60) of claim 95 wherein an index matching gel accomplishes the optical coupling.

98. The composite microscope objective (60) of claim 95 wherein an index matching fluid accomplishes the optical coupling.

99. The composite microscope objective (60) of claim 95 wherein optical contact bonding accomplishes the optical coupling.

100. The composite microscope objective (60) of claim 94 wherein said plano-concave back surface mirror is formed integrally with said flat, transparent substrate.

101. The composite microscope objective (60) of claim 93 wherein said aberration corrector plate is detached from said flat, transparent substrate.

102. A method for characterizing microscopic species using a microscope objective device comprising:

a) A concave mirror;

b) An aberration corrector plate made of optically transparent material wherein said aberration corrector plate is an ashperic lens with the zone of said plate means outside of the neutral zone wherein the thickness of said plate means is thinnest, having negative optical power, and the zone inside the neutral zone having positive optical power

c) A viewing zone located in between said concave mirror and said aberration corrector plate.

103. The method of claim 102 wherein an optical image of the viewing zone is formed outside the device.

104. The method of claim 103 wherein the viewing zone is located within a flow channel contained in a rectangularly-shaped cuvette means made of optically transparent material.

105. The method of claim 104 wherein said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

106. The method of claim 105 wherein a plano surface of said plano-concave back surface mirror means is optically coupled to a flat surface of said cuvette means.

107. The method of claim 106 wherein an optical adhesive material accomplishes the optical coupling.

108. The method of claim 106 wherein an index matching gel accomplishes the optical coupling.

109. The method of claim 106 wherein an index matching fluid accomplishes the optical coupling.

110. The method of claim 106 wherein optical contact bonding accomplishes the optical coupling.

111. The method of claim 106 wherein the plano-concave back surface mirror is formed integrally with said cuvette.

112. The method of claim 104 wherein said aberration corrector plate is a plano-aspherical lens.

113. The method of claim 112 wherein a plano-surface of said aberration corrector plate is optically coupled to a flat surface of said cuvette means opposite of said concave mirror.

114. The method of claim 113 wherein an index matching gel accomplishes the optical coupling.

115. The method of claim 113 wherein an index matching fluid accomplishes the optical coupling.

116. The method of claim 113 wherein optical contact bonding accomplishes the optical coupling.

117. The method of claim 113 wherein the plano-aspherical lens is formed integrally with said cuvette.

118. The method of claim 112 wherein said aberration corrector plate is detached from said cuvette.

119. The method of claim 102 wherein the viewing zone is inside a jet stream.

120. The method of claim 119 wherein said concave mirror is a front surface mirror.

121. The method of claim 102 wherein the viewing zone is located on a surface of a flat, transparent substrate.

122. The method of claim 121 wherein said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

123. The method of claim 122 wherein a plano surface of said plano-concave back surface mirror means is optically coupled to said flat, transparent substrate.

124. The method of claim 123 wherein an optical adhesive material accomplishes the optical coupling.

125. The method of claim 123 wherein an index matching gel accomplishes the optical coupling.

126. The method of claim 123 wherein an index matching fluid accomplishes the optical coupling.

127. The method of claim 123 wherein optical contact bonding accomplishes the optical coupling.

128. The method of claim 122 wherein said plano-concave back surface mirror is formed integrally with said flat, transparent substrate.

129. The method of claim 128 wherein said aberration corrector plate is detached from said flat, transparent substrate.

130. A fluidic subsystem (70) for supplying a liquid flow pulsation free to an outlet of the fluidic subsystem (70), the fluidic subsystem (70) comprising:

a) a liquid pump for supplying liquid drawn from a reservoir;

b) a T-coupling having at least one (1) inlet and two (2) outlets,

i. the inlet of said T coupling receiving liquid from said liquid pump;

ii. a first fraction of the liquid received by the inlet flowing via a first one of the outlets and via a bypass conduit back to the reservoir; and

iii. a second fraction of the liquid received by the inlet flowing via a second one of the outlets and via a particle filter to the outlet of the fluidic subsystem (70).

131. The fluidic subsystem (70) of claim 130 wherein the particle filter has inlet thereto for receiving liquid from the T-coupling, the inlet of the particle filter being disposed so that air becomes trapped within the particle filter at the inlet thereto.

132. The fluidic subsystem (70) of claim 131 wherein when said liquid pump is turned off air cannot enter into the bypass conduit.

133. The fluidic subsystem (70) of claim 132 further comprising a small capsule disposed between the second one of the outlets of said T-coupling and the particle filter for storing air ejected from the particle filter when the liquid pump is turned off.

134. The fluidic subsystem (70) of claim 132 further comprising a length of tubing disposed between the second one of the outlets of said T-coupling and the particle filter for storing air ejected from the particle filter when the liquid pump is turned off.

135. The fluidic subsystem (70) of claim 132 further comprising an adjustable valve located in the bypass conduit between the first one of the outlets of the T-coupling and the reservoir for restricting liquid flow therebetween.

136. The fluidic subsystem (70) of claim 132 further comprising an adjustable valve located between the second one of the outlets of the T-coupling and the outlet of the fluidic subsystem (70) for restricting liquid flow therebetween.

137. The fluidic subsystem (70) of claim 132 wherein the throughput of the liquid pump is adjustable.

138. A method for supplying a liquid flow pulsation free to an outlet of the fluidic subsystem (70), the method comprising:

a) a liquid pump for supplying liquid drawn from a reservoir;

b) a T coupling having at least one (1) inlet and two (2) outlets,

i. the inlet of said T coupling receiving liquid from said liquid pump;

ii. a first fraction of the liquid received by the inlet flowing via a first one of the outlets and via a bypass conduit back to the reservoir; and

iii. a second fraction of the liquid received by the inlet flowing via a second one of the outlets and via a particle filter to the outlet of the fluidic subsystem (70).

139. The method of claim 138 wherein during normal operation certain amount of air is trapped near the inlet portion of said filter cartridge means.

140. The method of claim 139 wherein said reservior means holds sufficient amount of liquid such that when said pump means is turned off, portion of the tubing between said T-coupling means and said reservoir means is still filled with liquid, preventing said trapped air from leaking into said bypass means.

141. The method of claim 140 wherein said reservoir means is a capsule.

142. The method of claim 140 wherein said reservior means is a piece of tubing.

143. The method of claim 140 wherein an adjustable flow restrictor menas is placed in the bypass route.

144. The method of claim 140 wherein an adjustable flow restrictor means is placed in the sheath route.

145. The method of claim 140 wherein the throughput of the sheath pump is adjustable.

146. A peristaltic pump 80 comprising:

a) a pump housing having an arcuate curved track formed therein that extends between a pump inlet and a pump outlet;

b) a plurality of rollers that are attached to a rotor, the rollers having a substantially equal angular spacing between each pair of immediately adjacent rollers, the rotor being rotatable together with the rollers attached thereto inside said pump housing; and

c) a compressible tube sandwiched between said rollers and the arcuate curved track of said pump housing, the arcuate curved track including:

i. an exit section wherein, as a roller rolls through the exit section, said compressible tube adjacent to said roller progressively expands from fully closed at a beginning of said exit section to fully open at the pump outlet where said roller breaks contact with said compressible tube; and

ii. at least one pumping section along the arcuate curved track between the pump inlet and the pump outlet wherein said compressible tube is compressed to fully closed by at least one of said rollers.

147. The peristaltic pump 80 of claim 146 wherein the arcuate curved track of said pump housing includes at least two (2) pumping sections, the arcuate curved track further including at least one recess section located between said pumping sections along the arcuate curved track, and wherein said compressible tube at said recess section becomes decompressed to full expansion then compressed to fully closed when one (1) of said rollers rolls through said recess section.

148. The peristaltic pump 80 of claim 147 includes a plurality of recess sections along said arcuate curved track upstream of the pump outlet, the angular spacing between the compression part of said recess section adjacent to the pump outlet and said exit section of said arcuate curved track being substantially the same as the angular spacing between each pair of immediately adjacent rollers.

149. The peristaltic pump 80 of claim 147 wherein said compression part of said recess section adjacent to the pump outlet has a shape complementing a shape of said exit section of said arcuate curved track to maintain the total fluid volume inside a section of said compressible tube extending from said recess section to the pump outlet substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

150. The peristaltic pump 80 of claim 147 having a plurality of recess sections respectively interspersed between immediately adjacent pairs of a plurality of pumping sections.

151. The peristaltic pump 80 of claim 149 wherein both:

a) angular spacing between adjacent pairs of recess sections; and

b) angular spacing between said exit section of said arcuate curved track and an adjacent recess section to said exit section

are substantially the same as the angular spacing between each pair of immediately adjacent rollers.

152. The peristaltic pump 80 of claim 150 wherein shapes of a plurality of recess sections of said arcuate curved track complement a shape of said exit section of said arcuate

curved track to maintain a fluid volume in sections of said compressible tube at the plurality of recess sections and said exit section substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

153. The peristaltic pump 80 of claim 146 wherein a speed of said rotor is programmably controlled to vary substantially in inverse proportion to the fluid volume change rate in said compressible tube due to its changing compression near the exit section of said arcuate curved track.

154. A method for delivering liquid using a peristaltic pump 80 comprising:

a) a pump housing with arcuate curved track;

b) A plurality of rollers attached to a rotor means rotatable inside said pump housing;

c) Said plurality of rollers spaced at substantially equal angular spacing from each other;

d) A compressible tube sandwiched between said rollers and the arcuate curved track of said pump housing;

e) Said arcuate curved track of said pump housing including an exit section, wherein said compressible tube being progressively decompressed to full expansion when one of said rollers rolls off the compressible tube at the pump outlet;

f) At least one pumping section along the arcuate curved track of said pump housing between the pump inlet and pump outlet wherein said compressible tube is compressed to full closure by one of said rollers.

155. The method of claim 154 wherein the arcuate curved track of said pump housing includes at least two (2) pumping sections, the arcuate curved track further including at least one recess section located between said pumping sections along the arcuate curved track, and wherein said compressible tube at said recess section becomes decompressed to full expansion then compressed to fully closed when one (1) of said rollers rolls through said recess section.

156. The method of claim 155 wherein the peristaltic pump 80 includes a plurality of recess sections along said arcuate curved track upstream of the pump outlet; The angular spacing between the compression part of said recess section adjacent to the pump outlet and

said exit section of said arcuate curved track being substantially the same as the angular spacing between each pair of immediately adjacent rollers.

157. The method of claim 155 wherein said compression part of said recess section adjacent to the pump outlet has a shape complementing a shape of said exit section of said arcuate curved track to maintain the total fluid volume inside a section of said compressible tube extending from said recess section to the pump outlet substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

158. The method of claim 155 having a plurality of recess sections respectively interspersed between immediately adjacent pairs of a plurality of pumping sections.

159. The method of claim 157 wherein both:

a) angular spacing between adjacent pairs of recess sections; and

b) angular spacing between said exit section of said arcuate curved track and an adjacent recess section to said exit section

are substantially the same as the angular spacing between each pair of immediately adjacent rollers.

160. The method of claim 158 wherein shapes of a plurality of recess sections of said arcuate curved track complement a shape of said exit section of said arcuate curved track to maintain a fluid volume in sections of said compressible tube at the plurality of recess sections and said exit section substantially invariant when one of said rollers progressively rolls off said exit section of the arcuate curved track.

161. The method of claim 154 wherein a speed of said rotor of the peristaltic pump 80 is programmably controlled to vary substantially in inverse proportion to the fluid volume change rate in said compressible tube due to its changing compression near the exit section of said arcuate curved track.

162. A WDM (90) for separating a beam of light emitted from an into multiple colored bands, the WDM (90) comprising:

a) a collimating optical element that magnifies an to produce an image of substantially the same size as the effective size of said collimating optical element;

b) at least one dichroic filter located between said collimating optical element and said image, wherein said dichroic filter separates the collimated beam of light into two (2) branches of distinctive colors;

c) a focusing optical element located in one of said branches, wherein the beam of light in said branch is focused to a spot having a diameter of less than 1.0 mm by said focusing optical element; and

d) an image relay optical element located near the image produced by said collimating optical element in the other branch, wherein said image relay optical element produces an image of said collimating optical element at substantially unit magnification.

163. The WDM (90) of claim 162 wherein at least one additional dichroic filter is located between said image relay optical element and the image produced by said image relay optical element, wherein said dichroic filter produces two (2) branches of the beam of light having distinctive colors.

164. The WDM (90) of claim 163 wherein another focusing optical element is located in one of said branches and focuses the beam of light in the branch into a spot having a diameter of less than 1.0 mm.

165. The WDM (90) of claim 164 wherein successive combinations of said image relay optical element, dichroic filter and focusing optical element are cascaded to produce additional focused spots having a diameter of less than 1.0 mm for multiple colored bands of said beam of light.

166. The WDM (90) of claim 164 wherein the dichroic filter is assembled using a template that include two (2) optically flat glass plates bonded together in optical contact, and wherein the dichroic filter is bonded to a filter holder using the template such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder.

167. The WDM (90) of claim 166 wherein the reference surface of the filter holder rests against an optically flat surface of an reference block included in the WDM (90) thereby providing consistent optical alignment when installing the dichroic filter into the WDM (90).

168. A method for separating beam of light emitted from an into colored bands using a WDM (90) comprising:

a) a collimating optical element that magnifies an to produce an image of substantially the same size as the effective size of said collimating optical element;

b) at least one dichroic filter located between said collimating optical element and said image, wherein said dichroic filter separates the collimated beam of light into two (2) branches of distinctive colors;

c) a focusing optical element located in one of said branches, wherein the beam of light of said branch is focused to a spot having a diameter of less than 1.0 mm by said focusing optical element; and

d) an image relay optical element located near the image produced by said collimating optical element in the other branch, wherein said image relay optical element produces an image of said collimating optical element at substantially unit magnification.

169. The method of claim 168 wherein at least one additional dichroic filter is located between said image relay optical element and the image produced by said image relay optical element, wherein said dichroic filter produces two (2) branches of beam of light having distinctive colors.

170. The method of claim 169 wherein another focusing optical element is located in one of said branches and focuses the beam of light in the branch into a spot having a diameter of less than 1.0 mm.

171. The method of claim 170 wherein successive combinations of said image relay optical element, dichroic filter and focusing optical element are cascaded to produce additional focused spots having a diameter of less than 1.0 mm for multiple colored bands of said beam of light.

172. The method of claim 170 wherein the dichroic filter is assembled using a template that include two (2) optically flat glass plates bonded together in optical contact, and wherein the dichroic filter is bonded to a filter holder using the template such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder.

173. The method of claim 172 wherein the reference surface of the filter holder rests against an optically flat surface of an reference block included in the WDM (90) thereby providing consistent optical alignment when installing the dichroic filter into the WDM (90).

174. An optical system for impinging beams of light into a viewing zone in which a sample flow carrying objects and a sheath flow pass through, comprising:

a first light source for emitting a first beam of light along a first beam path to illuminate objects in the viewing zone at a first location;

a second light source for emitting a second beam of light along a second beam path to illuminate objects in the viewing zone at a second location, wherein the wavelength of the second light source is different from the wavelength of the first light source;

a beam compressing optical element for reducing widths of the first and second beams of light on their major axes to a width less than the width of the sheath flow; and

a first chromatic compensation element located on at least one of the first beam path and the second beam path for compensating chromatic aberration in the viewing zone such that the first location and the second locations are on a common plane parallel to the direction of the sample flow.

175. The optical system of claim 174, further comprising a cuvette in which the viewing zone is located.

176. The optical system of any one of claims 174 and 175, wherein the sample flow and the sheath flow form a jet stream in which the viewing zone is located.

177. The optical system of any one of claims 174 to 176, further comprising a collimating lens for converting the first beam of light and the second beam of light into collimated elliptical beams of light, wherein the minor axes of the collimated elliptical beams of light are substantially parallel to the direction of the sample flow.

178. The optical system of any one of claims 174 to 177, wherein the beam compressing optical element reduces widths of the first and second beams of light on their major axes, wherein the major axes are substantially perpendicular to the direction of the sample flow.

179. The optical system of any one of claims 174 to 178, further comprising a cylindrical focusing element positioned adjacent to the viewing zone with an axis of the cylindrical focusing element being oriented perpendicular to the direction of the sample flow.

180. The optical system of claim 179, wherein the cylindrical focusing element focuses the first beam of light and the second beam of light on their minor axes in the viewing zone.

181. The optical system of any one of claims 174 to 180, further comprising a second chromatic compensation element, wherein the first chromatic compensation element is located on the first beam path, wherein the second chromatic compensation element is located on the second beam path.

182. The optical system of claim 181, wherein the first chromatic compensation element and the second chromatic compensation element have different optical properties.

183. The optical system of any one of claims 174 to 182, further comprising a first wavelength division multiplexer (WDM) and a second WDM to process light scattered from and fluoresced by objects at the first and second locations, respectively.

184. The optical system of any one of claims 174 to 183, further comprising a third light source for emitting a third beam of light along a third beam path to illuminate objects in the viewing zone at a third location, wherein the wavelength of the third light source is different from the wavelengths of the first and second light sources.

185. The optical system of claim 184, further comprising a third chromatic compensation element located on the third beam path for compensating chromatic aberration in the viewing zone such that the first location, the second location, and the third location are on the common plane parallel to the direction of the sample flow.

186. The optical system of any one of claims 184 and 185, further comprising a third wavelength division multiplexer (WDM) to process light scattered from and fluoresced by objects at the third location.

187. An optical system comprising:

a first light source for emitting a first beam of light to illuminate objects at a first location in a viewing zone;

a composite microscope objective for imaging light scattered from and fluoresced by the objects at the first location in the viewing zone at an image plane external to the composite microscope, wherein the composite microscope includes a concave mirror and an aberration corrector plate, wherein the aberration corrector plate is an aspheric lens that has a first zone with negative optical power and a second zone with positive optical power

radially inside the first zone, wherein the viewing zone is positioned between the concave mirror and the aberration corrector plate; and

a beam splitter for reflecting or transmitting scattered and fluoresced light, wherein the light source and the image plane are on two sides of the beam splitter.

188. The optical system of claim 187, wherein the aspheric lens further comprises a neutral zone between the first and second zones.

189. The optical system of claim 188, wherein the neutral zone is the thinnest portion of the aspheric lens.

190. The optical system of any one of claims 187 to 189, further comprising a cuvette with a flow channel, wherein the viewing zone is located in the flow channel.

191. The optical system of claim 190, further comprising a fluidic system to deliver the objects to the viewing zone in the flow channel.

192. The optical system of any one of claims 190 and 191, wherein the cuvette has a rectangular-shaped cross section and is made of optically transparent material.

193. The optical system of any one of claims 190 to 192, wherein the aspheric lens and the concave mirror are coupled to or separated from the cuvette.

194. The optical system of any one of claims 190 to 193, wherein the aspheric lens and the concave mirror are coupled to the cuvette by one or more of an optical adhesive material, an index matching gel, an index matching fluid, and an optical contact bonding.

195. The optical system of any one of claims 187 to 189, further comprising a jet stream, wherein the viewing zone is located in the jet stream.

196. The optical system of any one of claims 187 to 189, further comprising a substrate, wherein the viewing zone is located on the surface of the substrate.

197. The optical system of claim 196, wherein the substrate is flat and made of optically transparent material.

198. The optical system of any one of claims 187 to 197, wherein the concave mirror is a plano-concave back surface mirror or a front surface mirror made of an optically transparent material.

199. The optical system of any one of claims 187 to 198, wherein the aspheric lens is a plano-aspherical lens.

200. The optical system of any one of claims 187 to 199, wherein the first beam of light is reflected by the beam splitter and enters the composite microscope objective, wherein the scattered and fluoresced light propagates out of the composite microscope objective and transmits through the beam splitter.

201. The optical system of claim 200, wherein the optical axis of the first beam of light reflected by the beam splitter and entering into the composite microscope objective is substantially parallel to the optical axis of the scattered and fluoresced light propagating out of the composite microscope objective.

202. The optical system of any one of claims 187 to 199, wherein the first beam of light transmits through the beam splitter and enters the composite microscope objective, and wherein the scattered and fluoresced light propagates out of the composite microscope objective and is reflected by the beam splitter.

203. The optical system of claim 202, wherein the optical axis of the first beam of light transmitting through the beam splitter and entering into the composite microscope objective is substantially parallel to the optical axis of the scattered and fluoresced light propagating out of the composite microscope objective.

204. The optical system of any one of claims 187 to 203, further comprising a detector to detect the scattered and fluoresced light propagating out of the composite microscope objective.

205. The optical system of claim 204, further comprising an optical transmission member for propagating the scattered and fluoresced light received at the image plane to the detector.

206. The optical system of any one of claims 204 and 205, further comprising a wavelength division multiplexer (WDM) to process the scattered and fluoresced light to be detected by the detector, wherein the scattered and fluoresced light is separated into multiple colored bands.

207. The optical system of claim 206, wherein the wavelength division multiplexer (WDM) comprises:

i. a collimating optical element that produces an image of substantially the same size as the effective size of the collimating optical element;

ii. at least one dichroic filter located between the collimating optical element and the image, wherein the dichroic filter separates the collimated beam of light into two branches of distinctive colors;

iii. a focusing optical element located in one of the branches, wherein the beam of light in the branch is focused to a spot by the focusing optical element; and

iv. an image relay optical element located near the image produced by the collimating optical element in the other branch, wherein the image relay optical element produces an image of the collimating optical element at substantially unit magnification.

208. The optical system of claim 187, wherein the first light source emits coherent light or incoherent light.

209. The optical system of any one of claims 187 to 208, further comprising a second light source for emitting a second beam of light to illuminate objects at a second location in the viewing zone, wherein the first and second locations are spatially separated.

210. The optical system of claim 209, further comprising a first detector and a second detector to detect the scattered and fluoresced light by the objects at the first and second locations, respectively.

211. The optical system of claim 210, further comprising a first optical transmission member and a second optical transmission member for propagating the scattered and fluoresced light by the objects at the first and second locations received at the image plane to the first and the second detectors, respectively.

212. The optical system of any one of claims 210 and 211, further comprising a first wavelength division multiplexer (WDM) and a second WDM to process the scattered and fluoresced light to be detected by the first and second detectors, wherein the scattered and fluoresced light is separated into multiple colored bands.

213. A light detection system comprising:

a concave mirror for reflecting light that propagates from a viewing zone; and

a detector for measuring axial light loss produced by an object in the viewing zone by detecting light reflected by the concave mirror.

214. The light detection system of claim 213, wherein the detector is an axial light loss detector to measure the axial light loss produced by the object in order to determine the size of the object.

215. The light detection system of any one of claims 213 and 214, further comprising a first light source emitting a first beam of light to irradiate the object in the viewing zone, wherein the axis of irradiation is substantially perpendicular to the flow direction of the object.

216. The light detection system of claim 215, wherein the light reflected by the concave mirror includes forward scattered light and remaining light of the first beam of light, wherein the axial light loss of the first beam of light results from the object passing through the first beam of light, and wherein the optical axis of the remaining light is substantially paralleled to the axis of irradiation.

217. The light detection system of claim 216, wherein the forward scattered light and the remaining light have the same wavelength.

218. The light detection system of any one of claims 216 and 217, wherein the detector is in a heterodyne mode measuring coherent interference of the forward scattered light and the remaining light.

219. The light detection system of any one of claims 215 to 218, further comprising a second light emitting a second beam of light to irradiate the object in the viewing zone, wherein the wavelength of the second beam of light is different from the wavelength of the first beam of light.

220. The light detection system of claim 219, further comprising a filter upstream of the detector to separate the light irradiated by the first light source and reflected by the concave mirror from the light irradiated by the second light source and reflected by the concave mirror in order for the detector to measure them separately.

221. The light detection system of any one of claims 213 to 220, wherein the viewing zone is located within a microscope objective.

222. The light detection system of claim 221, further comprising a cylindrical lens coupled with the microscope objective, wherein the optical axis of the cylindrical lens is substantially perpendicular to the optical axis of the light reflected by the concave mirror.

223. The light detection system of any one of claims 221 and 222, further comprising a second concave mirror and an aberration corrector plate located on two sides of the viewing zone of the microscope objective, wherein the optical axes of the second concave mirror and the aberration corrector plate are substantially parallel to the optical axis of the light reflected by the concave mirror.

224. The light detection system of claim 223, wherein light side-scattered from and fluoresced by the object in the viewing zone is reflected by the second concave mirror and propagates out of the microscope objective via the aberration corrector plate, wherein the aberration corrector plate is an aspheric lens that has a first zone with negative optical power and a second zone with positive optical power radially inside the first zone.

225. The light detection system of claim 224, further comprising a second light detection system to detect and process the light side-scattered from and fluoresced by the object in the viewing zone of the microscope objective.

226. The light detection system of any one of claims 213 to 225, wherein the concave mirror is an ellipsoidal mirror.

227. The light detection system of any one of claims 213 to 225, wherein the concave mirror is a combination of a flat mirror and a lens.

228. A power monitoring system for adjusting power of a light source, comprising:

a first light source for emitting a first beam of light;

a second light source for emitting a second beam of light;

a first dichroic filter for reflecting the first beam of light and passing the second beam of light;

a second dichroic filter for reflecting the second beam of light;

a first detector for measuring residual power of the first and second beams of light downstream of the first dichroic filter on a time-division multiplexing basis; and

a control unit coupled with the first detector and the first and second light sources, wherein the control unit adjusts power of one or more of the first and second light sources based on measured residual power of the first and second beams of light by the first detector.

229. The power monitoring system of claim 228, wherein the first detector is positioned near the first dichroic filter.

230. The power monitoring system of any one of claims 228 and 229, wherein the first detector is coupled to the first dichroic filter.

231. The power monitoring system of any one of claims 228 to 230, wherein the residual power of the first and second beams of light includes power of the first beam of light passing through the first dichroic filter and power of the second beam of light reflected by the first dichroic filter.

232. The power monitoring system of any one of claims 228 to 230, wherein the first detector measures the residual power of the first beam of light when the second light source is off to reduce interference between the residual power of the first and second beams of light.

233. The power monitoring system of any one of claims 228 to 230, wherein the first detector measures the residual power of the second beam of light when the first light source is off to reduce interference between the residual power of the first and second beams of light.

234. The power monitoring system of any one of claims 228 to 233, wherein the control unit includes a feedback circuit to increase the power of the light source when residual power of the light source drops or to lower the power of the light source when the residual power of the light source increases.

235. The power monitoring system of any one of claims 228 to 234, further comprising a second detector positioned near the second dichroic filter to measure the residual power of the second beam of light downstream of the second dichroic filter, wherein the second detector is coupled to the control circuit.

236. The power monitoring system of claim 235, wherein the residual power of the second beam of light downstream of the second dichroic filter includes power of the second beam of light passing through the second dichroic filter.

237. The power monitoring system of any one of claims 228 to 236, further comprising a third light source for emitting a third beam of light and a third dichroic filter acting as a mirror to reflect the third beam of light, wherein the third light source is coupled to the control circuit.

238. The power monitoring system of claim 237, wherein the first detector measures residual power of the first, second, and third beams of light downstream of the first dichroic filter on a time-division multiplexing basis.

239. The power monitoring system of any one of claims 237 and 238, further comprising a second detector positioned near the second dichroic filter to measure residual power of the second and third beams of light downstream of the second dichroic filter on a time-division multiplexing basis, wherein the second detector is coupled to the control circuit.

240. The power monitoring system of claim 234, wherein the residual power of the second and third beams of light downstream of the second dichroic filter includes power of the second beam of light passing through the second dichroic filter and power of the third beam of light reflected by the second dichroic filter.

241. The power monitoring system of any one of claims 237 to 240, further comprising a third detector positioned near the third dichroic filter to measure residual power of the third beams of light downstream of the third dichroic filter, wherein the third detector is coupled to the control circuit.

242. The power monitoring system of claim 241, wherein the residual power of the third beam of light downstream of the third dichroic filter includes power of the third beam of light passing through the third dichroic filter.

243. The power monitoring system of any one of claims 228 to 242, wherein the second dichroic filter is one of a dichroic filter and a mirror.

244. An optical system comprising:

a) an objective adapted for imaging light scattered from and fluoresced by an illuminated object within a viewing zone comprising:

i. an aspheric lens with a first zone with negative optical power and a second zone inside the first zone with positive optical power; and

ii. a concave mirror for reflecting light scattered from and fluoresced by the illuminated object through the aspheric lens, wherein the viewing zone is located between said concave mirror and the aspheric lens;

b) an optical transmission member for propagating light received from the aspheric lens; and

c) a wavelength division multiplexer (WDM) for receiving light propagated by the optical transmission member, the wavelength division multiplexer (WDM) comprising:

i. a first optical element that produces a beam of light with an image of substantially the same size as the effective size of said first optical element;

ii. at least one dichroic filter located between said first optical element and said image, wherein said dichroic filter separates the beam of light into two branches of distinctive colors;

iii. a second optical element located in one of said branches, wherein the beam of light in said branch is focused to a spot by said second optical element; and

iv. an image relay optical element located near the image produced by said first optical element in the other branch, wherein said image relay optical element produces an image of said first optical element at substantially unit magnification.

245. The optical system of claim 244, wherein the aspheric lens further comprises a neutral zone between the first and second zones.

246. The optical system of claim 245, wherein the neutral zone is the thinnest portion of the aspheric lens.

247. The optical system of any one of claims 244 to 246, wherein the concave mirror comprises a spherical mirror.

248. The optical system of any one of claims 244 to 247, wherein the aspheric lens comprises a plano-aspheric lens.

249. The optical system of any one of claims 244 to 248, further comprising a cuvette with a flow channel, wherein the viewing zone is located in the flow channel.

250. The optical system of claim 249, further comprising a fluidic system to deliver objects to the viewing zone in the flow channel.

251. The optical system of any one of claims 249 and 250, wherein the cuvette has a rectangularly-shaped cross-section.

252. The optical system of any one of claims 249 to 251, wherein the aspheric lens and concave mirror are coupled to the cuvette.

253. The optical system of any one of claims 244 to 252, wherein the optical transmission member comprises an optical fiber.

254. The optical system of claim 253, wherein the optical fiber comprises a multimode fiber.

255. The optical system of any one of claims 244 to 254, wherein the first optical element is a collimating element.

256. The optical system of any one of claims 244 to 254, wherein the second optical element is a focusing element.

257. The optical system of any one of claims 244 to 256, wherein the image relay optical unit comprises a concave mirror.

258. The optical system of any one of claims 244 to 257, wherein the spot is less than 1.0 mm.

259. The optical system of any one of claims 244 to 258, further comprising a filter holder for holding the dichroic filter, the filter holder having a reference surface parallel to a surface of the dichroic filter.

260. The optical system of claim 259, further comprising a reference block with a flat surface, wherein the reference surface of the filter holder is pressed against the flat surface of the reference block.

261. The optical system of claim 260, wherein the reference surface of the filter holder and the surface of the dichroic filter are offset.

262. The optical system of any one of claims 244 to 261, wherein the image relay optical unit comprises a convex lens.

263. An optical system comprising:

a) a light source for emitting a beam of light to illuminate an object in a viewing zone;

b) a concave mirror for receiving and reflecting light scattered from and fluoresced by the illuminated object;

c) an aspheric lens with a first zone with negative optical power and a second zone inside the first zone with positive optical power,

wherein light reflected by the concave mirror passes through the aspheric lens, and wherein the viewing zone is located between said concave mirror and the aspheric lens;

d) an optical transmission member for receiving and propagating light from the aspheric lens; and

e) a multiplexer for receiving light from the optical transmission member and separating the light into at least two colors.

264. The system of claim 263, wherein the light source comprises a laser for generating a laser beam.

265. The system of claim 264, further comprising optical elements for collimating and shaping the laser beam.

266. The system of any one of claims 263 to 265, wherein the multiplexer comprises a wavelength division multiplexer (WDM) comprising:

i. a first optical element that receives light from the optical transmission member and produces an image of substantially the same size as the effective size of said first optical element;

ii. at least one dichroic filter located between said first optical element and said image, wherein said dichroic filter separates the light into two branches of different colors;

iii. a second optical element for focusing one of said branches of light to a spot; and

iv. an image relay optical unit located near the image produced by said first optical unit in the other branch, wherein said image relay optical unit produces an image of said first optical element at substantially unit magnification.

267. The system of claim 266, wherein the first optical element is a collimating element.

268. The system of any one of claims 266 and 267, wherein the second optical element is a focusing element.

269. The system of any one of claims 266 to 268, wherein the image relay optical unit comprises a concave mirror.

270. The system of any one of claims 266 to 269 wherein the image relay optical unit comprises a convex lens.

271. The system of any one of claims 266 to 270, wherein the spot is less than 1.0 mm.

272. The system of any one of claims 266 to 271, further comprising a filter holder for holding the dichroic filter, the filter holder having a reference surface parallel to a surface of the dichroic filter.

273. The system of claim 272, further comprising a reference block with a flat surface, wherein the reference surface of the filter holder is pressed against the flat surface of the reference block.

274. The system of claim 273, wherein the reference surface of the filter holder and the surface of the dichroic filter are offset.

275. An apparatus for imaging light scattered from and fluoresced by an illuminated object within a viewing zone comprising:

a) a fluid delivery system for delivering an object to a viewing zone;

b) a light source for illuminating the object in the viewing zone;

c) a concave mirror located on one side of the viewing zone for reflecting light scattered from and fluoresced by the illuminated object;

d) an aspheric lens located on another side of the viewing zone for receiving the light reflected by the concave mirror and forming an image at an image plane, the aspheric lens having a first zone with negative optical power and a second zone radially inside the first zone with positive optical power.

276. The apparatus of claim 275, further comprising a cuvette with a flow channel coupled to the fluid delivery system, wherein the viewing zone is in the flow channel.

277. The apparatus claim 276, wherein the concave mirror and the aspheric lens are coupled to the cuvette.

278. The apparatus of claim 275, wherein the viewing zone is located on a surface of a flat, transparent substrate.

279. The apparatus of claim 275, wherein the viewing zone is located inside a jet stream.

280. The apparatus of an one of claim 276 to 279, wherein a flow along the channel or jet stream comprises a sheath flow and a sample flow.

281. The apparatus of any one of claims 275 to 280, wherein the light source comprises a coherent light source.

282. The apparatus of claim 281, wherein the coherent light source comprises a laser diode.

283. The apparatus of any one of claims 275 to 282, further comprising a third zone located between the first and second zones, wherein the third zone has neutral optical power.

284. The apparatus of claim 283, wherein the third zone is the thinnest portion of the aspheric lens.

285. A method of gathering and imaging light scattered from or fluoresced by objects in a viewing zone comprising the steps of:

delivering an object to a viewing zone;

illuminating the object in the viewing zone to produce scattered and fluoresced light;

reflecting the scattered and fluoresced light using a concave mirror toward a transparent aberration corrector plate; and

correcting spherical aberrations in the reflected light with the transparent aberration corrector plate, wherein the transparent aberration corrector plate has a first zone with negative optical power and a second zone radially inside the first zone with positive optical power.

286. The method of claim 285, wherein the step of delivering an object comprises forming a jet stream in the viewing area, said jet stream containing at least one object.

287. The method of any one of claims 285 and 286, wherein the step of delivering an object comprises:

forming a liquid sheath flow in the viewing zone; and

injecting a sample flow carrying at least one object into the sheath flow.

288. The method of any one of claims 285 to 287, wherein the step of illuminating the object comprises directing a laser beam at the object.

289. The method according to any one of claims 285 to 288, wherein the step of reflecting light comprises reflecting light with a spherical mirror.

290. The method of any one of claims 285 to 289, further comprising propagating the light to a remote location using an optical fiber.

291. The method of claim 290, wherein the step of propagating the light occurs after the step of correcting spherical aberrations.

292. The method of any one of claims 290 and 291, further comprising

collimating the propagated beam of light to produce a collimated beam of light;

filtering the collimated beam of light to produce a first beam of a color of interest and a first reflected beam;

focusing the first beam to form a first spot smaller than that of the first beam; and

detecting the first spot.

293. The method of claim 292, further comprising:

filtering the second reflected beam to produce a second beam of a color of interest and a second reflected beam;

focusing the second beam to form a second spot smaller than that of the second beam; and

detecting the second spot.

294. An optical method for impinging beams of light into a viewing zone comprising:

directing a first beam of light to illuminate objects in a viewing zone to produce scattered and fluoresced light;

reflecting the scattered and fluoresced light using a concave mirror toward an aberration corrector plate;

correcting aberrations in the reflected light with the aberration corrector plate, wherein the aberration corrector plate has a first zone with negative optical power a second zone radially inside the first zone with positive optical power; and

reflecting or transmitting the corrected light using a beam splitter.

295. The optical method of claim 294, wherein the directing the first beam of light includes reflecting the first beam of light into the viewing zone using the beam splitter when the corrected light is transmitted through the beam splitter.

296. The optical method of claim 294, wherein the directing the first beam of light includes transmitting the first beam of light into the viewing zone using the beam splitter when the corrected light is reflected by the beam splitter.

297. The optical method of any one of claims 294 to 296, wherein the optical axis of the first beam of light entering into the viewing zone is substantially parallel to the optical axis of the corrected light.

298. The optical method of any one of claims 294 to 297, further comprising detecting the corrected light.

299. The optical method of any one of claims 294 to 298, further comprising propagating the corrected light to a remote location using an optical fiber.

300. The optical method of any one of claims 294 to 299, further comprising processing the corrected light using a wavelength division multiplexer (WDM) to separate the corrected light into multiple colored bands.

301. The optical method of claim 300, wherein the wavelength division multiplexer (WDM) comprises:

i. a collimating optical element that produces an image of substantially the same size as the effective size of the collimating optical element;

ii. at least one dichroic filter located between the collimating optical element and the image, wherein the dichroic filter separates the collimated beam of light into two branches of distinctive colors;

iii. a focusing optical element located in one of the branches, wherein the beam of light in the branch is focused to a spot by the focusing optical element; and

iv. an image relay optical element located near the image produced by the collimating optical element in the other branch, wherein the image relay optical element produces an image of the collimating optical element at substantially unit magnification.

302. The optical method of any one of claims 294 to 301, wherein the first beam of light is coherent light or incoherent light.

303. An optical method for detecting light comprising:

reflecting light that propagates from a viewing zone using a concave mirror; and

measuring axial light loss produced by an object in the viewing zone by detecting light reflected by the concave mirror.

304. The optical method of claim 303, further comprising directing a first beam of light to irradiate the object in the viewing zone, wherein the axis of irradiation is substantially perpendicular to the flow direction of the object.

305. The optical method of any one of claims 303 and 304, wherein the light reflected by the concave mirror includes forward scattered light and remaining light of the first beam of light, wherein the axial light loss of the first beam of light results from the object passing through the first beam of light, and wherein the optical axis of the remaining light is substantially paralleled to the axis of irradiation.

306. The optical method of claim 305, wherein the forward scattered light and the remaining light have the same wavelength.

307. The optical method of any one of claims 305 and 306, further comprising measuring coherent interference of the forward scattered light and the remaining light.

308. The optical method of any one of claims 303 to 307, further comprising directing a second light to irradiate the object in the viewing zone, wherein the wavelength of the second beam of light is different from the wavelength of the first beam of light.

309. The optical method of claim 308, further comprising separating the light irradiated by the first light source and reflected by the concave mirror from the light irradiated by the second light source and reflected by the concave mirror using a filter in order to measure them on a time-division multiplexing basis.

310. The optical method of any one of claims 303 to 309, wherein the viewing zone is located within a microscope objective.

311. The optical method of claim 310, further comprising a cylindrical lens coupled with the microscope objective, wherein the optical axis of the cylindrical lens is substantially perpendicular to the optical axis of the light reflected by the concave mirror.

312. The optical method of any one of claims 310 and 311, further comprising a second concave mirror and an aberration corrector plate located on two sides of the viewing zone of the microscope objective, wherein the optical axes of the second concave mirror and the aberration corrector plate are substantially parallel to the optical axis of the light reflected by the concave mirror.

313. The optical method of claim 312, wherein light side-scattered from and fluoresced by the object in the viewing zone is reflected by the second concave mirror and propagates out of the microscope objective via the aberration corrector plate, wherein the aberration corrector plate is an aspheric lens that has a first zone with negative optical power and a second zone with positive optical power radially inside the first zone.

314. The optical method of claim 313, further comprising a second light detection system to detect and process the light side-scattered from and fluoresced by the object in the viewing zone of the microscope objective.

315. The optical method of any one of claims 303 to 314, wherein the concave mirror is an ellipsoidal mirror.

316. The optical method of any one of claims 303 to 314, wherein the concave mirror is a combination of a flat mirror and a lens.

317. A composite microscope objective (60) adapted for imaging light scattered from and fluoresced by an object present within a viewing zone, the composite microscope objective comprising:

a) a viewing zone;

b) a concave mirror arrangement;

c) an exit area; and

d) illumination beam forming arrangement;

wherein the viewing zone is arranged between the concave mirror arrangement and the exit area;

wherein the concave mirror is arranged to reflect scattered and fluoresced light impinging from an object present in the viewing zone to the exit area;

wherein the illumination beam forming arrangement is arranged so that an illumination beam entering the illumination beam forming arrangement is pre-definitely formed at the viewing zone.

318. The composite microscope objective of claim 317, wherein the concave mirror arrangement, the viewing zone and the exit area are arranged along a first axis/x-axis.

319. The composite microscope objective of any one of claims 317 and 318, wherein an optical image of the viewing zone is formed outside the composite microscope objective.

320. The composite microscope objective of any one of claims 317 to 319, wherein the illumination beam forming arrangement is arranged so that an illumination beam impinges the viewing zone along a second axis/z-axis substantially perpendicularly to said first axis/x-axis.

321. The composite microscope objective of any one of claims 317 to 320, further comprising a cuvette,

wherein the cuvette is of an optical transparent material, wherein the viewing zone is formed in the cuvette.

322. The composite microscope objective of claim 321, wherein the cuvette includes a channel, wherein the channel extends along a third axis/y-axis being substantially perpendicular to the first axis/x-axis and the second axis/z-axis, so that a liquid flow in the channel flows along the third axis/y-axis, wherein the viewing zone is located within the channel.

323. The composite microscope objective of any one of claims 317 to 322, wherein the cuvette is of rectangular cross section in a plane of the first axis/x-axis and second axis/z-axis.

324. The composite microscope objective of any one of claims 317 to 323, wherein the channel is of rectangular cross section in a plane of the first axis/x-axis and second axis/z-axis.

325. The composite microscope objective of any one of claims 317 to 320, further comprising a jet stream,

wherein the viewing zone is arranged in the jet stream.

326. The composite microscope objective of any one of claims 317 to 320, wherein the viewing zone is located on a surface of a flat, transparent substrate.

327. The composite microscope objective of any one of claims 317 to 326, wherein the illumination beam forming arrangement is adapted to compress an illumination beam, so that the illumination beam in the viewing zone has a compressed dimension along the third axis/y-axis.

328. The composite microscope objective of claim 327, wherein the illumination beam forming arrangement is a cylindrical lens having a cylindrical axis parallel to the first axis/x-axis.

329. The composite microscope objective of any one of claims 317 to 328, further comprising an aberration corrector arrangement,

wherein the aberration corrector arrangement is arranged in the exit area.

330. The composite microscope objective of claim 329, wherein the aberration corrector arrangement is an aspheric lens made of optically transparent material.

331. The composite microscope objective of any one of claims 329 and 330, wherein said aberration corrector arrangement comprises:

i. a first zone with negative optical power;

ii. a second zone radially inside the first zone with positive optical power; and

iii. a neutral zone between the first zone and the second zone,

wherein the neutral zone is thinner than each of the first zone and the second zone, so that light reflected from the concave mirror arrangement passing through said aberration corrector arrangement forms a focal area.

332. The composite microscope objective of any one of claims 317 to 331, wherein the concave mirror arrangement, the viewing zone and the aberration corrector arrangement form a reversed Schmidt camera.

333. The composite microscope objective of any one of claims 317 to 332, wherein the concave mirror arrangement is formed by plane-convex lens.

334. The composite microscope objective of any one of claims 317 to 332, wherein said concave mirror is a plano-concave back surface mirror made from an optically transparent material.

335. The composite microscope objective of claim 334, wherein a plano-surface of said plano-concave back surface mirror is optically coupled to a flat surface of said cuvette.

336. The composite microscope objective of clam 334, wherein a plano-surface of said plano-concave back surface mirror is optically coupled to said flat, transparent substrate.

337. The composite microscope objective of any one of claims 317 to 332, wherein said concave mirror is a front surface mirror.

338. The composite microscope objective of any one of claims 317 to 332, wherein at least one of the concave mirror arrangement, the aberration corrector arrangement and the illumination beam forming arrangement is attached to the cuvette by one selected from a group, the group consisting of index matching gel, index matching fluid, optical adhesive material and optical contact bonding.

339. The composite microscope objective of any one of claims 317 to 338, further comprising an illumination system,

wherein the illumination system comprises:

a laser source, a collimating optical arrangement to form a collimated laser beam and a beam shaping arrangement being adapted to shape a collimated beam, wherein the beam shaping arrangement includes the illumination beam forming arrangement.

340. The composite microscope objective of claim 339, wherein the laser source is a laser diode and the collimating arrangement is arranged with respect to the laser diode so as to form a collimated beam.

341. The composite microscope objective of claim 339, wherein the laser diode and the collimation optical arrangement are adapted to form a beam having an elliptical cross section having a major axis and a minor axis, wherein the minor axis is oriented substantially along the third axis/y-axis and the major axis is oriented substantially along the first axis/x-axis.

342. The composite microscope objective of any one of claims 317 to 341, wherein the beam shaping arrangement includes a major axis optical beam compressing arrangement being adapted to compress at least the major axis of the collimated elliptical beam.

343. The composite microscope objective of any one of claims 317 to 342, wherein the illumination beam forming arrangement is adapted to compress at least the minor axis of the collimated elliptical beam.

344. The composite microscope objective of any one of claims 339 to 343, wherein the illumination system is an optical system according to any one of claims 174 to 187.

345. The composite microscope objective of any one of claims 339 to 344, wherein the viewing zone is movable along the second axis/z-axis with respect to the illumination system so as to vary a focus of the compressed elliptical beam within the viewing zone along the second axis/z-axis.

346. The composite microscope objective of any one of claims 339 to 342, wherein the cuvette is movable with respect to the laser source so as to spatially vary a focus of the laser source in the channel.

347. The composite microscope objective of any one of claims 339 to 342, further comprising a control unit being adapted to control the movement of components of the composite microscope objective along the second axis/z-axis so as to spatially vary a focus of the laser source in the channel.

348. A wavelength division multiplexer (WDM) for separating light emitted from a light source into multiple colored bands, the wavelength division multiplexer comprising:

a) an imaging optical arrangement,

b) a dichroic filter arrangement,

c) a semiconductor photo detector,

d) a focusing optical arrangement

wherein the imaging optical arrangement forms a beam of light from the light emitted from a light source and produces an image of substantially the same size as the effective size of said imaging optical arrangement;

wherein the dichroic filter arrangement is located between said imaging optical arrangement and said image, and separates the beam of light into a first branch and a second branch of distinctive colors;

wherein the semiconductor photo detector is located in the first branch;

wherein the focusing optical arrangement is located between the dichroic filter arrangement and the semiconductor photo detector so as to focus the beam of light onto the semiconductor photo detector.

349. The wavelength division multiplexer according to claim 348, wherein the focusing optical element arrangement is located in or in proximity to an image plane of said image.

350. The wavelength division multiplexer according to any one of claims 348 and 349, wherein focusing optical arrangement and the semiconductor photo sensor are arranged to each other that the beam of light is focused to a spot having a diameter of less than 1.0 mm, particularly of less than 0.6 mm.

351. The wavelength division multiplexer according to any one of claims 348 to 350, further comprising an image relay optical arrangement,

wherein the image relay optical arrangement is located in or in proximity to an image plane produced by said imaging optical arrangement in the second branch, wherein said image relay optical arrangement is adapted to produce an image of said imaging optical arrangement in a third branch having substantially the same size as the image in the second branch.

352. The wavelength division multiplexer according to claim 351, wherein the image relay optical arrangement is a concave mirror.

353. The wavelength division multiplexer according to any one of claims 351 and 352, further comprising an additional dichroic filter arrangement,

wherein the additional dichroic filter arrangement is located between said image relay optical arrangement and an image produced by said image relay optical arrangement, wherein said additional dichroic filter arrangement from the third branch produces a fourth branch and a fifth branch of the beam of light having distinctive colors.

354. The wavelength division multiplexer according to claim 353, further comprising an additional focusing optical arrangement and an additional semiconductor photo detector,

wherein the additional focusing optical arrangement is located in the fourth branch and focuses the beam of light in the fourth branch so as to focus the beam of light onto the additional semiconductor photo detector.

355. The wavelength division multiplexer according to any one of claims 348 to 354, further comprising a plurality of image relay optical arrangements, a plurality of dichroic filter arrangements, a plurality of semiconductor photo detectors and a plurality of focusing optical arrangements, wherein each of the plurality of focusing optical arrangements is arranged between a respective one of the plurality of dichroic filter

arrangements and a respective one of the plurality of semiconductor photo detectors so as to form a cascaded arrangement.

356. The wavelength division multiplexer according to claim 355, wherein the additional focused spots having a diameter of less than 1.0 mm, particularly of less than 0.6 mm for multiple colored bands of said beam of light.

357. The wavelength division multiplexer according to any one of claims 348 to 356, wherein the plurality of dichroic filter arrangements are arranged in a common plane.

358. The wavelength division multiplexer according to any one of claims 348 to 357, further comprising a plan-parallel optical basis having a first surface and second surface parallel thereto,

wherein the plurality of dichroic filter arrangements are arranged parallel, preferably in abutment to the first surface of the plan-parallel optical basis.

359. The wavelength division multiplexer according to any one of claims 348 to 358, wherein the dichroic filter arrangements are assembled using a template that includes two optically flat glass plates bonded together in optical contact, wherein the dichroic filter arrangements are bonded to a filter holder using the template such that a coated filter surface of the dichroic filter arrangements are indented and optically parallel to a reference surface of the filter holder.

360. The wavelength division multiplexer according to claim 359, wherein the reference surface of the filter holder rests against an optically flat surface of an reference block included in the wavelength division multiplexer thereby providing consistent optical alignment when installing the dichroic filter arrangements into the wavelength division multiplexer.

361. The wavelength division multiplexer according to any one of claims 358 to 360, wherein the respective image relay optical arrangements are formed into the second surface of the plan-parallel optical basis.

362. The wavelength division multiplexer of any one of claims 348 to 361, wherein at least one of the semiconductor photo detectors is an avalanche photo diode detector.

363. The wavelength division multiplexer of any one of claims 348 to 362, wherein at least one of the semiconductor photo detectors is a carbon nanotube detector

ABSTRACT

The disclosed flow cytometer includes a wavelength division multiplexer (WDM). The WDM includes an extended light source providing light that forms an object, a collimating optical element that captures light from the extended light source and projects a magnified image of the object as a first light beam, and a first focusing optical element configured to focus the first light beam to a size smaller than the object of the extended light source to a first semiconductor detector. The disclosed flow cytometer further includes a composite microscope objective to direct light emitted by a particle in a flow channel in a viewing zone of the composite microscope to the extended light source, a fluidic system and a peristaltic pump configured to supply liquid sheath and liquid sample to the flow channel, and a laser diode system to illuminate the particle in the flow channel.

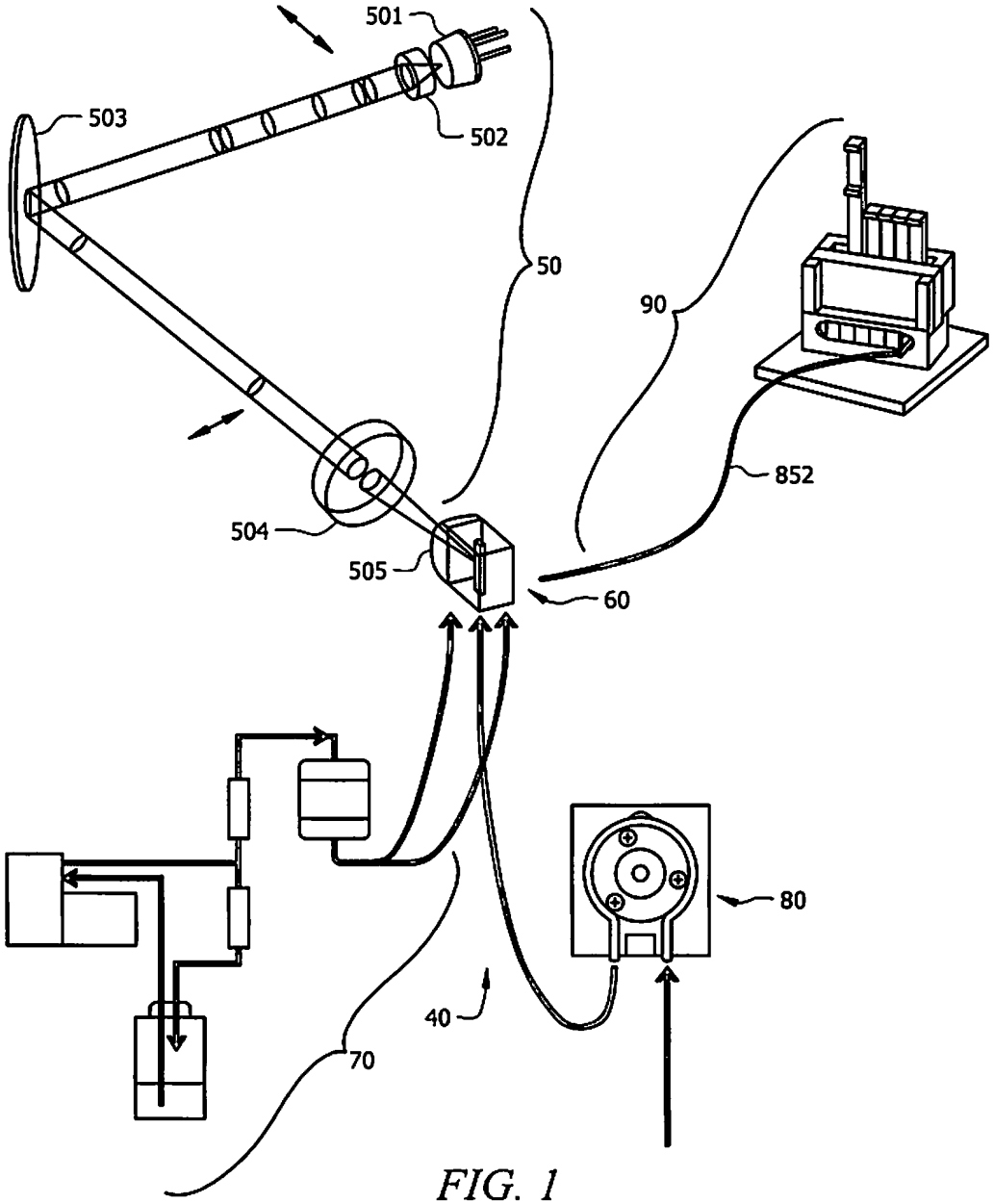


FIG. 1

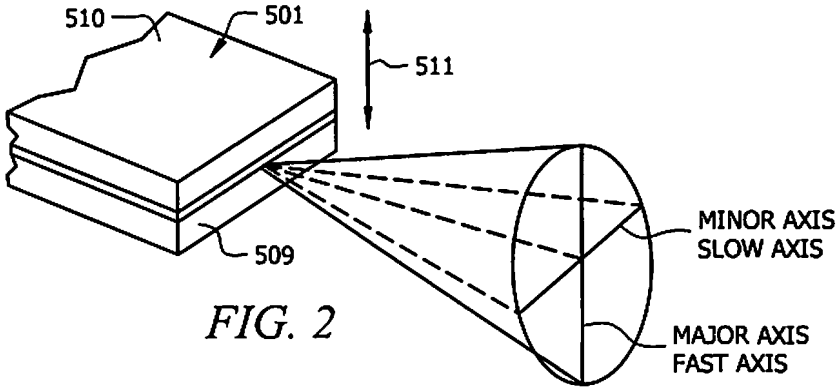


FIG. 2

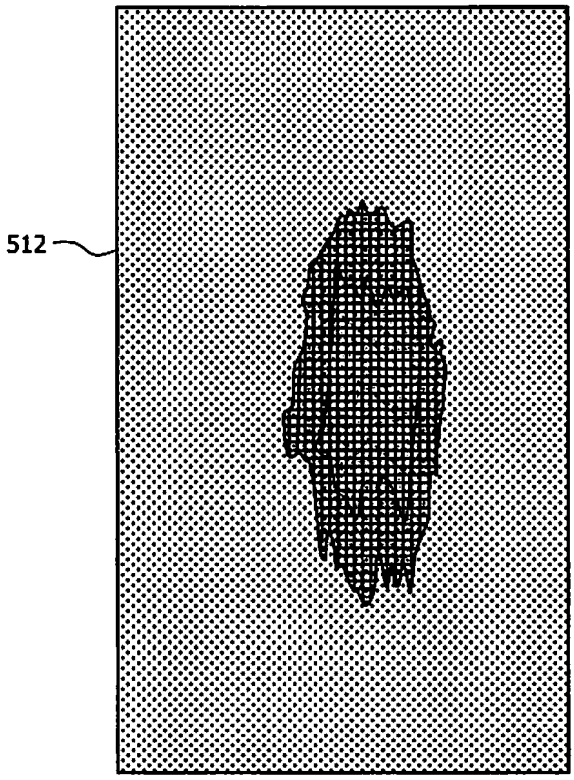


FIG. 2A

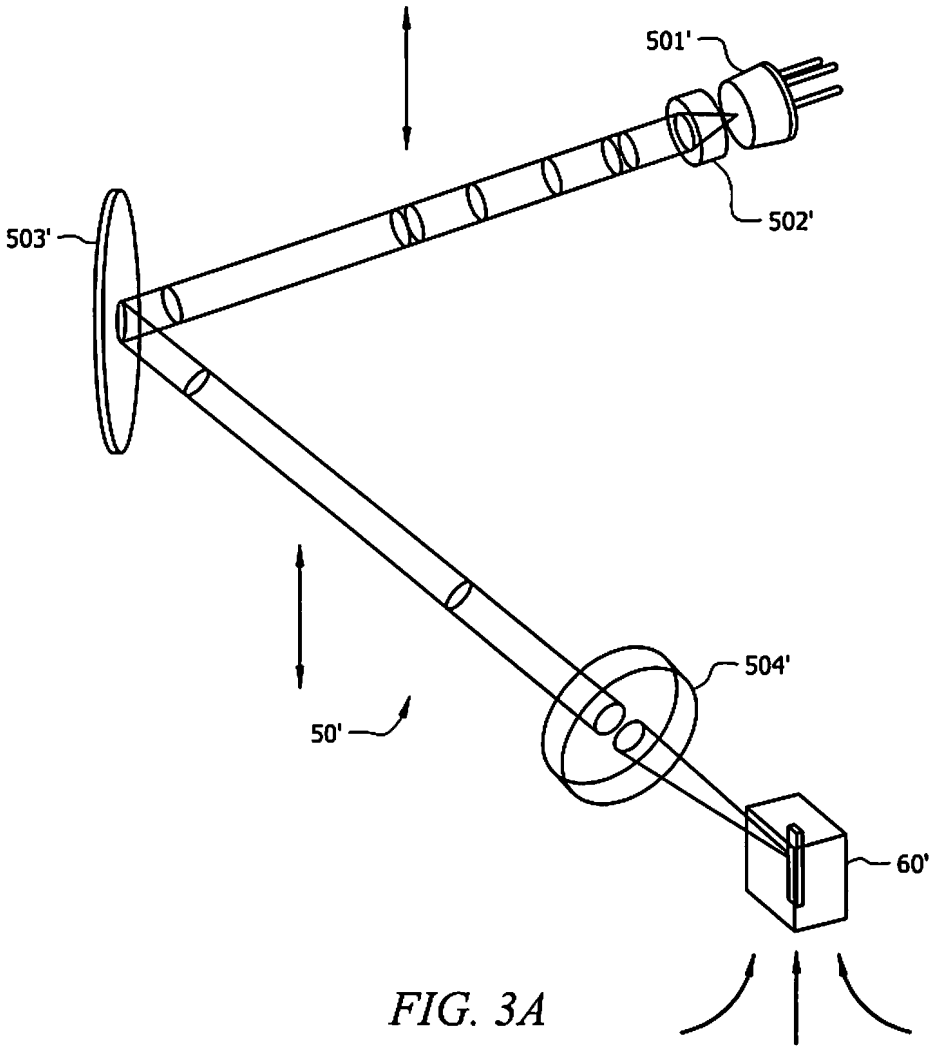


FIG. 3A

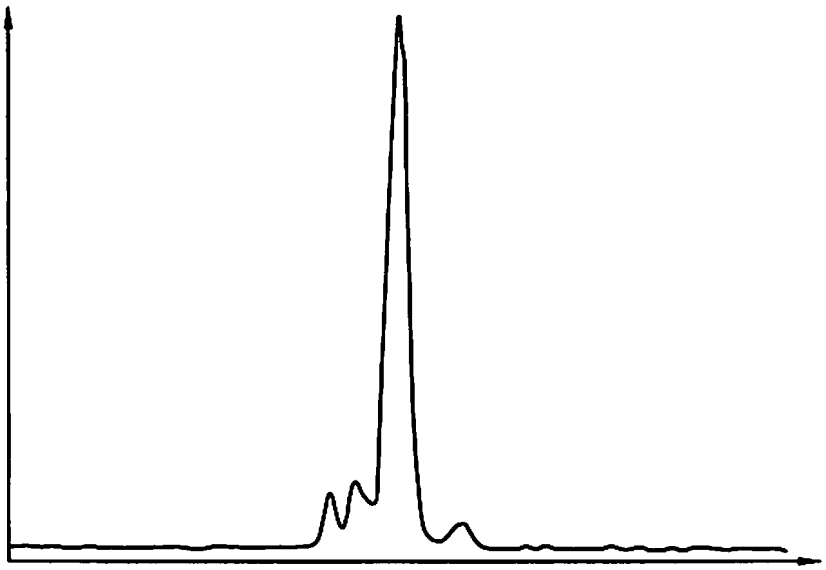


FIG. 3B

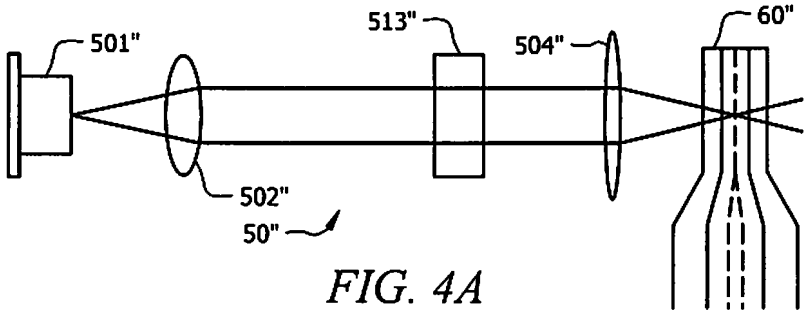


FIG. 4A

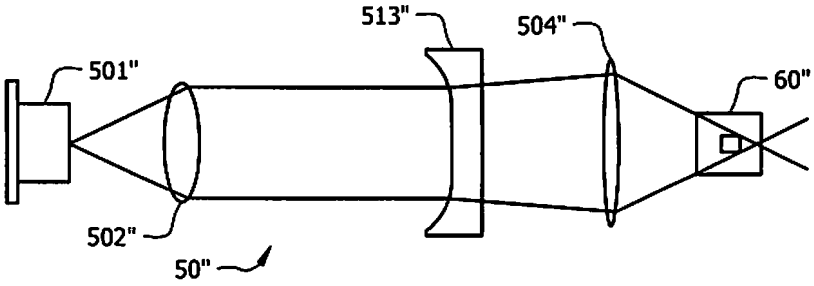


FIG. 4B

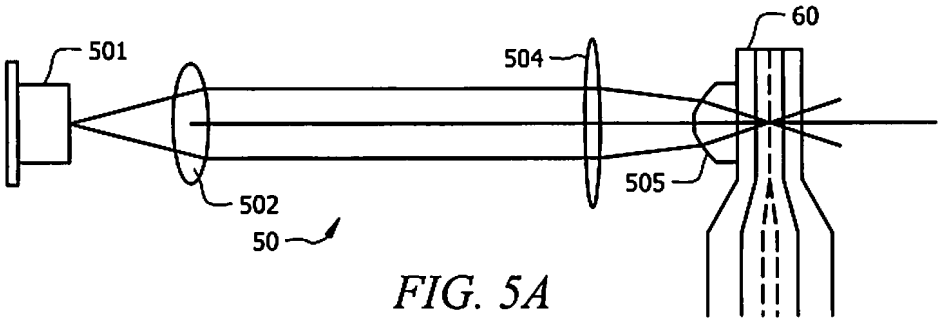


FIG. 5A

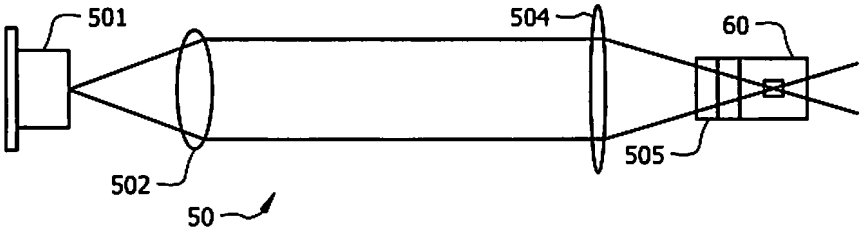


FIG. 5B

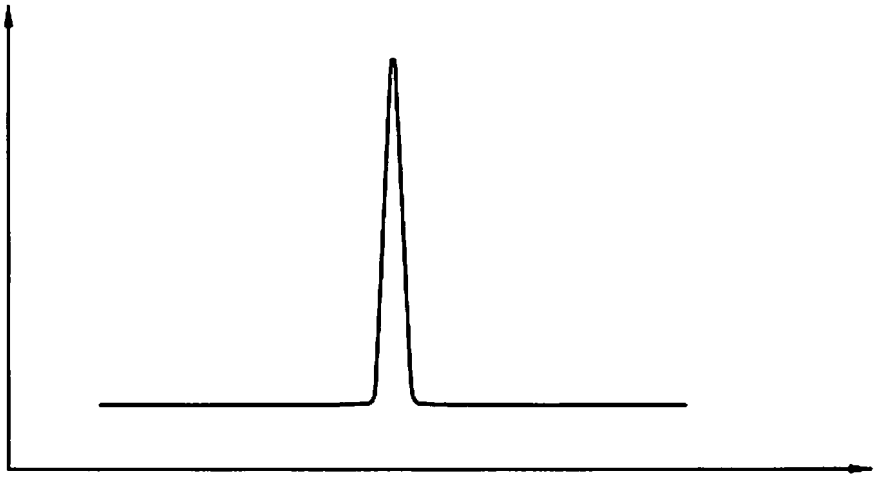


FIG. 5C

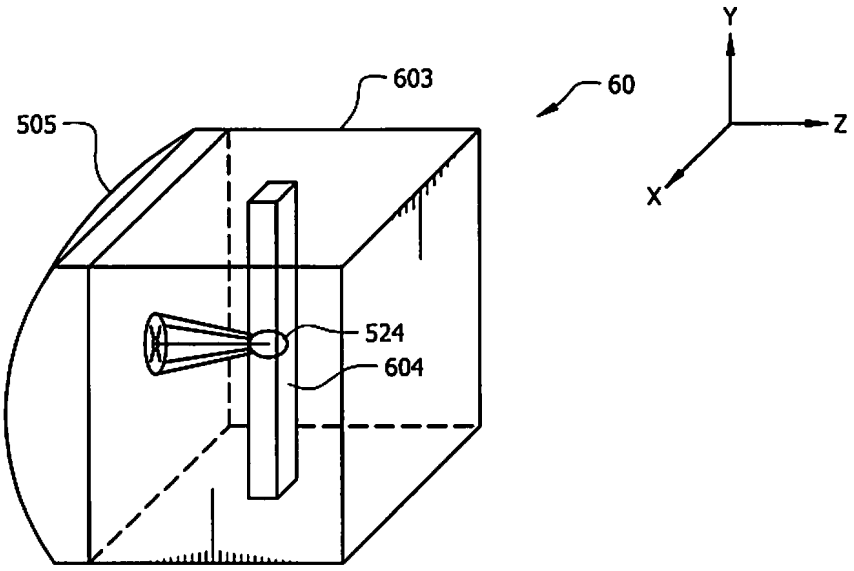


FIG. 5D

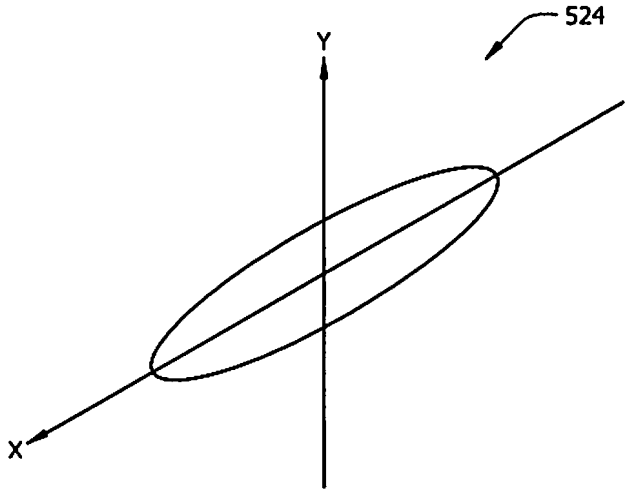


FIG. 5E

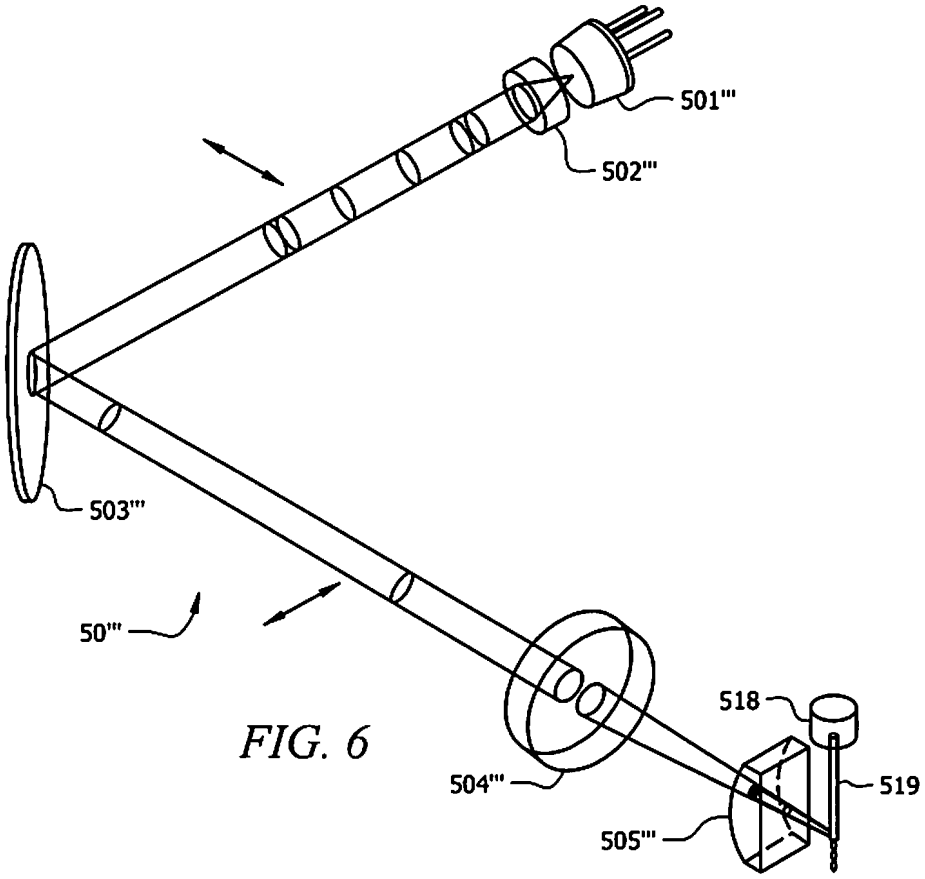


FIG. 6

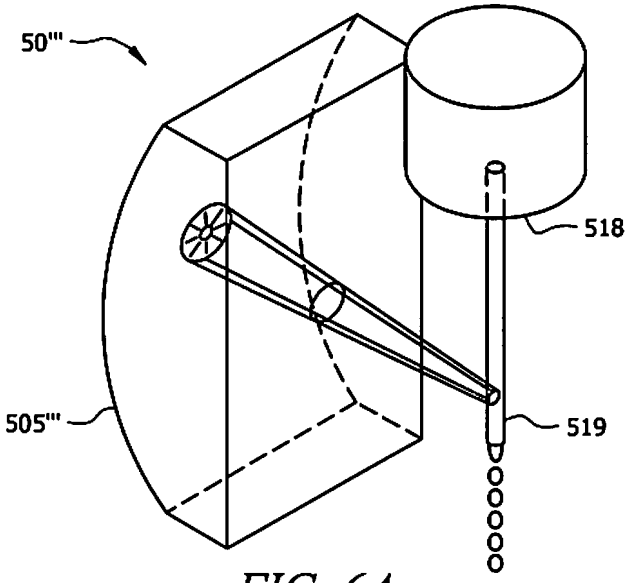


FIG. 6A

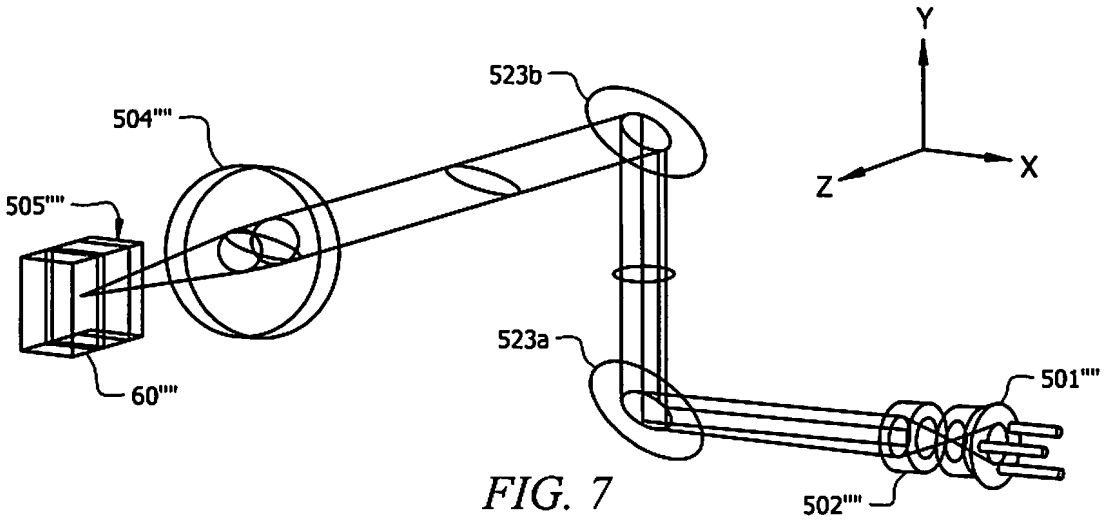


FIG. 7

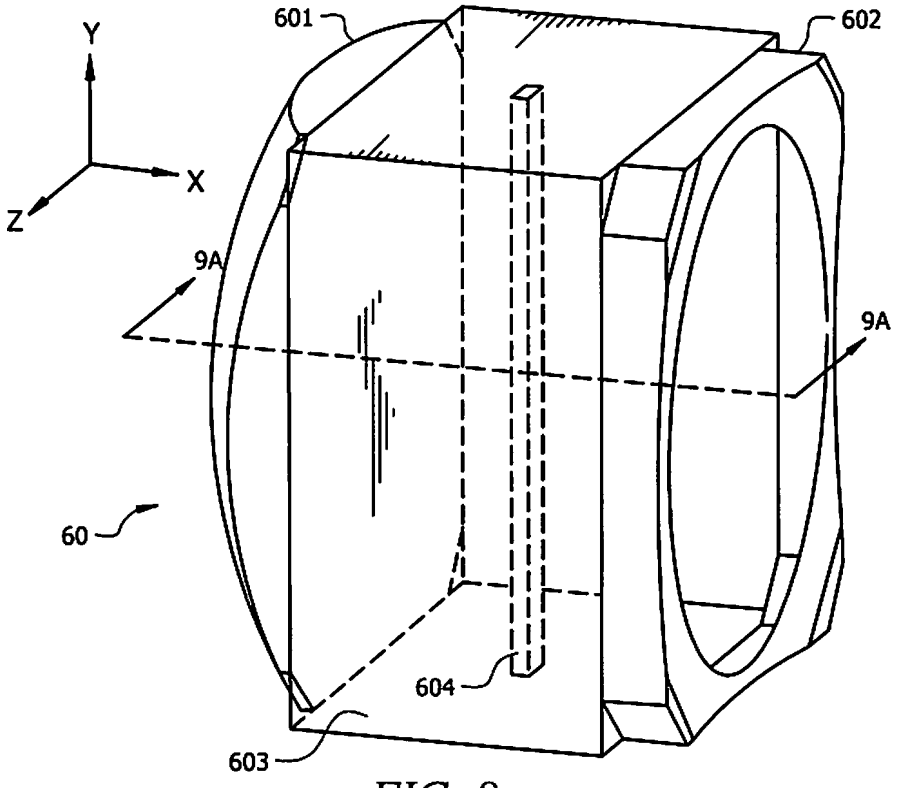


FIG. 8

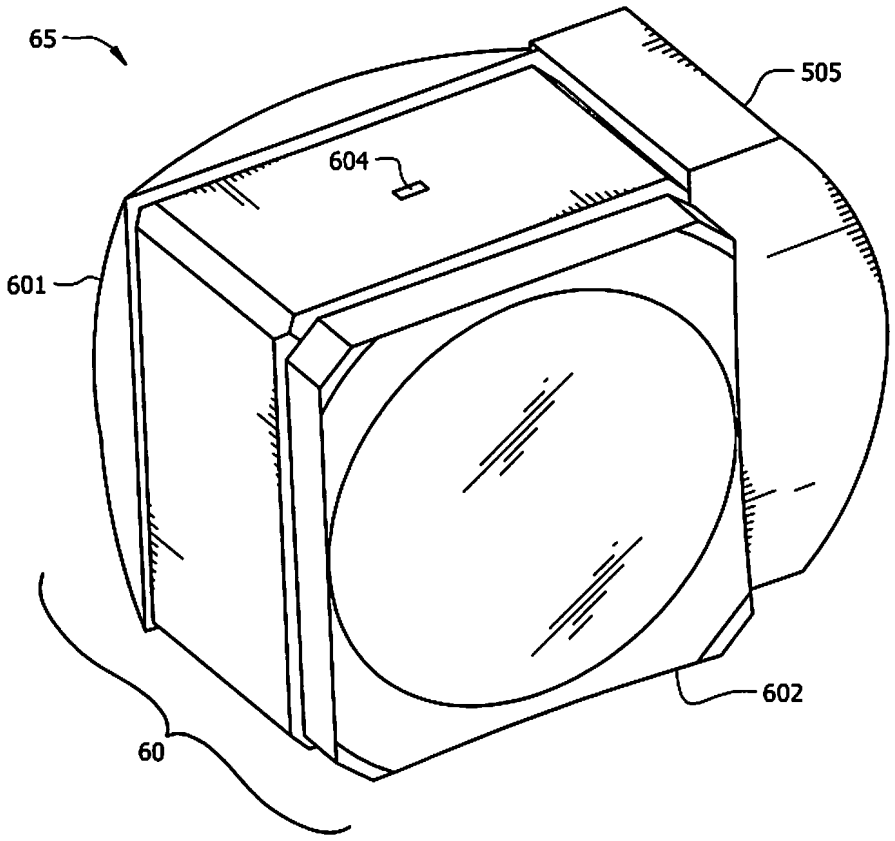


FIG. 8A

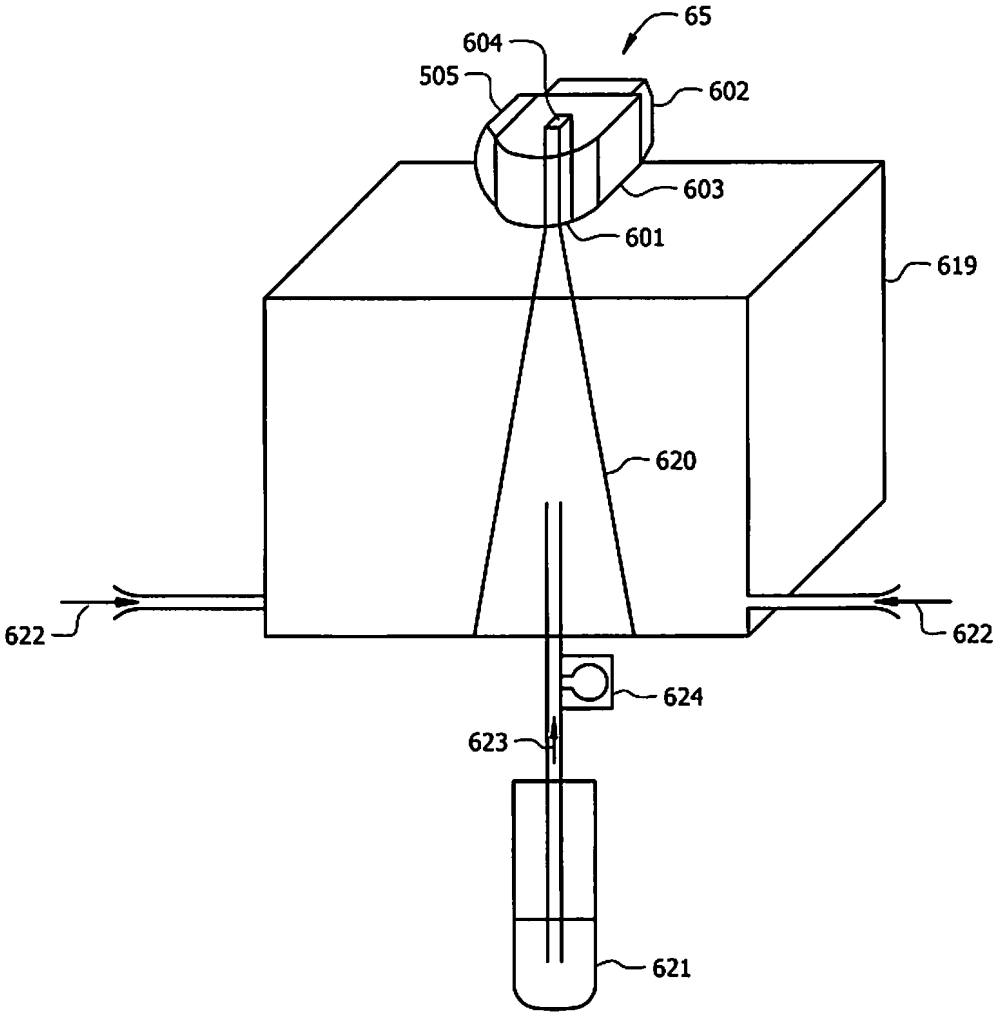


FIG. 8B

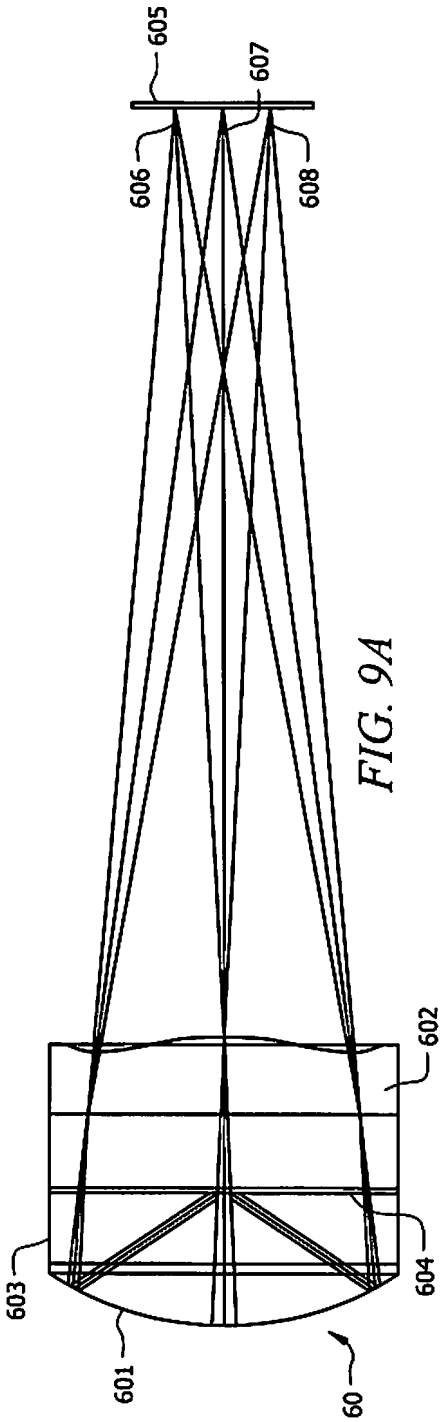


FIG. 9A

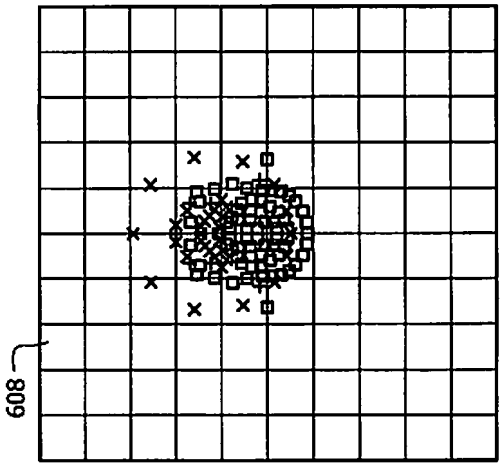


FIG. 9B3

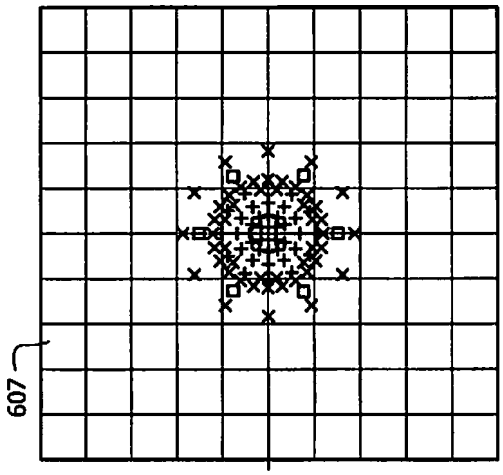


FIG. 9B2

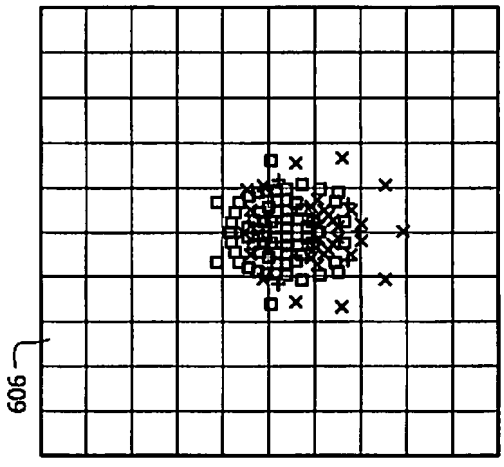


FIG. 9B1

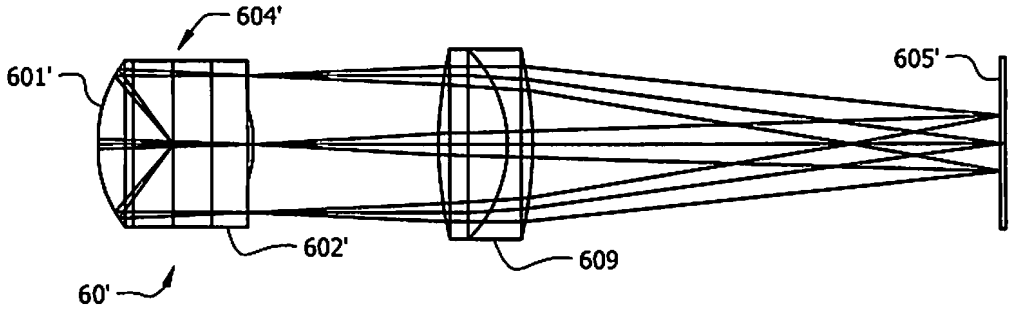


FIG. 10

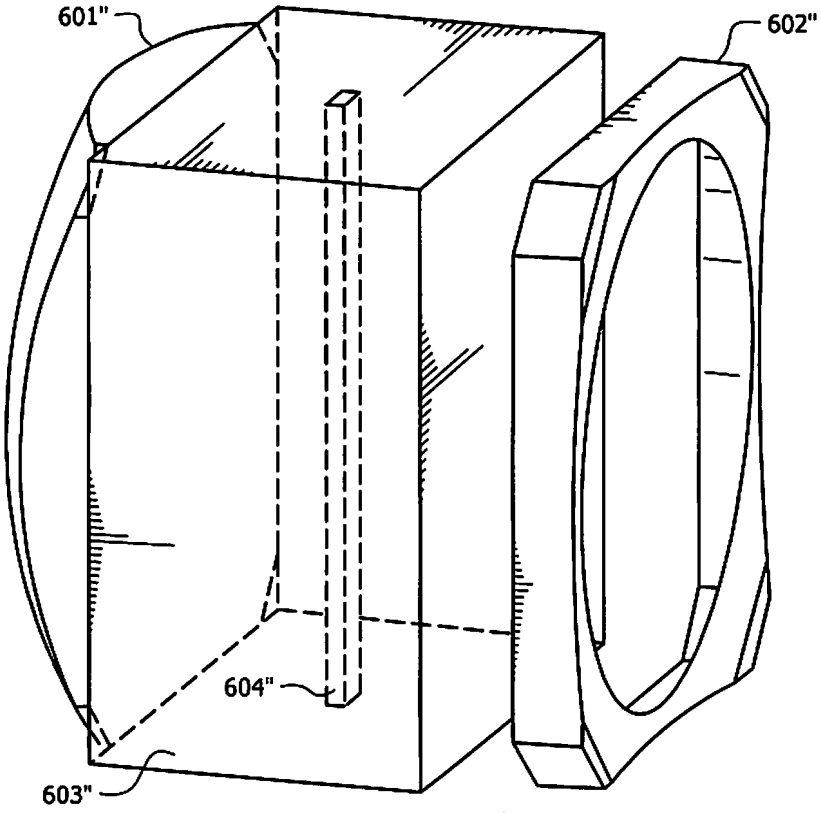


FIG. 11

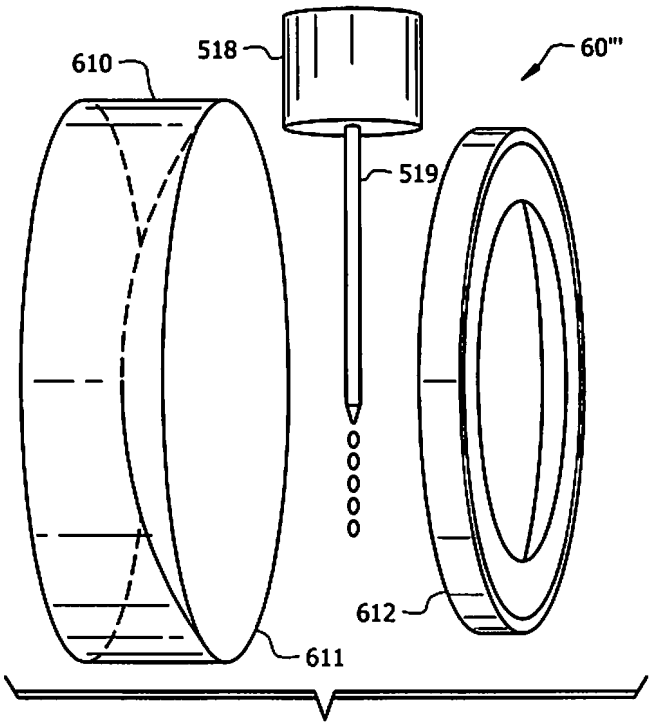


FIG. 12

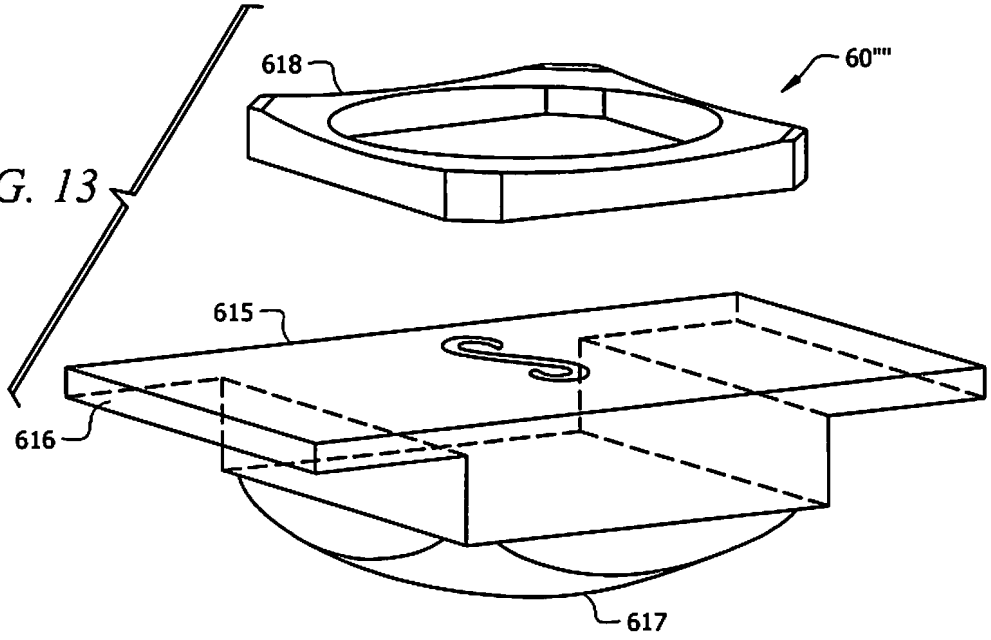


FIG. 13

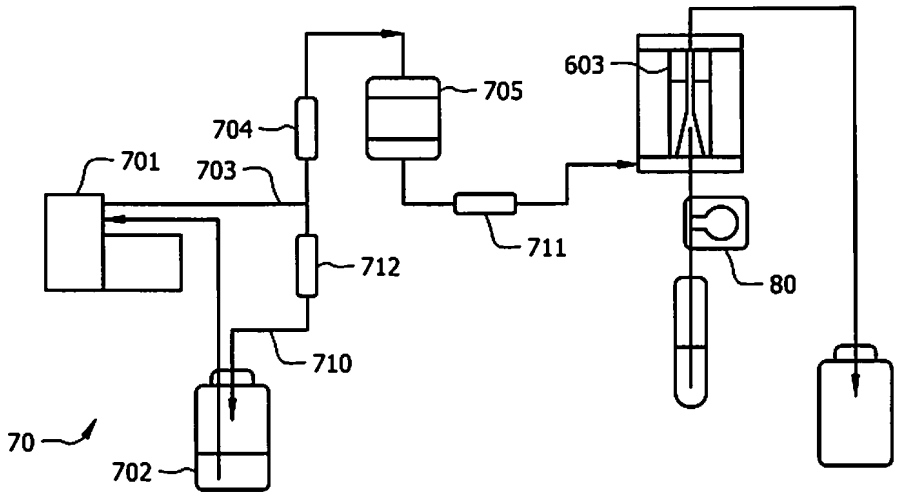

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FIG. 14

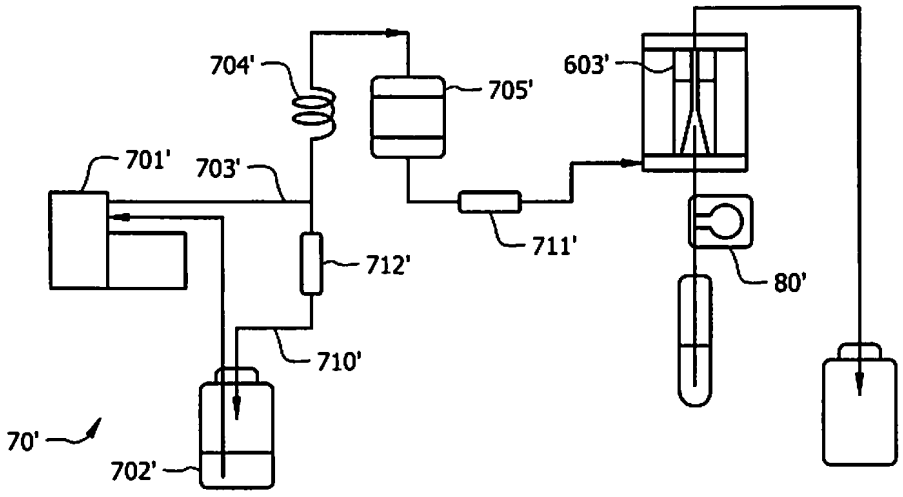

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FIG. 15

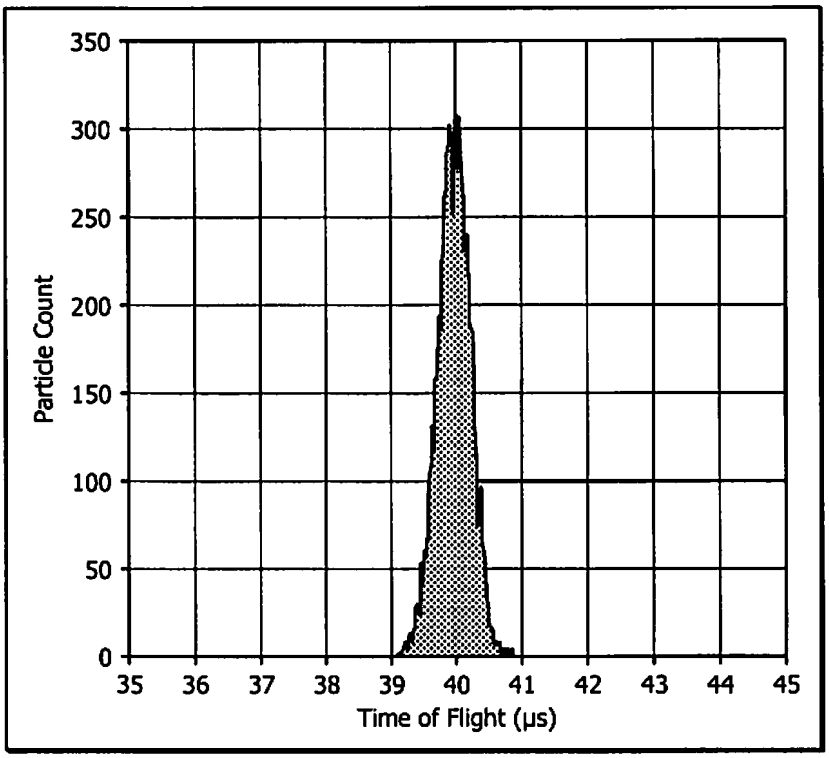


FIG. 16A

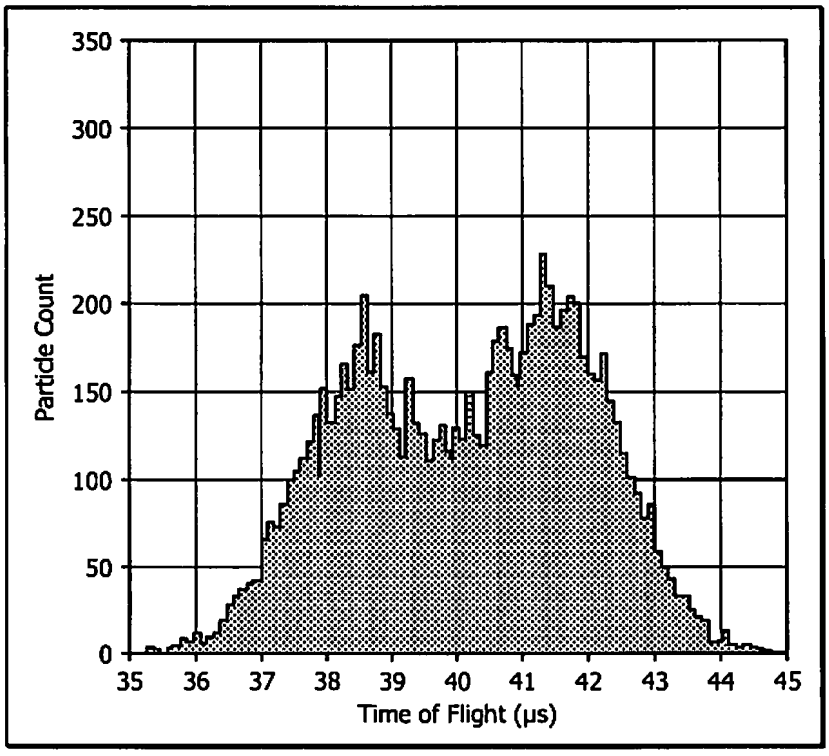


FIG. 16B

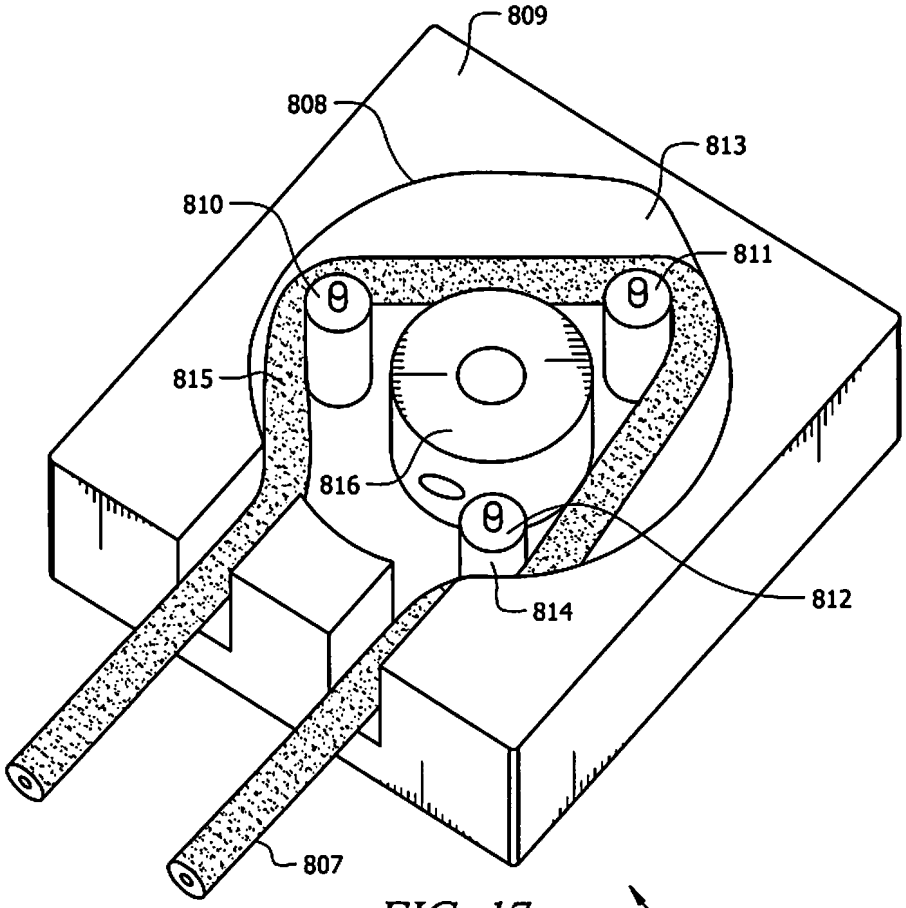


FIG. 17

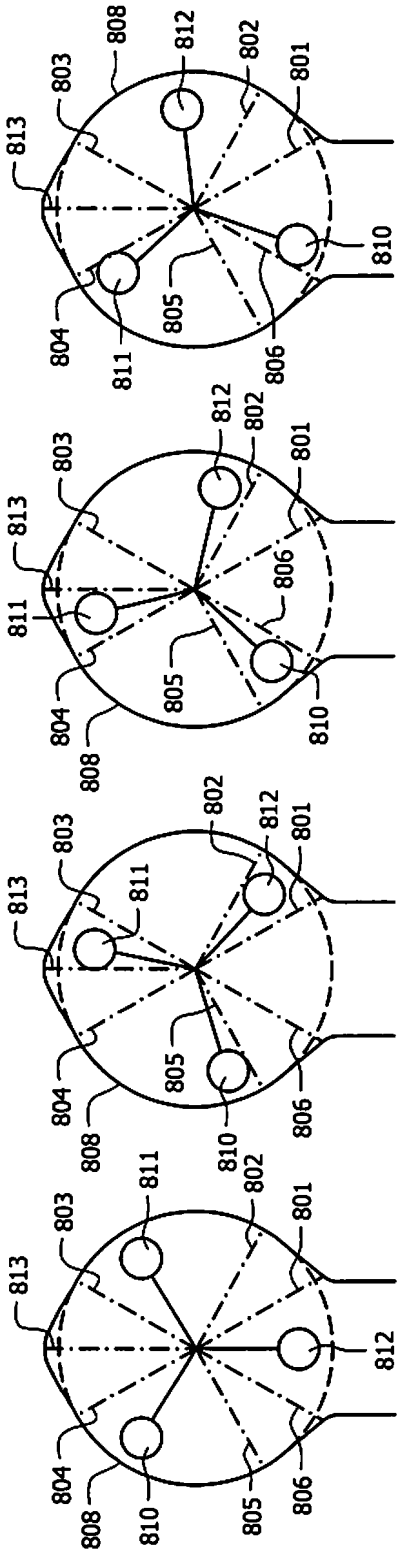


FIG. 18D

FIG. 18C

FIG. 18B

FIG. 18A

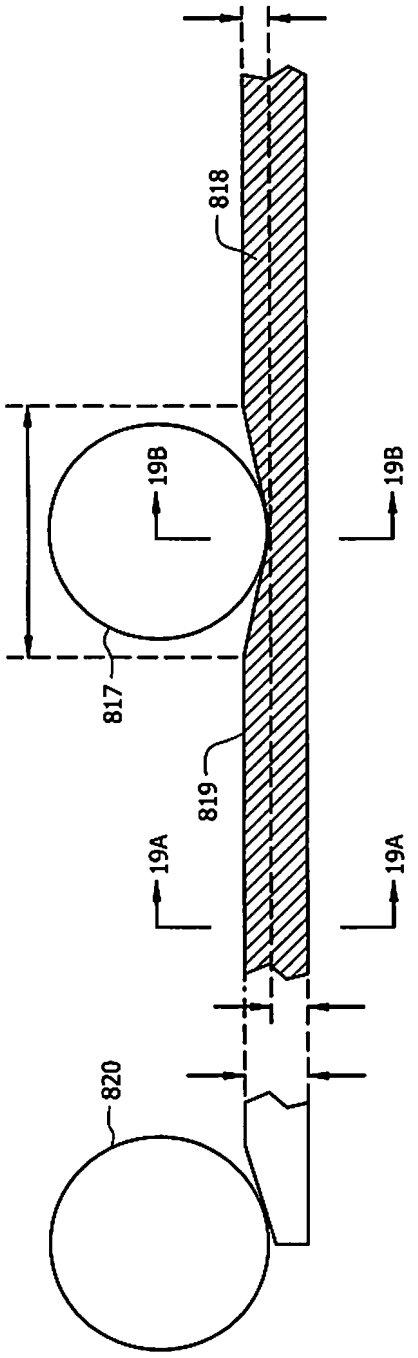


FIG. 19

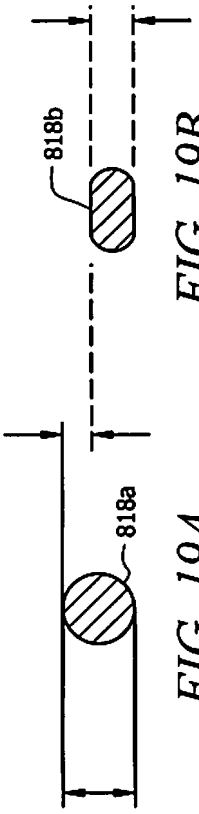


FIG. 19B

FIG. 19A

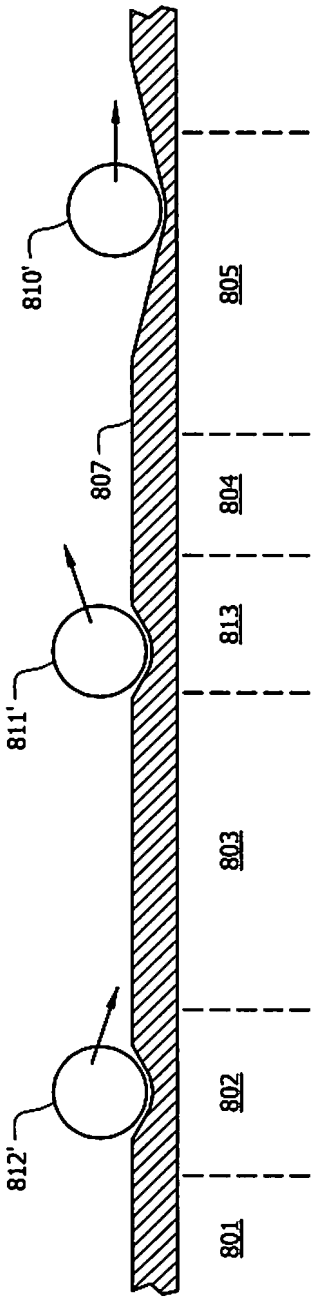


FIG. 20A

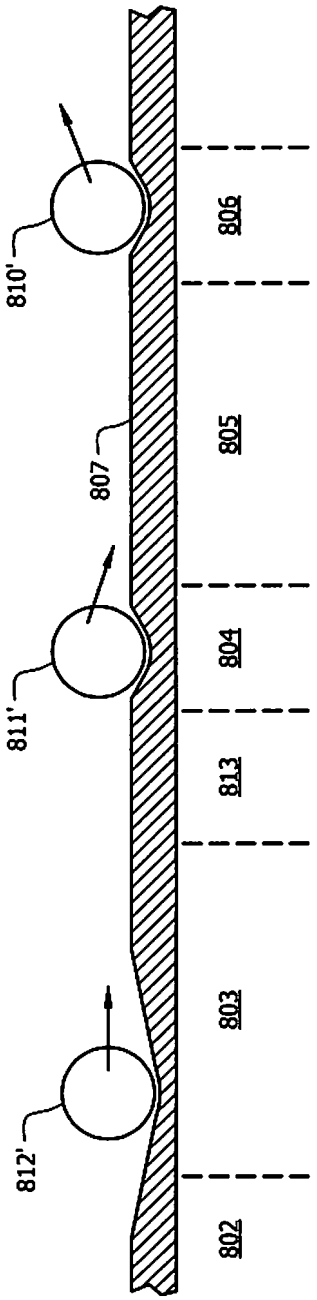


FIG. 20B

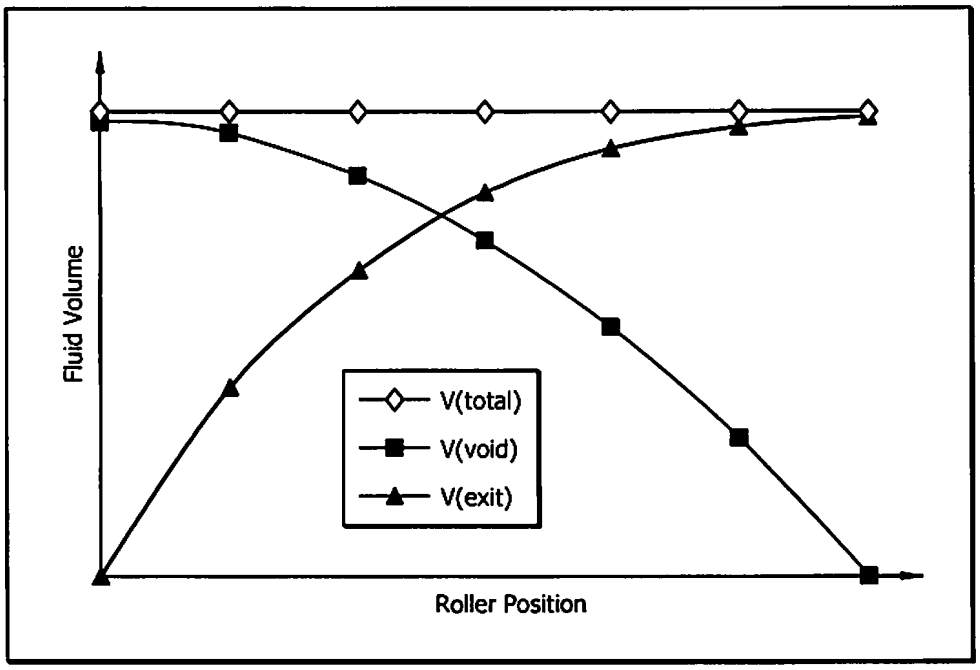


FIG. 21

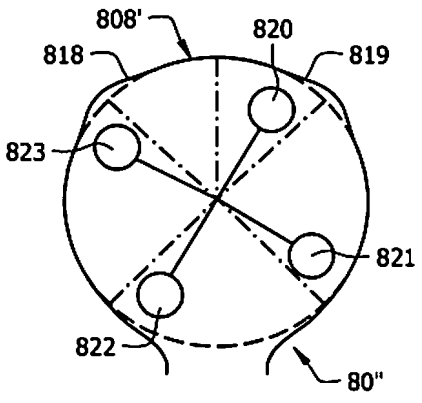


FIG. 22

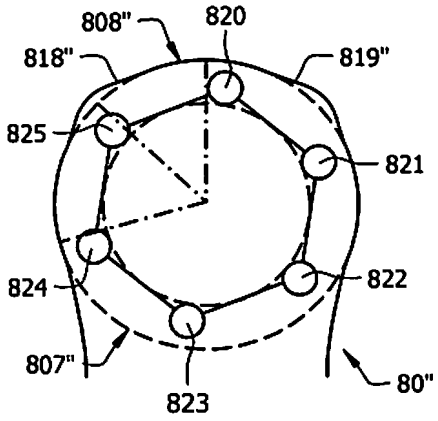


FIG. 23

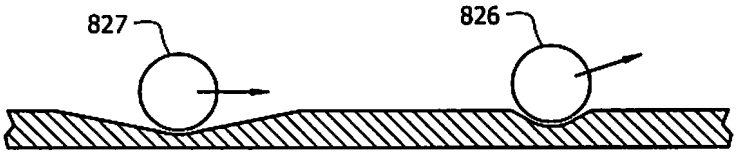


FIG. 24A

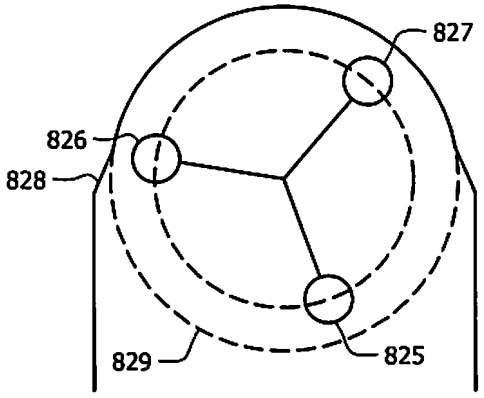


FIG. 24B

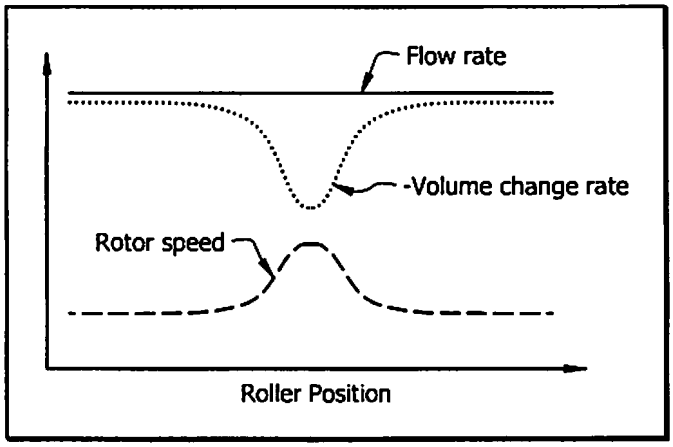


FIG. 24C

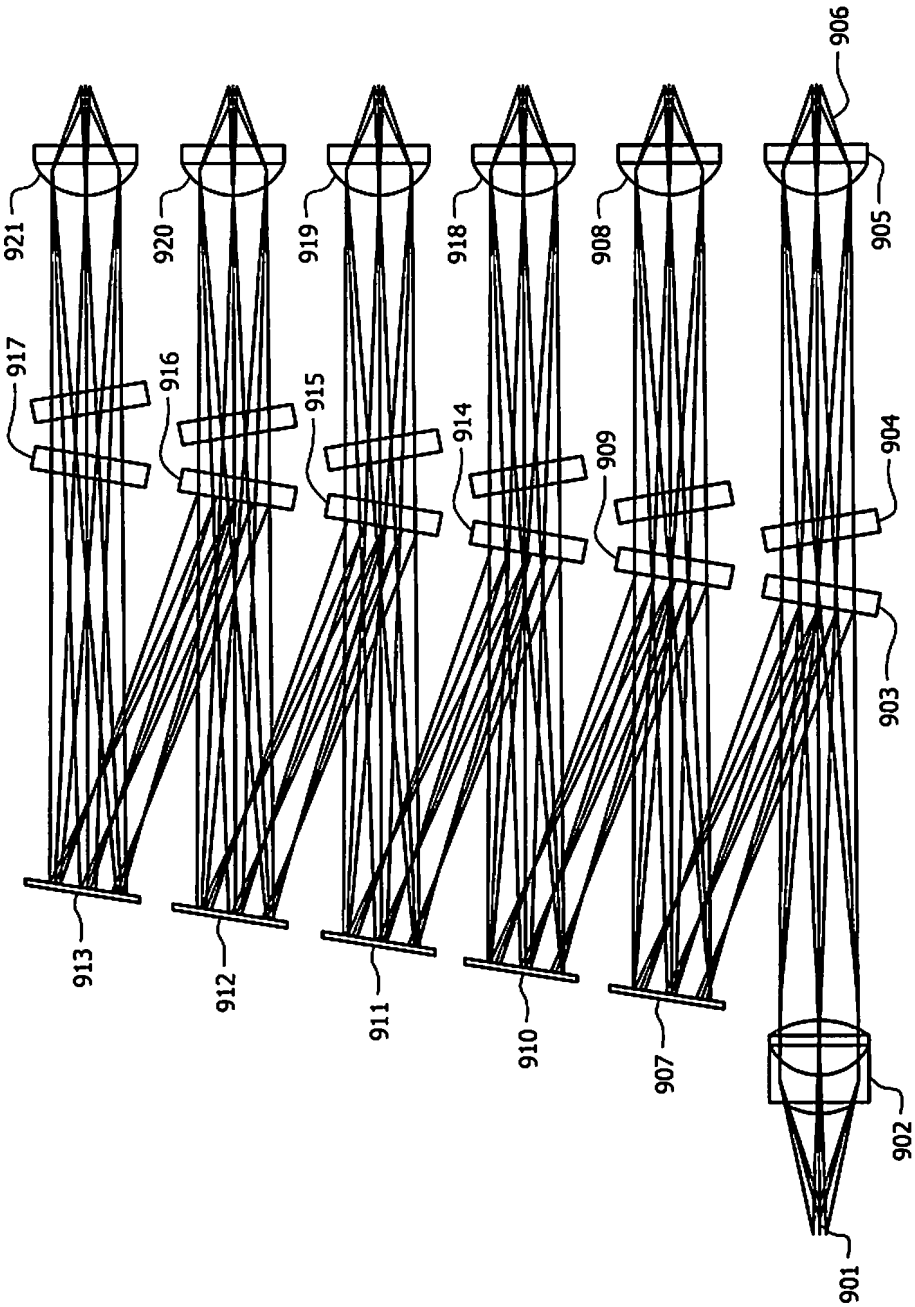

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FIG. 25

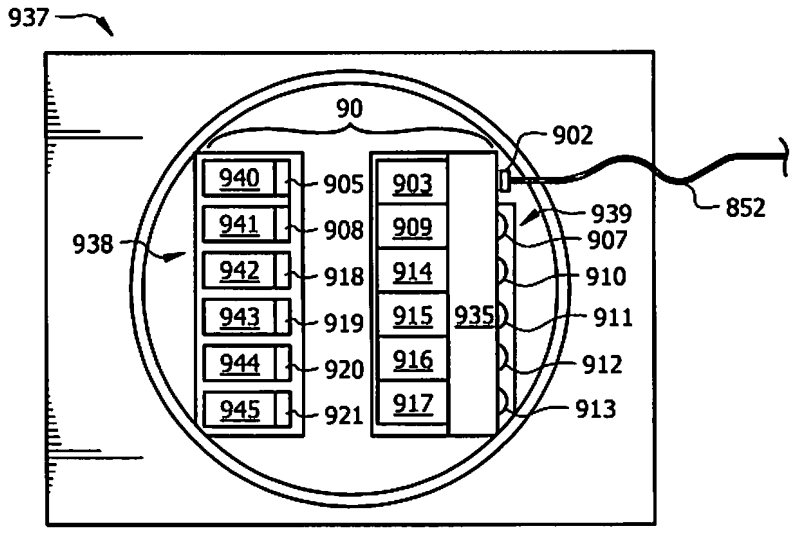


FIG. 25A

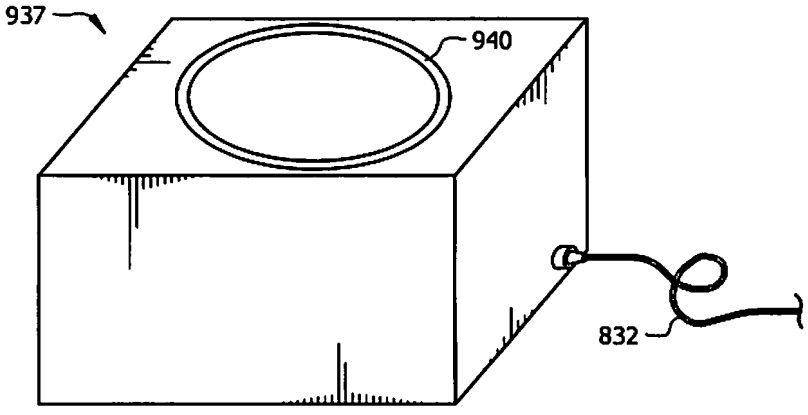


FIG. 25B

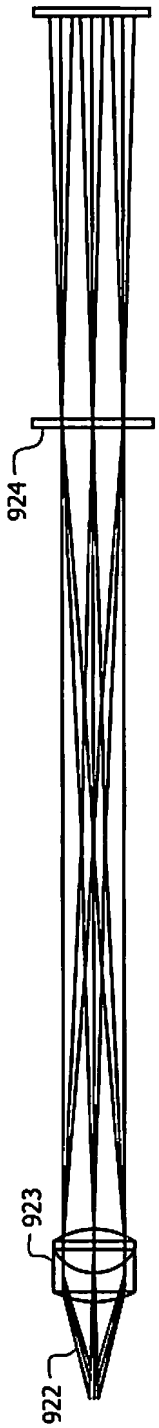


FIG. 26

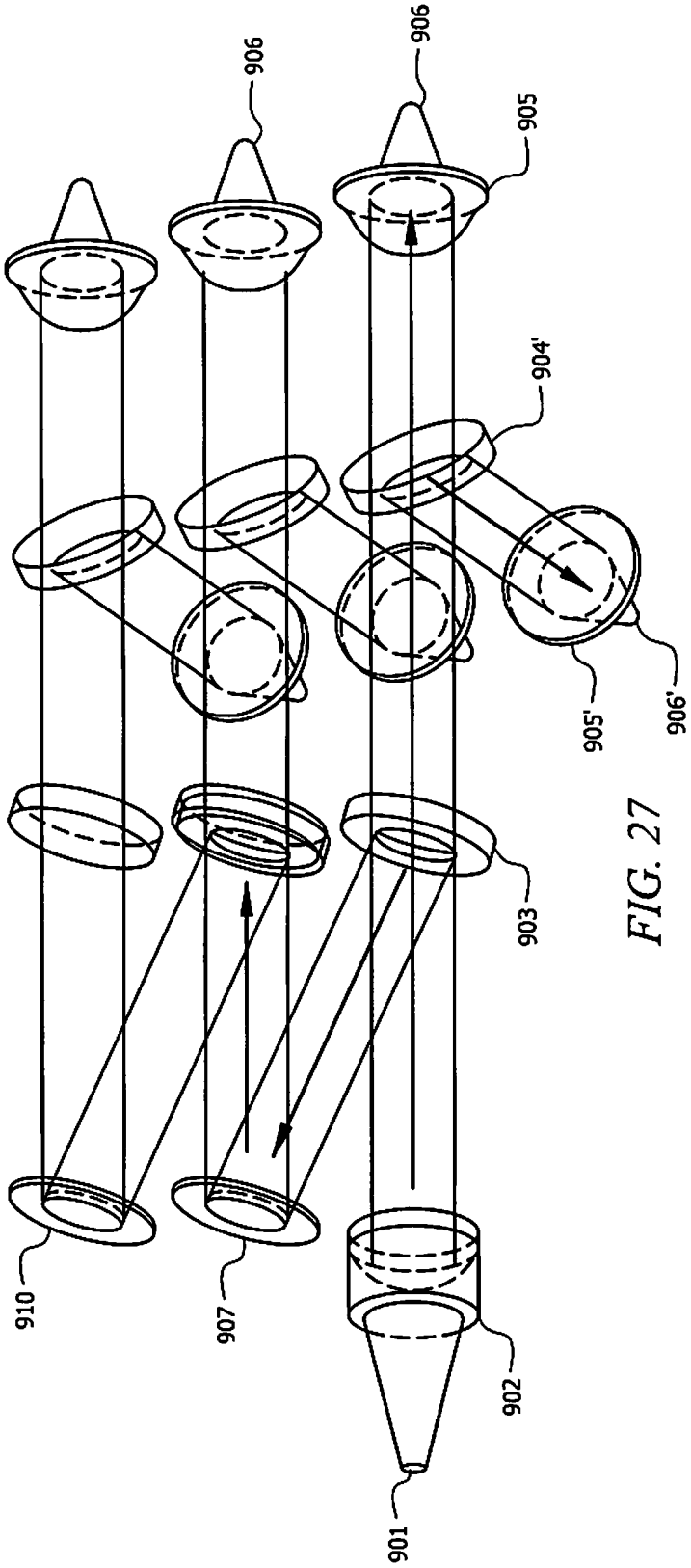


FIG. 27

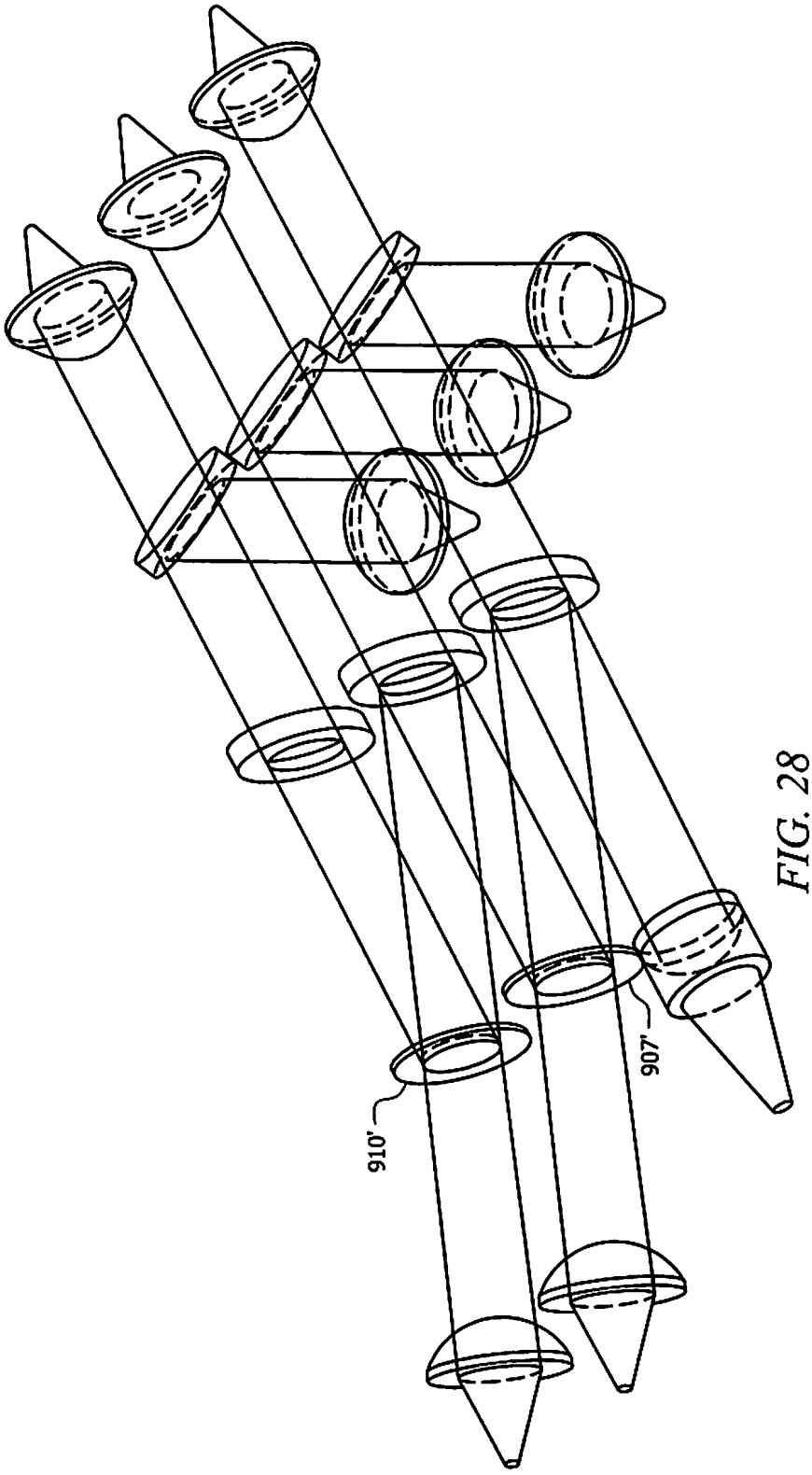


FIG. 28

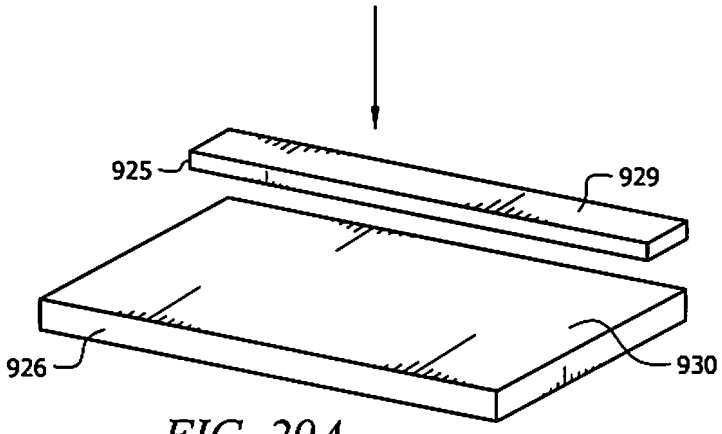


FIG. 29A

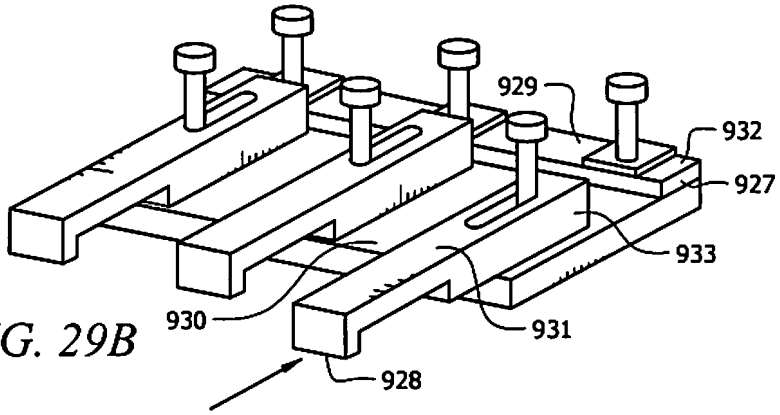


FIG. 29B

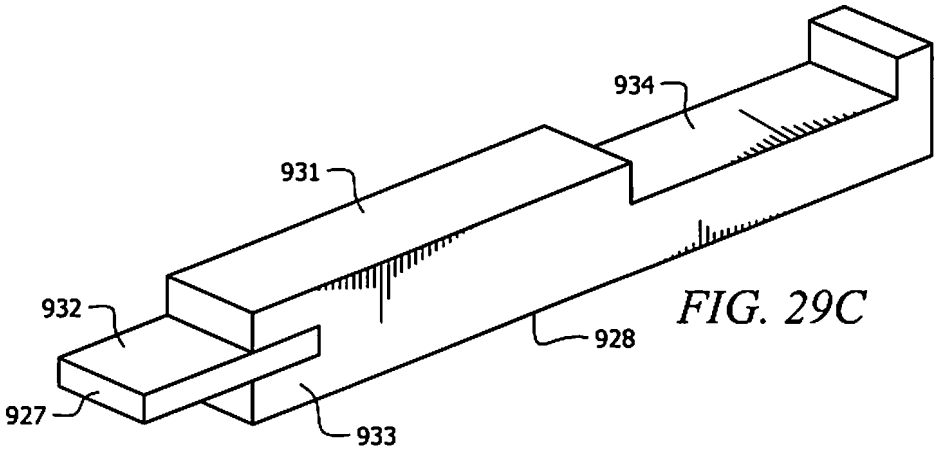


FIG. 29C

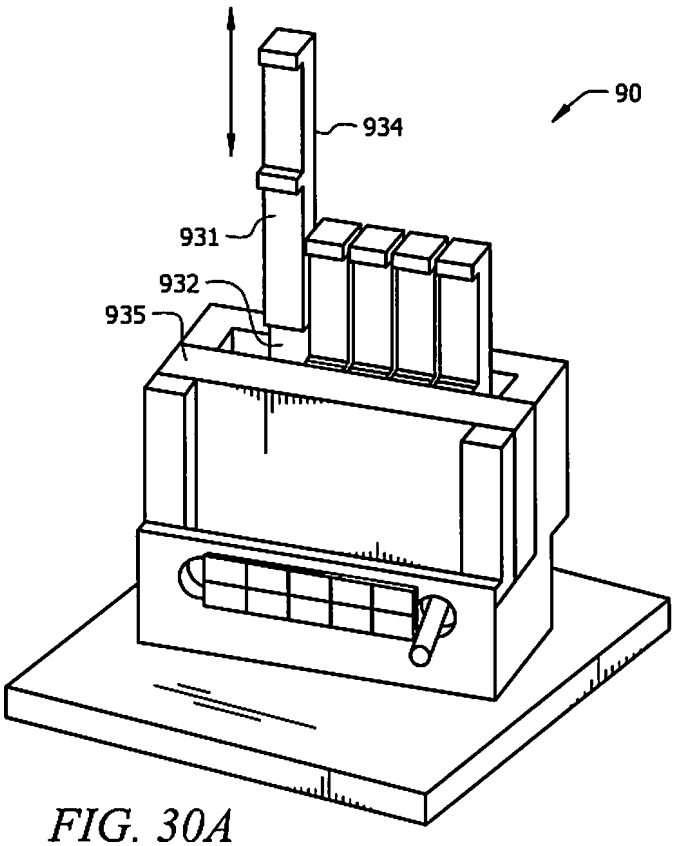


FIG. 30A

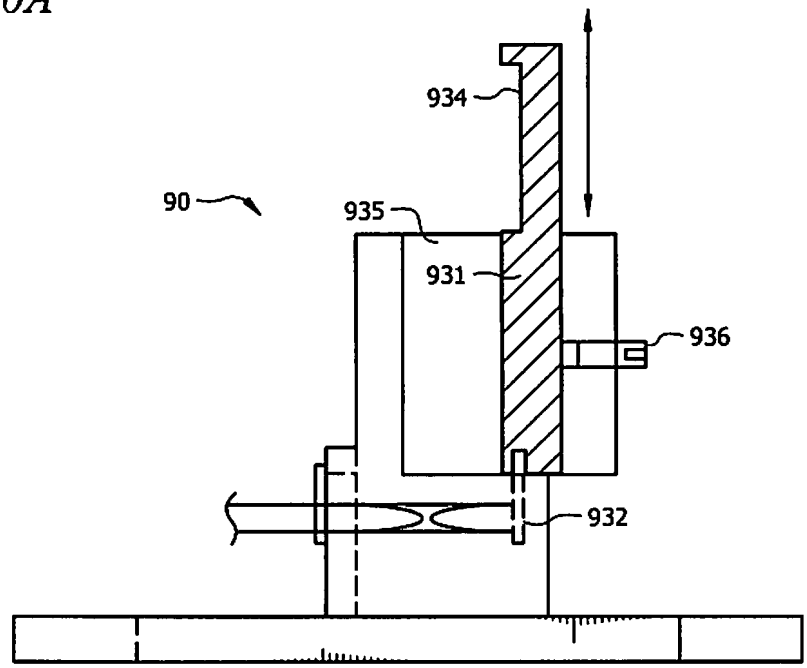


FIG. 30B

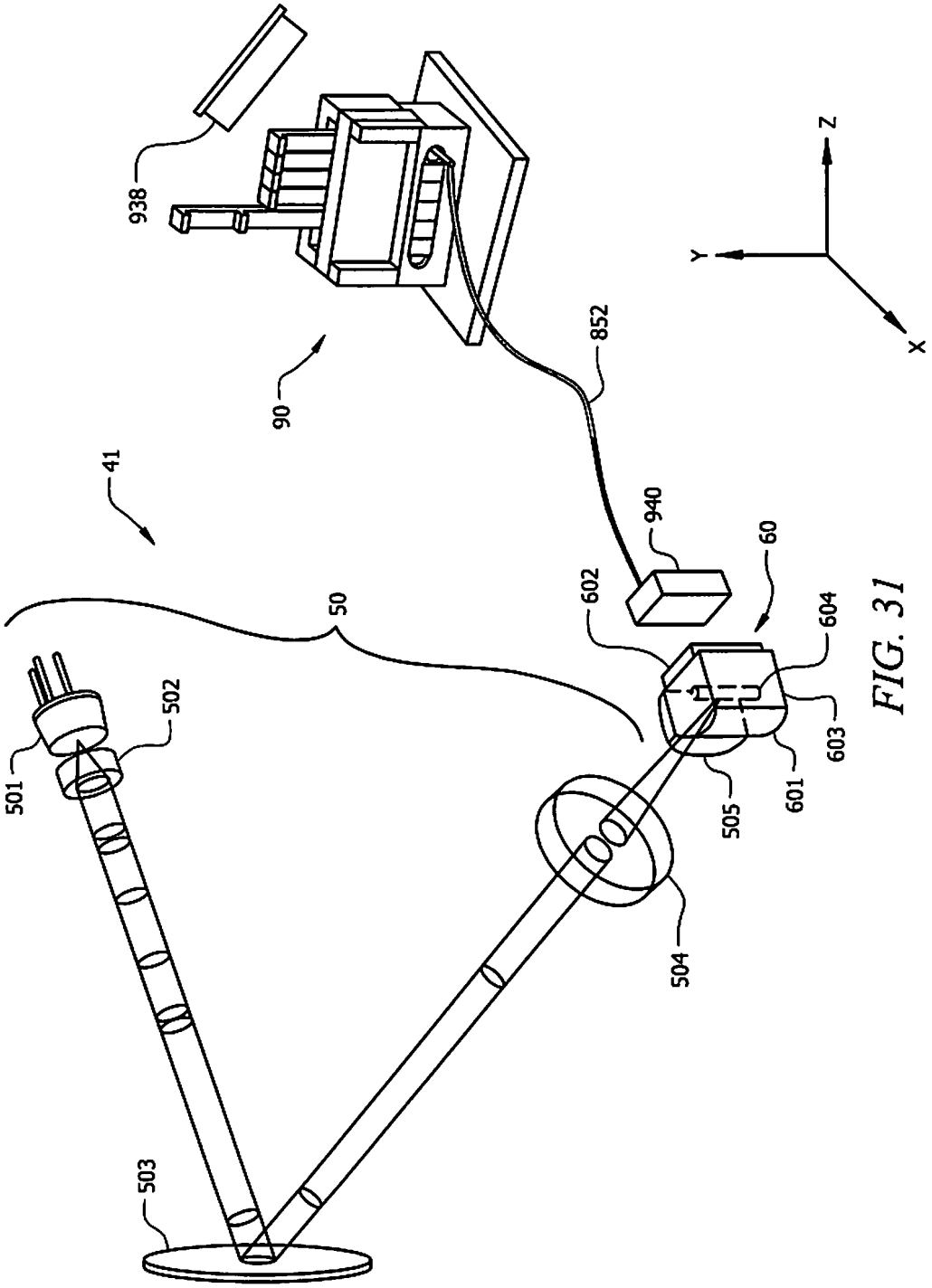


FIG. 31

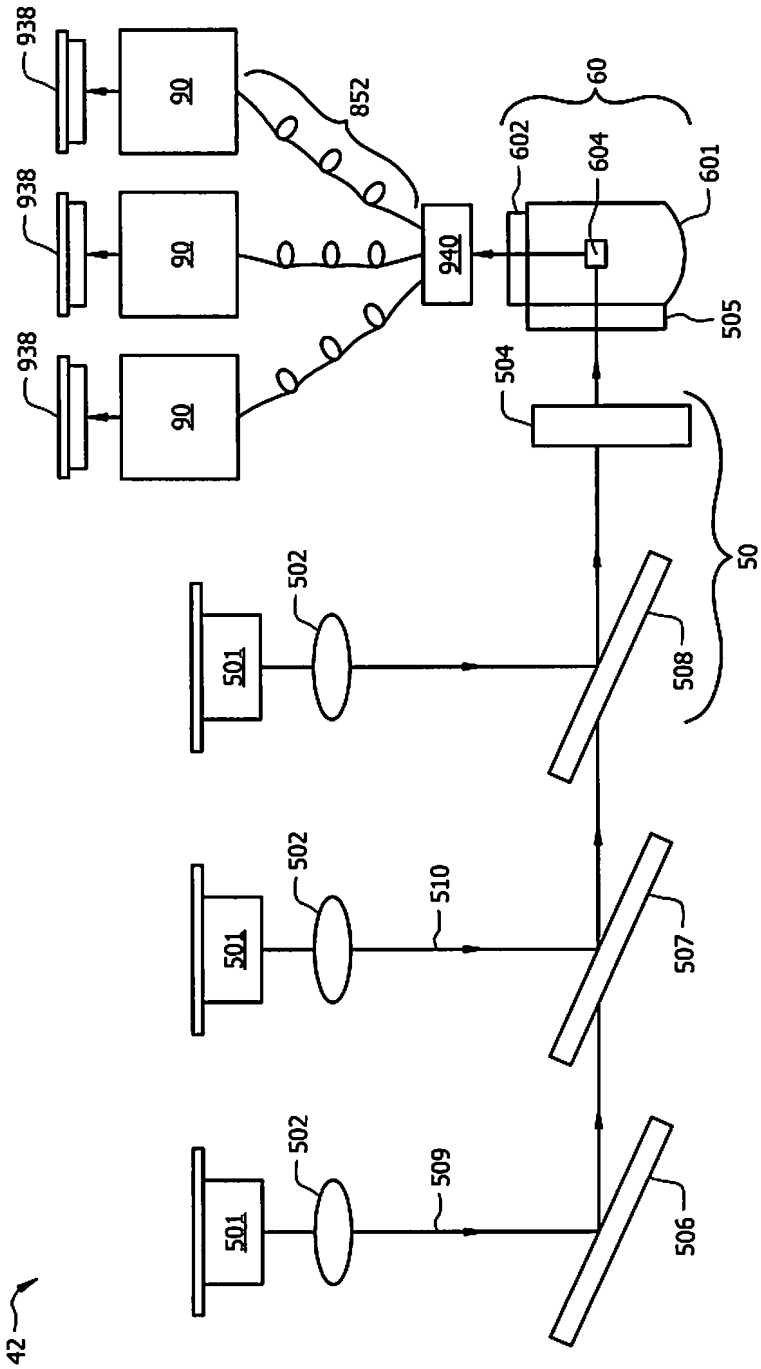

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FIG. 32

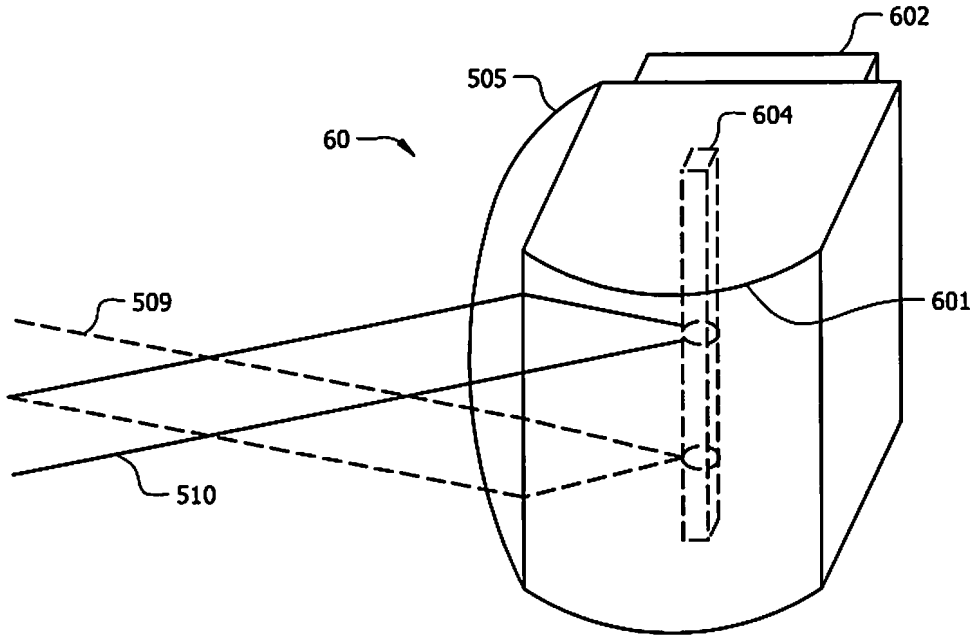


FIG. 33

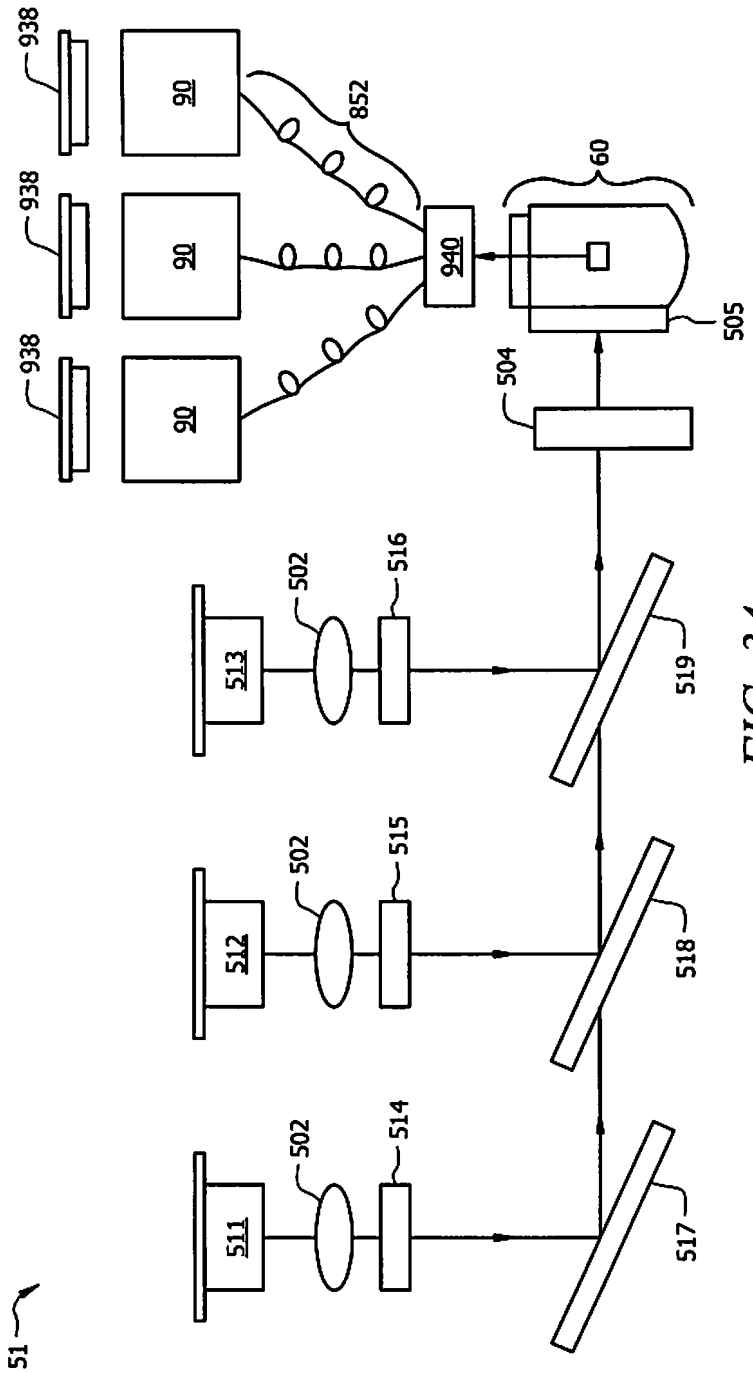

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FIG. 34

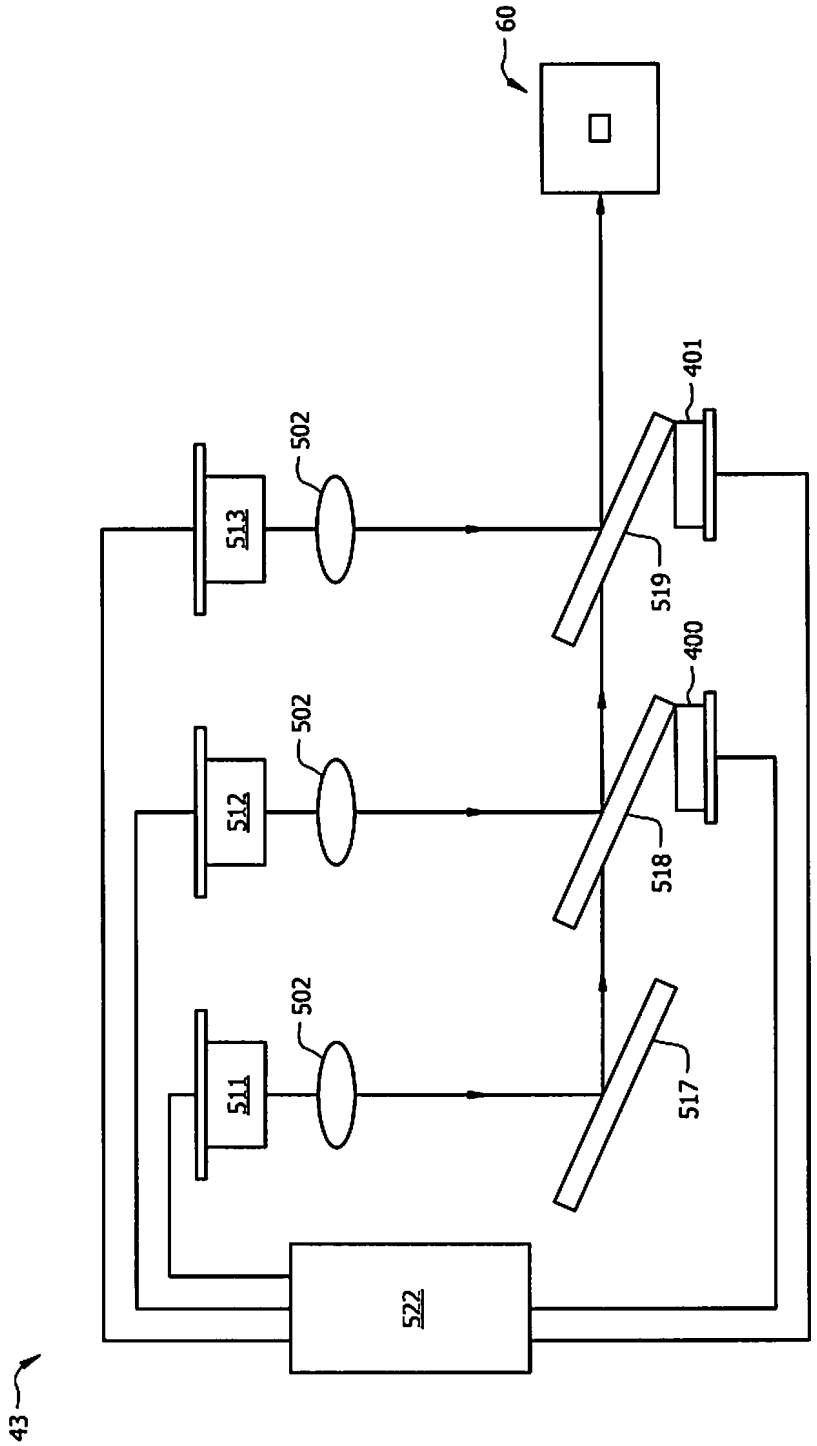


FIG. 35

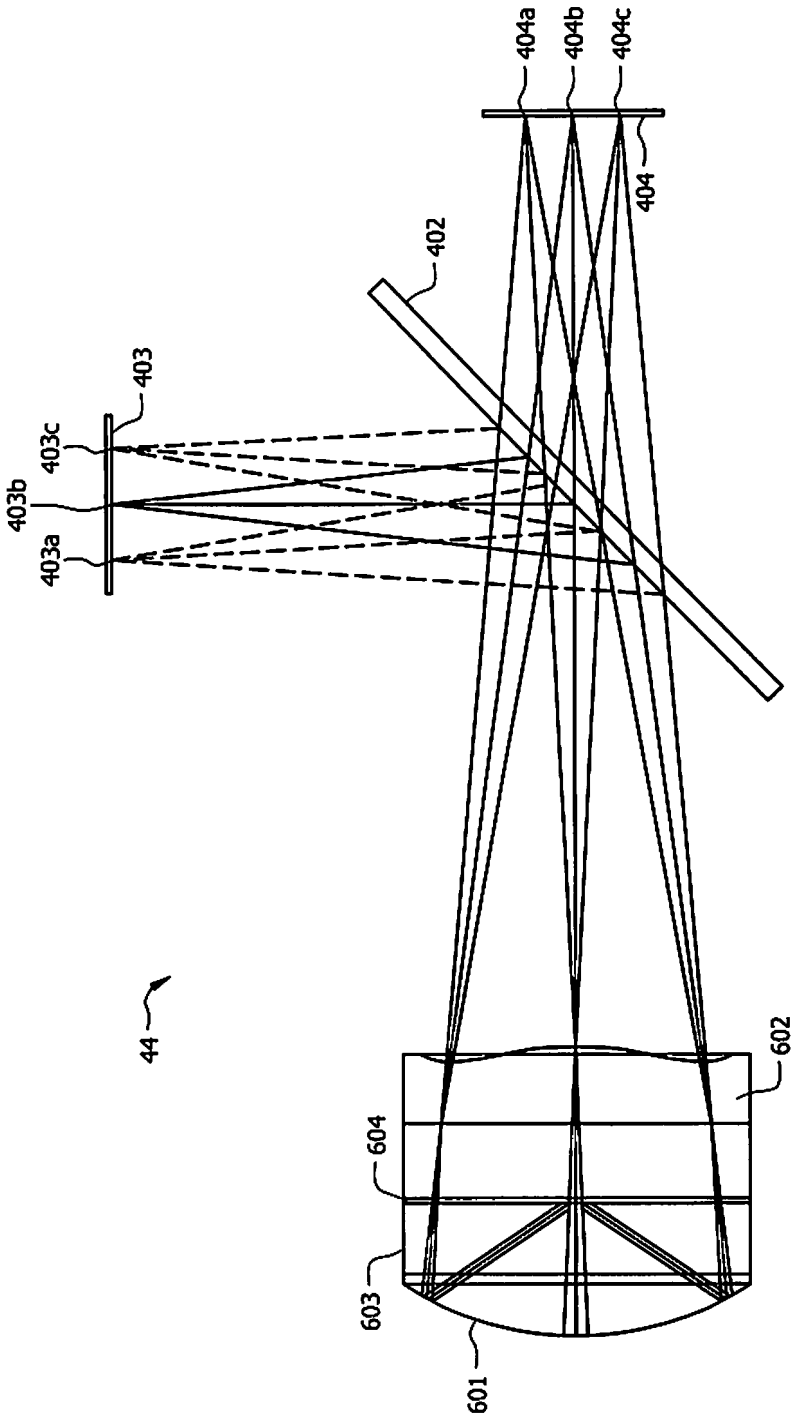


FIG. 36

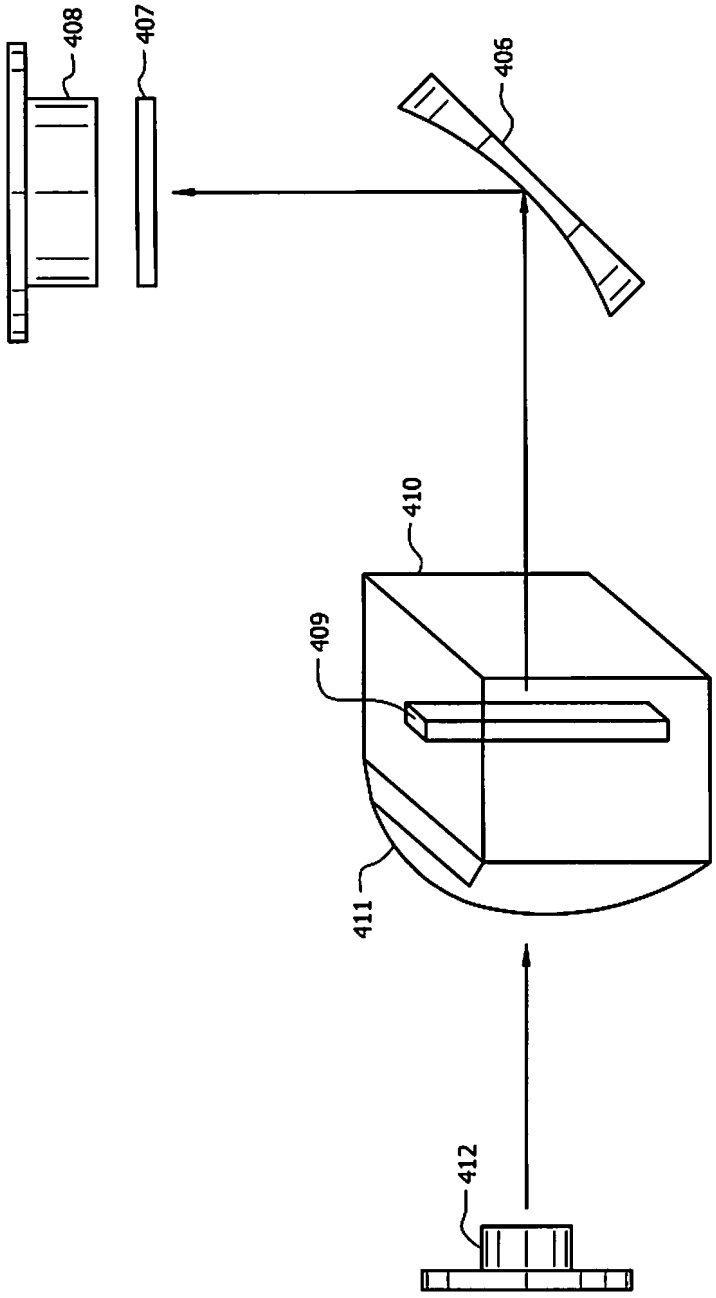


FIG. 37

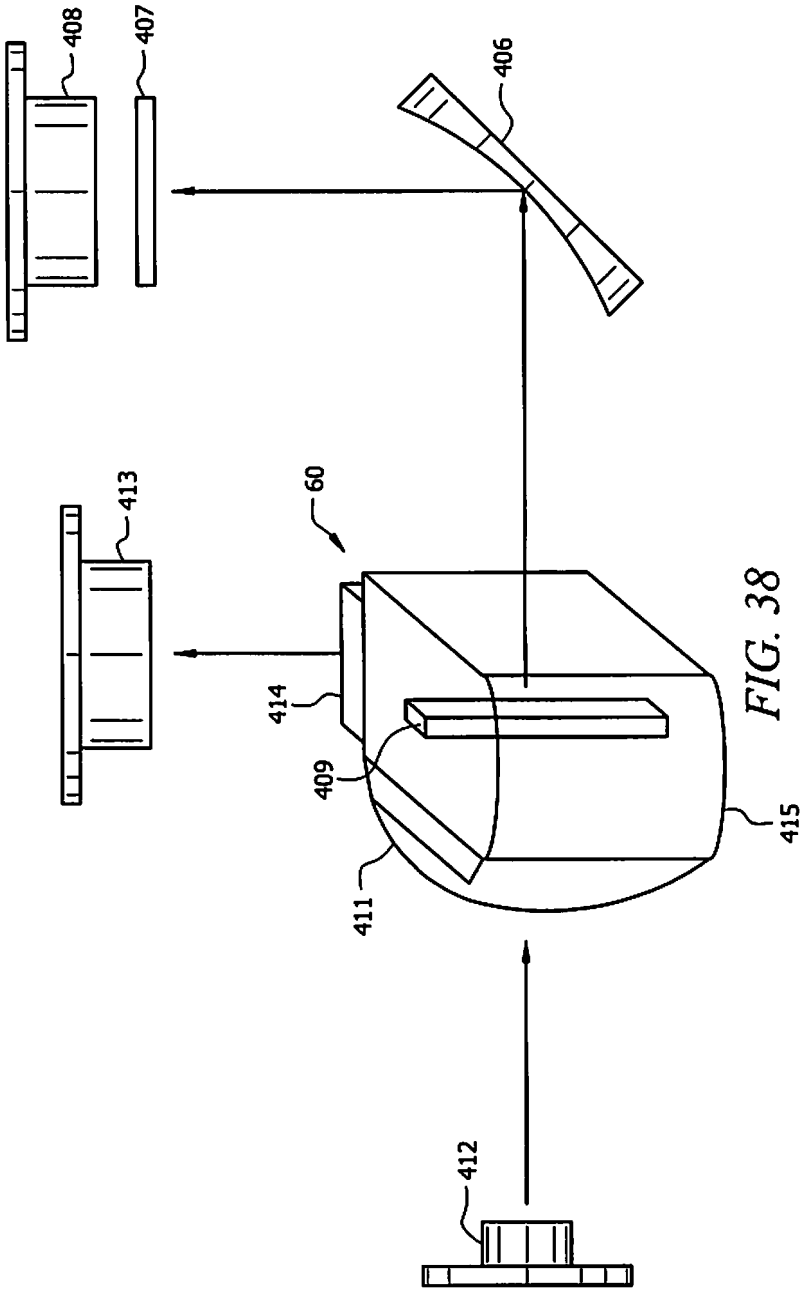


FIG. 38

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 14/555,102

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	23 minus 20 =	* 3			OR	x 80 =	240
INDEPENDENT CLAIMS (37 CFR 1.16(h))	5 minus 3 =	* 2				x 420 =	840
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						400
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	3080

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US

CONFIRMATION NO. 4589

32425
NORTON ROSE FULBRIGHT US LLP
98 SAN JACINTO BOULEVARD
SUITE 1100
AUSTIN, TX 78701-4255

NOTICE

OC000000072981295

Date Mailed: 01/23/2015

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Yong Qin Chen

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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/555,102	11/26/2014	2877	3220	BECC.P0002US	23	5

CONFIRMATION NO. 4589

32425
NORTON ROSE FULBRIGHT US LLP
98 SAN JACINTO BOULEVARD
SUITE 1100
AUSTIN, TX 78701-4255

FILING RECEIPT



Date Mailed: 01/23/2015

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Yong Qin Chen, San Jose, CA;

Applicant(s)

IRIS INTERNATIONAL, INC., Chatsworth, CA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/911,859 12/04/2013
and is a CIP of PCT/US2013/043453 05/30/2013
which claims benefit of 61/653,245 05/30/2012
and claims benefit of 61/653,328 05/30/2012
and claims benefit of 61/715,819 10/18/2012
and claims benefit of 61/715,836 10/19/2012
and claims benefit of 61/816,819 04/29/2013

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 01/22/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/555,102**

Projected Publication Date: 04/30/2015

Non-Publication Request: No

Early Publication Request: No
Title

FLOW CYTOMETER

Preliminary Class

356

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
14/555,102	November 26, 2014

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 129381

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

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OR

☐ The address associated with Customer Number: 129381

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

IRIS INTERNATIONAL, INC.

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature		Date (Optional)	
Name	Daniel B. Kim		
Title	Assistant Secretary, Iris International, Inc.		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Electronic Acknowledgement Receipt

EFS ID:	21802303
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	32425
Filer:	Gina N. Shishima/Ben Horton
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US
Receipt Date:	18-MAR-2015
Filing Date:	26-NOV-2014
Time Stamp:	10:29:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Oath or Declaration filed	Declaration.PDF	120952 7a9a78a188b28db62430ed7704d4cd8b35afeaab	no	1

Warnings:	
Information:	

2	Power of Attorney	POA-1.pdf	2911420 eb2a9388165ec23c84d5ed8710e63d22a872cd49	no	1

Warnings:	
Information:	
Total Files Size (in bytes):	3032372

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	FLOW CYTOMETER

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/555,102

filed on November 26, 2014.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

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LEGAL NAME OF INVENTOR

Inventor: Yong Qin Chen Date (Optional): 3/9/2015

Signature: 

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US

CONFIRMATION NO. 4589

129381
NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

POA ACCEPTANCE LETTER

OC000000074192728

Date Mailed: 03/25/2015

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 03/18/2015.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/zmoguss/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040

CONFIRMATION NO. 4589

129381
NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

PUBLICATION NOTICE



Title:FLOW CYTOMETER

Publication No.US-2015-0115174-A1
Publication Date:04/30/2015

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

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Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP	Serial No.: 14/555,102
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	A1	20020141902	10/3/02	Ozasa et al.	422	82.09	3/27/02
	A2	20030142720	7/31/03	Bradburn et al.	372	108	5/24/02
	A3	20040218184	11/4/04	Joregenson et al.	356	419	2/27/04
	A4	20050084402	4/21/05	Vanek	417	477.3	10/27/04
	A5	20060221325	10/5/06	Wells	356	73	3/30/05
	A6	20060245964	11/2/06	Koslov	417	477.1	6/29/06
	A7	20060256335	11/16/06	Chen	356	369	5/3/06
	A8	20060292021	12/28/06	Tu	417	477.1	11/7/02
	A9	20070124947	6/7/07	Munroe et al.	33	286	10/25/06
	A10	20080024758	1/31/08	Tabata	356	39	7/26/07
	A11	20080241911	10/2/08	Ueno et al.	435	287.1	3/28/08
	A12	20090091746	4/9/09	Fukuda et al.	356	73	10/1/08
	A13	20090190128	7/30/09	Cerni et al.	356	336	11/24/08
	A14	20110014075	1/20/11	Reif et al.	417	477.12	7/1/10
	A15	20110032522	2/10/11	Graves et al.	356	338	10/12/10
	A16	20120156074	6/21/12	Kusch et al.	417	477.3	2/4/10
	A17	20120274925	11/1/12	Chen et al.	356	73	11/1/12
	A18	20120281204	11/8/12	Hoshishima et al.	356	72	1/13/11
	A19	20120282126	11/8/12	Brandt et al.	417	477.9	11/4/10
	A20	20120287419	11/15/12	Sharpe et al.	356	51	5/11/12
	A21	20130010181	1/10/13	Baba	348	360	3/14/11
	A22	20130020498	1/24/13	Ebi et al.	250	435	7/18/12
	A23	20130050782	2/28/13	Heng et al.	358	494	2/17/12

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	A24	20130070243	3/21/13	Goehde	356	338	11/9/12
	A25	20130094102	4/18/13	Baba	359	784	7/13/10
	A26	20130204538	8/8/13	Rich	702	21	10/25/11
	A27	2385495	9/25/45	Brian	250	487.1	8/21/43
	A28	2669709	2/16/54	Glancy et al.	348	784	10/19/60
	A29	2764147	9/25/56	Brunner	126	247	2/23/51
	A30	315667	4/14/85	Serdinko	417	475	10/20/84
	A31	3411293	11/19/68	Akins	60	454	10/20/66
	A32	3542491	11/24/70	Newman	417	295	5/27/69
	A33	3661460	5/9/72	Elking et al.	356	36	8/28/70
	A34	3726613	4/10/73	von Casimir	417	477.1	10/12/70
	A35	3826593	7/30/74	von Casimir	417	53	5/11/73
	A36	3873204	3/25/75	Friedman et al.	356	39	11/29/72
	A37	3946239	3/23/76	Salzman et al.	250	461.2	1/24/75
	A38	3953727	4/27/76	d'Auria et al.	398	88	1/17/75
	A39	3989381	11/2/76	Fulwyler	356	39	5/5/75
	A40	4073574	2/14/78	Clarke et al.	359	728	10/1/76
	A41	4188543	2/12/80	Brunsting et al.	250	458.1	4/24/78
	A42	4189236	2/19/80	Hogg et al.	356	317	3/20/78
	A43	4244045	1/6/81	Nosu et al.	398	86	1/31/79
	A44	4482994	11/13/84	Ishikawa	398	86	6/7/82
	A45	4515274	5/7/85	Hollinger et al.	209	3.1	12/2/81
	A46	4564342	1/14/86	Weber et al.	417	477.6	7/24/84

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	A47	4576556	3/18/86	Thompson	417	477.12	4/2/80
	A48	4623225	11/18/86	Forkner	359	669	6/29/84
	A49	4727020	2/23/88	Recktenwald	435	6.11	2/25/85
	A50	4745285	5/17/88	Recktenwald et al.	250	458.1	8/21/86
	A51	4778253	10/18/88	Siga et al.	359	819	3/26/86
	A52	4834630	5/30/89	Godwin	417	475	2/16/88
	A53	4871249	10/3/89	Watson	356	73	7/1/88
	A54	4920275	4/24/90	Itoh	250	574	12/30/88
	A55	4950136	8/21/90	Haas et al.	417	477.7	8/14/89
	A56	4976590	12/11/90	Baldwin	417	53	6/8/88
	A57	4997275	3/5/91	Gaucher et al.	356	72	9/23/88
	A58	5050963	9/24/91	Murakami	359	808	10/9/90
	A59	5142462	8/25/92	Kashima	362	268	4/27/90
	A60	5177641	1/5/93	Kobayashi et al.	359	820	10/30/90
	A61	5230614	7/27/93	Zanger et al.	417	477.9	6/3/92
	A62	5245318	9/14/93	Tohge et al.	340	611	12/15/92
	A63	5251060	10/5/93	Uenishi et al.	359	328	9/30/92
	A64	5299066	3/29/94	Rombult	359	819	8/12/92
	A65	5317162	5/31/94	Pinsky et al.	250	461.2	9/9/92
	A66	5369476	11/29/94	Bowers et al.	399	49	1/28/92
	A67	5373395	12/13/94	Adachi	359	652	5/10/93
	A68	5396487	3/7/95	Abe et al.	359	819	10/31/91
	A69	5467225	11/14/95	Manabe	359	661	11/1/94

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	A70	5470211	11/28/95	Knott et al.	417	477.9	8/11/94
	A71	5475210	12/12/95	Taguchi et al.	250	205	6/8/93
	A72	5548395	8/20/96	Kosaka	356	73	8/31/92
	A73	5583683	12/10/96	Scobey	398	79	6/15/95
	A74	5615200	3/25/97	Hoshino et al.	369	112.04	4/14/95
	A75	5746585	5/5/98	McDunn et al.	417	477.11	12/31/96
	A76	5748372	5/5/98	Kitagawa	359	565	4/17/96
	A77	5777674	7/7/98	Ohmuro	348	338	4/10/95
	A78	5781351	7/14/98	Murakami et al.	359	808	5/31/96
	A79	5786915	7/28/98	Scobey	398	82	10/22/96
	A80	5788927	8/4/98	Farrell et al.	422	63	6/30/96
	A81	5805363	9/8/98	Okuda et al.	359	819	7/17/97
	A82	5850292	12/15/98	Braun	356	419	11/13/97
	A83	5915925	6/29/99	North Jr.	417	36	1/7/97
	A84	5971713	10/26/99	North Jr.	417	36	12/29/98
	A85	6008920	12/28/99	Hendrix	398	79	3/11/98
	A86	6017194	1/25/00	North Jr.	417	36	12/31/98
	A87	6102678	8/15/00	Peclat	417	477.7	4/3/98
	A88	6135734	10/24/00	Isozumi et al.	417	542	9/23/98
	A89	6200101	3/13/01	North Jr.	417	36	7/6/99
	A90	6252719	6/26/01	Eichenbaum	359	634	3/19/99
	A91	6315952	11/13/01	Sklar et al.	422	63	6/10/99
	A92	6510007	1/21/03	Blasenheim	359	659	8/21/01

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	A93	6542306	4/1/03	Goodman	359	634	3/16/01
	A94	6572255	6/3/03	Husher	366	132	4/24/01
	A95	6608682	8/19/03	Ortyn et al.	356	419	10/12/01
	A96	6618143	9/9/03	Roche et al.	356	339	10/2/01
	A97	6638481	10/28/03	Sklar et al.	422	63	9/19/01
	A98	6647175	11/11/03	LoRegio et al.	385	24	10/8/01
	A99	6683314	1/27/04	Oostman Jr. et al.	250	461.2	8/28/01
	A100	6713019	3/30/04	Ozasa et al.	422	82.09	3/27/02
	A101	6748133	6/8/04	Liu et al.	385	24	11/26/01
	A102	6767188	7/27/04	Vrane et al.	417	40	8/15/02
	A103	6768593	7/27/04	Jutamulia	359	641	7/24/03
	A104	6788409	9/7/04	Goodwin	356	339	9/3/02
	A105	6794671	9/21/04	Nicoli et al.	250	574	7/17/02
	A106	6813017	11/2/04	Hoffman et al.	356	317	10/19/00
	A107	6839367	1/4/05	Nagamatsu et al.	372	34	3/16/01
	A108	6870679	3/22/05	Randall et al.	359	580	6/10/02
	A109	6870976	3/22/05	Chen et al.	385	14	3/13/01
	A110	6897954	5/24/05	Bishop et al.	356	317	12/20/02
	A111	6941047	9/6/05	Capewell et al.	385	47	3/1/04
	A112	6954722	10/11/05	Parks et al.	702	194	10/17/03
	A113	6975400	12/13/05	Ortyn et al.	356	419	7/28/03
	A114	7038778	5/2/06	Yamauchi	356	419	8/13/02
	A115	7072540	7/4/06	Szapiel et al.	385	24	4/22/03

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	A116	7110192	9/19/06	Sauter et al.	359	754	1/12/05
	A117	7113266	9/26/06	Wells	356	73	3/30/05
	A118	7127356	10/24/06	Nicoli et al.	702	26	5/18/04
	A119	7129505	10/31/06	Oostman Jr. et al.	250	458.1	10/17/03
	A120	7212343	5/1/07	He et al.	359	618	7/11/03
	A121	7260328	8/21/07	Kropp	398	82	2/24/03
	A122	7262838	8/28/07	Fritz	356	73	1/16/04
	A123	7268953	9/11/07	Matthae et al.	359	656	6/7/06
	A124	7305018	12/4/07	Otoma	372	50.1	12/29/05
	A125	7381565	6/3/08	Kurabayashi et al.	436	63	3/20/06
	A126	7385682	6/10/08	Chu et al.	356	73	9/7/06
	A127	7430048	9/30/08	Reel et al.	356	432	2/16/06
	A128	7450229	11/11/08	Ortyn et al.	356	326	5/4/05
	A129	7453915	11/18/08	Imai	372	50.1	12/5/06
	A130	7456960	11/25/08	Cerni et al.	356	336	11/15/05
	A131	7496463	2/24/09	Nicoli et al.	702	104	9/12/06
	A132	7505131	3/17/09	Roth	356	317	3/6/08
	A133	7507588	3/24/09	Mehrpouyan et al.	436	518	4/14/06
	A134	7561267	7/14/09	Luo et al.	356	336	12/22/06
	A135	7580120	8/25/09	Hamada et al.	356	73	4/6/06
	A136	7623243	11/24/09	Kato	356	419	9/13/04
	A137	7645127	1/12/10	Hagen et al.	417	477.12	6/29/06
	A138	7668422	2/23/10	Kropp	385	47	3/12/04

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	A139	7758324	6/20/10	Baumann et al.	417	477.2	5/12/05
	A140	7768120	8/3/10	Takashima	257	706	12/27/07
	A141	7781227	8/24/10	Mehrpouyan et al.	436	523	3/4/09
	A142	7787197	8/31/10	Chen	359	793	9/4/08
	A143	7835000	11/16/10	Graves et al.	356	338	11/3/06
	A144	7876436	1/25/11	Chu	356	338	10/31/07
	A145	7894047	2/22/11	Hamada et al.	356	39	7/24/09
	A146	7952981	3/31/11	Suzuki	369	121	10/23/08
	A147	7981661	7/19/11	Rich	435	286.5	4/17/07
	A148	8009189	8/30/11	Ortyn et al.	348	80	6/3/08
	A149	8049185	11/1/11	Hayashi et al.	250	459.1	2/4/09
	A150	8149525	4/3/12	Do	359	797	3/28/07
	A151	8157547	4/17/12	Vrielink	417	477.3	4/23/07
	A152	8187888	5/29/12	Rich	436	63	7/8/11
	A153	8213472	7/3/12	Hirai et al.	372	36	1/23/08
	A154	8229707	7/24/12	Olson et al.	702	189	7/4/11
	A155	8233146	7/31/12	Chen	356	246	1/5/10
	A156	8253938	8/28/12	Vacca et al.	356	337	5/26/11
	A157	8270098	9/18/12	Fukuta et al.	359	784	2/19/09
	A158	8284402	10/9/12	Frazier et al.	356	419	2/26/10
	A159	8337096	12/25/12	Shen et al.	385	89	11/30/09
	A160	8345237	1/1/13	Tsukii et al.	356	338	12/23/11
	A161	8405048	3/26/13	Hayashi	250	458.1	2/4/09

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	A162	8432541	4/30/13	Rich	356	246	11/4/10
	A163	8436371	5/7/13	Medendorp Jr., et al.	257	88	5/24/07
	A164	8436993	5/7/13	Kaduchak et al.	356	337	2/3/12
	A165	8488244	7/16/13	Li et al.	359	618	2/17/11
	A166	8507279	8/13/13	Ball et al.	436	10	6/2/10

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	B1	CN 102087198	6/8/11	China	Chinese (English abstract)
	B2	CN 102818794	12/12/12	China	Chinese (English abstract)
	B3	CN 102818795	12/12/12	China	Chinese (English abstract)
	B4	CN 102818796	12/12/12	China	Chinese (English abstract)
	B5	CN 201917509U	8/3/11	China	Chinese (English abstract)
	B6	CN 201936066U	8/17/11	China	Chinese (English abstract)
	B7	CN 201984209U	9/21/11	China	Chinese (English abstract)
	B8	EP 0889319	1/7/99	EPO	German (English abstract)
	B9	EP 1004907	4/22/09	EPO	English
	B10	EP 1953526	8/6/08	EPO	English
	B11	EP 2381475	3/4/15	EPO	English
	B12	EP 459764A2	12/4/91	EPO	English
	B13	EP 459764B1	12/11/96	EPO	English
	B14	EP 757346A1	2/5/97	EPO	English
	B15	EP 757346B1	11/14/01	EPO	English
	B16	EP 758755A2	2/19/97	EPO	English
	B17	JP 05333245	12/17/93	Japan	Japanese (English abstract)
	B18	JP 829726	2/2/96	Japan	Japanese (English translation)
	B19	WO 0057173	9/28/00	WIPO	English
	B20	WO 0127590	4/19/01	WIPO	English
	B21	WO 0140764	6/7/01	WIPO	English
	B22	WO 03012403	2/13/03	WIPO	French (English abstract)
	B23	WO 2005033654	4/14/05	WIPO	English
	B24	WO 2006013316	2/9/06	WIPO	English
	B25	WO 2009095358	8/6/09	WIPO	German (English abstract)
	B26	WO 2009098867	8/13/09	WIPO	Japanese (English abstract)

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Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	B27	WO 2011026942	3/10/11	WIPO	English
	B28	WO 2012056217	5/3/12	WIPO	English
	B29	WO 2012177367	12/27/12	WIPO	English
	B30	WO 2013013229	1/24/13	WIPO	English
	B31	WO 2013093035	6/27/13	WIPO	English
	B32	WO 9307471	4/15/93	WIPO	English
	B33	WO 9316368	8/19/93	WIPO	English
	B34	WO 9429695	12/22/94	WIPO	English
	B35	WO 9714066A2	4/17/97	WIPO	English

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C1	International Search Report and Written Opinion for PCT/US2013/043453 mailed December 20, 2013.
	C2	International Search Report and Written Opinion for PCT/US2014/067682 mailed April 9, 2015.
	C3	Brogioli et al., "Heterodyne near-field scattering," *Applied Physics Letters* 81(22):4109-4111, 2002.
	C4	Schmidt, B., "Ein lichtstarkes komafreies Spiegelsystem," *Mitteilungen der Haburger Sternwarte in Bergedorf* 7(36):15-17, 1932.
	C5	Smiley, C. H., "The Schmidt Camera," *Popular Astronomy* 44:415-421, 1936.

Espacenet

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Flow cytometry

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Abstract of CN102087198 (A)

The invention discloses a flow cytometry. The flow cytometry comprises a light source irradiation system, a liquid flow system, a light split system, a signal detection, analysis and processing system, and a personal computer (PC) display control system; the liquid flow system comprises a flowing chamber; linearly flowing cells are accommodated in the flowing chamber; a solid laser is used as an excitation light source of the light source irradiation system, and laser passes through a reflecting mirror and a plano-convex lens to be converged and irradiated on the flowing chamber, is integrated and then is further converged on the optical part of the flowing chamber to be irradiated on the linearly flowing cells; the signal detection, analysis and processing system comprises a lateral scattering detector, a yellow fluorescence channel detector, a near-infrared fluorescence channel detector, a forward scattering detector and a signal processing module; and all detectors are multi-pixel photon counters (MPPC). The flow cytometry has the characteristics of large dynamic detection range, high sensitivity, small size and compactness and convenience in maintenance.

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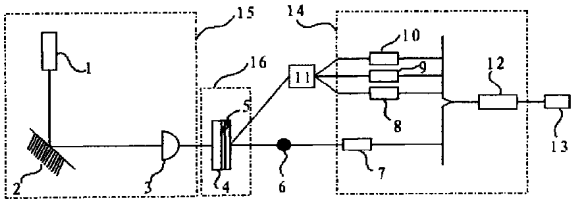
权利要求书 1 页 说明书 3 页 附图 1 页

(54)发明名称

一种流式细胞仪

(57)摘要

本发明公开了一种流式细胞仪，包括光源照射系统、液流系统、分光系统、信号探测分析处理系统和 PC 机显示控制系统，液流系统包括流动室，流动室内具有直线流动的细胞，一固体激光器作为光源照射系统的激发光源，激光经过反射镜、平凸透镜会聚照到流动室上，经过集成在流动室的光学部分进一步会聚，使激光照射在直线流动的细胞上，信号探测分析处理系统包括侧向散射探测器、黄色荧光通道探测器、近红外荧光通道探测器、前向散射探测器和信号处理模块，以上各探测器皆为多像素光子计数器（MPPC）。本发明的流式细胞仪具有较大的动态探测范围，高的灵敏度，体积小而紧凑，便于维护的特点。



CN 102087198 A

1. 一种流式细胞仪，包括光源照射系统（15）、液流系统（16）、分光系统（11）、信号探测分析处理系统（14）和 PC 机显示控制系统（13），所述液流系统（16）使细胞样品在鞘流的包裹下作直线流动，所述光源照射系统（15）对所述液流系统（16）中荧光染料标记的感兴趣的细胞流进行激光照射，激发得到所标记的荧光的发射信号，通过所述分光系统（11）进入所述信号探测分析处理系统（14）进行信号探测、分析和输出，所述 PC 机显示控制系统（13）对所述信号探测分析处理系统（14）的信息进行显示和存储并反馈控制所述光源照射系统（15）、液流系统（16）以及信号探测分析处理系统（14），所述液流系统（16）包括流动室（4），所述流动室（4）内具有直线流动的细胞（5），一固体激光器（1）作为光源照射系统的激发光源，激光经过反射镜（2）、平凸透镜（3）会聚照到流动室（4）上，经过集成在流动室（4）的光学部分进一步会聚，使激光照射在直线流动的细胞（5）上，其特征在于：所述信号探测分析处理系统包括分别设置在所述分光系统（11）后的一侧向散射探测器（8）、一黄色荧光通道探测器（9）和一近红外荧光通道探测器（10），还包括一前向散射探测器（7），以及一连接所述前向散射探测器（7）、侧向散射探测器（8）、黄色荧光通道探测器（9）和近红外荧光通道探测器（10）的信号处理模块（12）。

2. 根据权利要求 1 所述的流式细胞仪，其特征在于：所述前向散射探测器（7）与所述液流系统（16）之间还设置有一挡光板（6）。

3. 根据权利要求 1 所述的流式细胞仪，其特征在于：所述侧向散射探测器（8）、黄色荧光通道探测器（9）和近红外荧光通道探测器（10）组成探测器模块（18），所述流动室（4）、分光系统（11）与探测器模块（18）组成一点对点成像系统（17）。

4. 根据权利要求 1 或 2 或 3 所述的流式细胞仪，其特征在于：所述前向散射探测器（7）、侧向散射探测器（8）、黄色荧光通道探测器（9）和近红外荧光通道探测器（10）皆为多像素光子计数器。

一种流式细胞仪

技术领域

[0001] 本发明属于一种生物医学检测仪器，具体的涉及一种流式细胞仪。

背景技术

[0002] 流式细胞仪（flow cytometer，FCM）是集光学、电子学、流体力学、细胞化学、生物学、免疫学以及激光和计算机等多门学科和技术于一体的先进科学技术设备。它被广泛应用于临床医学、细胞学、生物学、微生物学、制药学、生殖学等领域，是现代科学研究中的先进仪器之一，被誉为实验室的“CT”。它对快速直线流动状态中的单列细胞或生物颗粒进行逐个、多参数、快速的定性、定量分析或分选的技术，具有检测速度快、测量参数多、采集数据量大、分析全面、分选纯度高、方法灵活等特点。

[0003] 目前商用的流式细胞仪采用气体激光器、固体激光器等，具有体积大，结构复杂，维护费用高的缺点，有些流式细胞仪采用单一的激发光源，限制了细胞荧光染料的选择。流式细胞仪的探测器一般采用光电倍增管（PMT），它具有大光敏面积，低噪声等效功率（NEP）的优点，但是体积庞大，量子效率低下，反向偏压过高，仅能够工作在 UV 和可见光谱范围内，对外部磁场抵抗力薄弱。

[0004] 商用流式细胞仪的液体控制系统价格昂贵，体积庞大，结构复杂。

发明内容

[0005] 为克服现有技术中的不足，本发明的目的在于提供一种具有较大的动态探测范围，高的灵敏度，体积小而紧凑，便于维护，真正实现便携式的流式细胞仪。

[0006] 为了解决上述技术问题，实现上述目的，本发明通过如下技术方案实现：

[0007] 一种流式细胞仪，包括光源照射系统、液流系统、分光系统、信号探测分析处理系统和 PC 机显示控制系统，所述液流系统使细胞样品在鞘流的包裹下作直线流动，所述光源照射系统对所述液流系统中荧光染料标记的感兴趣的细胞流进行激光照射，激发得到所标记的荧光的发射信号，通过所述分光系统进入所述信号探测分析处理系统进行信号探测、分析和输出，所述 PC 机显示控制系统对所述信号探测分析处理系统的信息进行显示和存储并反馈控制所述光源照射系统、液流系统以及信号探测分析处理系统，所述液流系统包括流动室，所述流动室内具有直线流动的细胞，一固体激光器作为光源照射系统的激发光源，激光经过反射镜、平凸透镜会聚照到流动室上，经过集成在流动室的光学部分进一步会聚，使激光照射在直线流动的细胞上。

[0008] 进一步的，所述信号探测分析处理系统包括分别设置在所述分光系统后的一侧向散射探测器、一黄色荧光通道探测器和一近红外荧光通道探测器，还包括一前向散射探测器，以及一连接所述前向散射探测器、侧向散射探测器、黄色荧光通道探测器和近红外荧光通道探测器的信号处理模块。

[0009] 优选的，所述侧向散射探测器、黄色荧光通道探测器和近红外荧光通道探测器组成探测器模块，所述括流动室、分光系统与探测器模块组成一点对点成像系统。

[0010] 进一步的，所述前向散射探测器与所述液流系统之间还设置有一挡光板。

[0011] 进一步的，所述前向散射探测器、侧向散射探测器、黄色荧光通道探测器和近红外荧光通道探测器皆为多像素光子计数器 (MPPC)。

[0012] 通过以上技术的运用，本发明具有以下优点：

[0013] 1、采用多像素光子计数器 (MPPC)，它有多个雪崩二极管 (APD) 组成的阵列，从每个 APD 的像素输出的总和构成了 MPPC 的输出，具有很好的光子计数性能，低的偏置电压，极好的时间分辨率，高增益、高灵敏度，体积小的优点，可以使仪器小而紧凑，灵敏度高。

[0014] 2、采用点对点成像系统，可以使流动室细胞的散射光和被激发产生的微弱荧光更好的被探测器响应。

[0015] 上述说明仅是本发明技术方案的概述，为了能够更清楚了解本发明的技术手段，并可依照说明书的内容予以实施，以下以本发明的较佳实施例并配合附图详细说明如后。本发明的具体实施方式由以下实施例及其附图详细给出。

附图说明

[0016] 下面结合附图和实施方式对本发明作进一步详细的说明。

[0017] 图 1 是本发明的流式细胞仪的一实施例的结构示意图。

[0018] 图 2 是本发明的流式细胞仪的一实施例的点对点成像系统结构示意图。

[0019] 图中标号说明：1、固体激光器，2、反射镜，3、平凸透镜，4、流动室，5、直线流动的细胞，6、挡光板，7、前向散射探测器，8、侧向散射探测器，9、黄色荧光通道探测器，10、近红外荧光通道探测器，11、分光系统，12、信号处理分析模块，13、PC 机显示控制系统，14、信号处理系统，15、光源照射系统，16、液流系统，17、点对点成像系统，18、探测器模块。

具体实施方式

[0020] 参见图 1 所示，一种流式细胞仪，包括光源照射系统 15、液流系统 16、分光系统 11、信号探测分析处理系统 14 和 PC 机显示控制系统 13，所述液流系统 16 使细胞样品在鞘流的包裹下作直线流动，所述光源照射系统 15 对所述液流系统 16 中荧光染料标记的感兴趣的细胞流进行激光照射，激发得到所标记的荧光的发射信号，通过所述分光系统 11 进入所述信号探测分析处理系统 14 进行信号探测、分析和输出，所述 PC 机显示控制系统 13 对所述信号探测分析处理系统 14 的信息进行显示和存储并反馈控制所述光源照射系统 15、液流系统 16 以及信号探测分析处理系统 14，所述液流系统 16 包括流动室 4，所述流动室 4 内具有直线流动的细胞 5。

[0021] 本实施例的流式细胞仪采用 532nm 的固体激光器 1 为激发光源，激光经过反射镜 2、平凸透镜 3 会聚照到流动室 4 上，经过集成在流动室的光学部分进一步会聚，使激光照射在直线流动的细胞 5 上。

[0022] 所述信号探测分析处理系统包括分别设置在所述分光系统 11 后的一侧向散射探测器 8、一黄色荧光通道探测器 9 和一近红外荧光通道探测器 10，还包括一前向散射探测器 7，以及一连接所述前向散射探测器 7、侧向散射探测器 8、黄色荧光通道探测器 9 和近红外荧光通道探测器 10 的信号处理模块 12。

[0023] 进一步的，所述前向散射探测器 7、侧向散射探测器 8、黄色荧光通道探测器 9 和近

红外荧光通道探测器 10 皆为多像素光子计数器。

[0024] 进一步的，前向散射光比较强，容易使前向散射探测器 7 饱和，故辅以小的挡光板 6 让少量前向散射光进入前向散射探测器 7 进行探测。

[0025] 进一步的，侧向散射光、激发产生的荧光经过分光系统 11 中的透镜进行会聚，然后经过滤光片使散射光，荧光进入侧向散射探测器 8、黄色荧光通道探测器 9 和近红外荧光通道探测器 10，再经过透镜会聚到探测器上，其中侧向散射探测器 8 为散射光 532nm 波长的探测通道、黄色荧光通道探测器 9 为黄色荧光 585nm 波长的探测通道、近红外荧光通道探测器 10 为近红外 780nm 波长的探测通道。

[0026] 优选的，参见图 2 所示，所述侧向散射探测器 8、黄色荧光通道探测器 9 和近红外荧光通道探测器 10 组成探测器模块 18，所述括流动室 4、分光系统 11 与探测器模块 18 组成一点对点成像系统 17，所述流动室 4 的被观测点可以经过分光系统 11 上的光学组件分别在侧向散射探测器 8、黄色荧光通道探测器 9、近红外荧光通道探测器 10 的感光面成像。被观测细胞的散射光，微弱荧光经过所述光学组件可以更好的会聚到探测器感光面上，完成微弱信号的探测。

[0027] 上述实施例只是为了说明本发明的技术构思及特点，其目的是在于让本领域内的普通技术人员能够了解本发明的内容并据以实施，并不能以此限制本发明的保护范围。凡是根据本发明内容的实质所作出的等效的变化或修饰，都应涵盖在本发明的保护范围内。

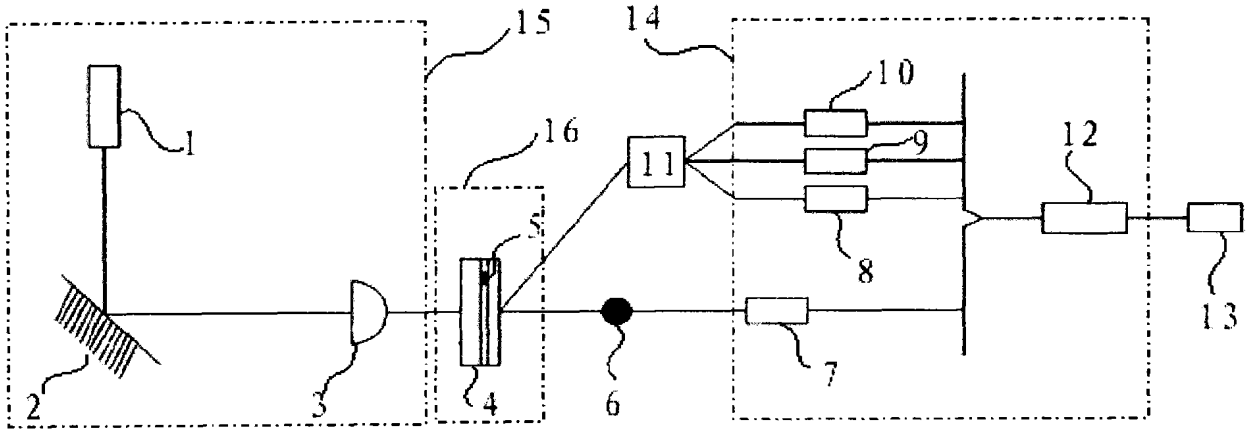


图 1

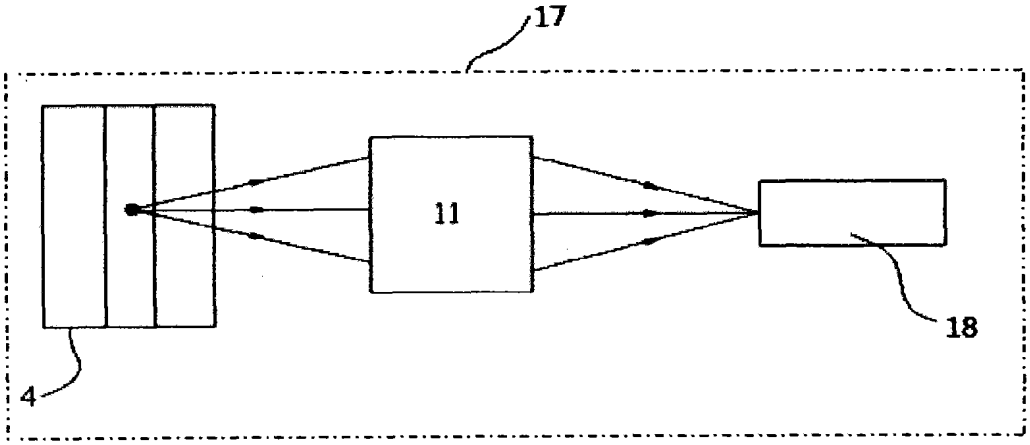


图 2

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Bibliographic data: CN102818794 (A) — 2012-12-12

Biological fluorescence microscopic detection instrument

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The invention provides a biological fluorescence microscopic detection instrument which comprises an excitation light illumination unit, excitation light, a fluorescence isolation unit, a reflecting unit, a microimaging unit, a fluorescence detection unit, a precision displacement platform and a control unit. An illumination pinhole is arranged between a first beam expander and a second beam expander of the excitation light illumination unit and located at a focal point of the first beam expander, an imaging detection pinhole is arranged between an imaging lens and a photomultiplier of the fluorescence detection unit and located at a focal point of the imaging lens, and therefore, the transverse resolution of the instrument is effectively improved; meanwhile, an annular beam shaping assembly is arranged between a tube lens and a microobjective of the microimaging unit, which enables the axial resolution of the biological fluorescence microscopic detection instrument to be improved.

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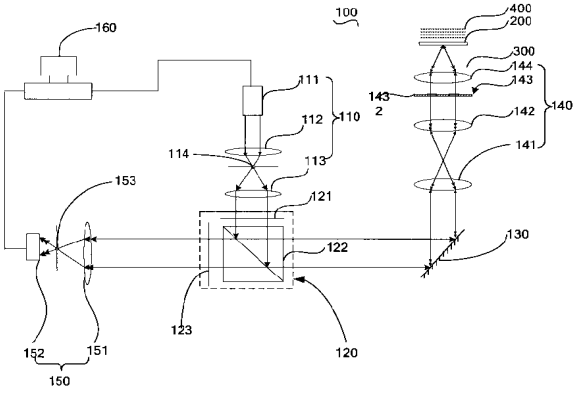
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(54)发明名称

生物荧光显微检测仪器

(57)摘要

本发明提供的生物荧光显微检测仪器包括激发光照明单元、激发光和荧光隔离单元、反射单元、显微成像单元、荧光探测单元及控制单元。激发光照明单元的第一扩束镜及第二扩束镜之间且位于第一扩束镜的焦点处设有照明针孔，荧光探测单元的成像镜头和光电倍增管之间且位于成像镜头的焦点处设有成像探测针孔，有效提高了该仪器的横向分辨率；同时，在显微成像单元的筒镜与显微物镜之间设置环形光束整形组件，提高了该生物荧光显微检测仪器在轴向的分辨率。



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1. 一种生物荧光显微检测仪器，其特征在于，包括激发光照明单元、激发光和荧光隔离单元、反射单元、显微成像单元、荧光探测单元及控制单元；

所述激发光照明单元包括激光光源、第一扩束镜、第二扩束镜；所述第一扩束镜及第二扩束镜之间设有照明针孔，且所述照明针孔位于所述第一扩束镜的焦点处；所述激发光和荧光隔离单元包括激发光滤色片、二色镜、荧光滤色片；所述反射单元为平面反射镜；所述显微成像单元包括中继透镜、筒镜、环形光束整形组件、显微物镜，所述环形光束整形组件包括设于所述筒镜及显微物镜之间的环形滤光片，所述环形滤光片具有将激光截止的内环区域和透射激光的环带区域，该内环区域和环带区域均可透射荧光；所述内环区域的半径不小于刚好能发生全内反射时所述显微物镜入瞳位置的临界半径；所述荧光探测单元包括成像镜头、光电倍增管，该成像镜头与所述光电倍增管之间设有成像探测针孔，所述成像探测针孔位于所述成像镜头的焦点处；待观察对象与所述照明针孔和成像探测针孔处于共轭位置上；所述光电倍增管可探测荧光，并将所述荧光转换成电信号；

所述第一扩束镜、照明针孔、第二扩束镜、激发光滤色片、二色镜、反射单元、中继透镜、筒镜、环形滤光片及显微物镜依次沿始于所述激光光源的光轴线设置，且所述显微物镜、环形滤光片、筒镜、中继透镜、反射单元、二色镜、荧光滤色片、成像镜头、成像探测针孔及光电倍增管依次沿始于由待观察对象激发的荧光光轴线设置；

所述控制单元与所述光电倍增管电性连接，用于采集所述电信号并生成待观察物质中一个点图像。

2. 根据权利要求 1 所述的生物荧光显微检测仪器，其特征在于，所述环带区域的宽度可调节。

3. 根据权利要求 1 所述的生物荧光显微检测仪器，其特征在于，所述显微物镜为无穷远消像差型大数值孔径的浸油物镜。

4. 根据权利要求 1 所述的生物荧光显微检测仪器，其特征在于，所述控制单元还与所述激光光源电性连接，用于控制激光的波长和功率。

生物荧光显微检测仪器

技术领域

[0001] 本发明涉及显微检测仪器设计及制造领域，尤其是涉及一种生物荧光显微检测仪器。

背景技术

[0002] 全内反射显微成像技术和共聚焦显微成像技术各有优缺点：全内反射显微成像具有很高的轴向分辨率，但其横向分辨率较低；共聚焦显微成像具有很高的横向分辨率，但其轴向分辨率较差。如何在一台显微镜中同时利用全内反射显微成像高的轴向分辨率和共聚焦显微成像高的横向分辨率尤为重要。

[0003] 发明专利申请 CN201080024155.9 中提出了将全内反射荧光图像和共焦图像简单且正确地重合的图像处理装置、程序和显微镜，该发明中包含了两套显微镜，一套是全内反射显微镜，另一套是共聚焦显微镜，但两套显微镜是分开工作的，通过光路切换的方法先获得一套显微镜的图像，再获得另一套显微镜的图像，最后计算机再利用两套图像中的基准点将两套图像进行了重合。该方法虽然生成了全内反射荧光图像和共焦图像的重合图像，由于两种显微镜是先后工作的，在物理上并不能同时得到三维高分辨率图像；另外该发明要先后操作两个显微镜拍摄图像，并要对图像进行重合，显微镜结构比较复杂，控制系统的构成也比较复杂。

发明内容

[0004] 本发明的目的是：提供一种集成全内反射显微成像技术和共聚焦显微成像技术的生物荧光显微检测仪器，该生物荧光显微检测仪器在横向及轴向上具有很高的分辨率。

[0005] 本发明的技术方案是：生物荧光显微检测仪器包括激发光照明单元、激发光和荧光隔离单元、反射单元、显微成像单元、荧光探测单元及控制单元；

[0006] 所述激发光照明单元包括激光光源、第一扩束镜、第二扩束镜；所述第一扩束镜及第二扩束镜之间设有照明针孔，且所述照明针孔位于所述第一扩束镜的焦点处；所述激发光和荧光隔离单元包括激发光滤色片、二色镜、荧光滤色片；所述反射单元为平面反射镜；所述显微成像单元包括中继透镜、筒镜、环形光束整形组件、显微物镜，所述环形光束整形组件包括设于所述筒镜及显微物镜之间的环形滤光片，所述环形滤光片具有将激光截止的内环区域和透射激光的环带区域，该内环区域和环带区域均可透射荧光；所述内环区域的半径不小于刚好能发生全内反射时所述显微物镜入瞳位置的临界半径；所述荧光探测单元包括成像镜头、光电倍增管，该成像镜头与所述光电倍增管之间设有成像探测针孔，所述成像探测针孔位于所述成像镜头的焦点处；待观察对象与所述照明针孔和成像探测针孔处于共轭位置上；所述光电倍增管可探测荧光，并将所述荧光转换成电信号；

[0007] 所述第一扩束镜、照明针孔、第二扩束镜、激发光滤色片、二色镜、反射单元、中继透镜、筒镜、环形滤光片及显微物镜依次沿始于所述激光光源的光轴线设置，且所述显微物镜、环形滤光片、筒镜、中继透镜、反射单元、二色镜、荧光滤色片、成像镜头、成像探测针孔

及光电倍增管依次沿始于由待观察对象激发的荧光光轴线设置；

[0008] 所述控制单元，与所述光电倍增管电性连接，用于采集所述电信号并生成待观察物质中一个点图像。

[0009] 下面对上述技术方案进一步解释：

[0010] 所述环带区域的宽度可调节。

[0011] 所述显微物镜为无穷远消像差型大数值孔径的浸油物镜。

[0012] 所述控制单元还与所述激光光源电性连接，用于控制激光的波长和功率。

[0013] 本发明的优点是：

[0014] 1. 本发明提供的生物荧光显微检测仪器在第一扩束镜及第二扩束镜之间且位于第一扩束镜的焦点处设有照明针孔，在成像镜头和光电倍增管之间且位于成像镜头的焦点处设有成像探测针孔，有效提高了该仪器的横向分辨率；同时，在筒镜及显微物镜之间设置环形滤光片，提高了该生物荧光显微检测仪器在轴向的分辨率。

[0015] 2. 本发明提供的生物荧光显微检测仪器由于入射激光光束经显微成像单元聚焦在区域面积约为艾利斑大小的点状区域内，即只有一个面积很小厚度很薄区域内的荧光物质能够被激发，而其它区域内的荧光物质不会被激发，即从源头上消除了杂散光的来源，因而具有很高的成像信噪比。

附图说明

[0016] 图 1 为本发明实施例提供的生物荧光显微检测仪器结构示意图。

[0017] 图 2 为本发明实施例提供的环形滤光片的结构示意图。

[0018] 图 3 为本发明实施例提供的环形光束通过显微物镜的光路传播示意图。

[0019] 其中：激发光照明单元 110、激光光源 111、第一扩束镜 112、第二扩束镜 113、照明针孔 114、激发光和荧光隔离单元 120、激发光滤色片 121、二色镜 122、荧光滤色片 123、反射单元 130、显微成像单元 140、中继透镜 141、筒镜 142、环形光束整形组件 143、显微物镜 144、环形滤光片 1432、荧光探测单元 150、成像镜头 151、光电倍增管 152、成像探测针孔 153、控制单元 160。

具体实施方式

[0020] 请参考图 1 至图 3。图 1 中标有单向箭头的光路为激光传播光路；标有双向箭头的光路表示为荧光传播光路。

[0021] 实施例：生物荧光显微检测仪器 100 包括激发光照明单元 110、激发光和荧光隔离单元 120、反射单元 130、显微成像单元 140、荧光探测单元 150 及控制单元 160。

[0022] 激发光照明单元 110 包括激光光源 111、第一扩束镜 112、第二扩束镜 113。在第一扩束镜 112 和第二扩束镜 113 之间且位于第一扩束镜 112 的焦点处设有照明针孔 114。

[0023] 激发光和荧光隔离单元 120 包括激发光滤色片 121、二色镜 122、荧光滤色片 123。激发光滤色 121 片用于接收激光，滤除激光中偏离中心波长的光束，并透射激光中中心波长处的光束。二色镜 122 接收并反射经激发光滤色片 121 透射的激光，并透射荧光。荧光滤色片 123 接收并透射经二色镜 122 透射的荧光，并截止激光。

[0024] 反射单元 130 为平面反射镜，用于反射激光和荧光。

[0025] 显微成像单元 140 包括中继透镜 141、筒镜 142、环形光束整形组件 143、显微物镜 144。环形光束整形组件 143 包括环形滤光片 1432。该环形滤光片 1432 设置于筒镜 142 及显微物镜 144 之间，具有将激光截止的内环区域 A 和透射激光的环带区域 B。内环区域 A 和环带区域 B 均可透射荧光。

[0026] 荧光探测单元 150 包括成像镜头 151、光电倍增管 152。在成像镜头 151 与光电倍增管 152 之间且位于成像镜头 151 的焦点处设有成像探测针孔 153。待观察对象与照明针孔 114 和成像探测针孔 153 处于共轭位置上。光电倍增管 152 可探测荧光，并将荧光转换成电信号。

[0027] 其中，第一扩束镜 112、照明针孔 114、第二扩束镜 113、激发光滤色片 121、二色镜 122、反射单元 130、中继透镜 141、筒镜 142、环形滤光片 1432、显微物镜 144 依次沿始于激光光源 111 的光轴线设置；显微物镜 144、环形滤光片 1432、筒镜 142、中继透镜 141、反射单元 130、二色镜 122、荧光滤色片 123、成像镜头 151、成像探测针孔 153、光电倍增管 152 依次沿始于由待观察对象激发的荧光光轴线设置。

[0028] 激光光源 111 发射的准直激光依次经第一扩束镜 112、照明针孔 114、第二扩束镜 113 扩束后形成平行的激光束；该平行激光束依次经激发光滤色片 121、二色镜 122、反射单元 130、中继透镜 141、筒镜 142 后经环形光束整形组件 143 整形为环形光束，该环形光束经显微物镜 144 聚集于待观察对象处，并激发待观察对象产生荧光；该荧光光束依次经显微物镜 144、环形滤光片 1432、筒镜 142、中继透镜 141、反射单元 130、二色镜 122、荧光滤色片 123、成像镜头 151 后聚焦于成像探测针孔 153 处，光电倍增管 152 探测该荧光束并将其转换成电信号。

[0029] 控制单元 160 将光电倍增管 152 输出的微弱电信号进行放大，并对放大后的电信号进行实时采样。由于任意时刻，光电倍增管 152 只能收集一个点位置荧光物质的光信息，并将光信号转换成电信号，控制单元 160 将采集到的电信号生成一个定点位置的待观察对象图像。控制单元 160 还电性连接于激光光源 111，用于控制入射激光波长选取和功率设置。

[0030] 在该生物荧光显微检测仪器 100 中，显微物镜 144 为无穷远消像差型大数值孔径的浸油物镜，由于环形光束的内环半径（图 2 中 A 区域的半径）不小于显微物镜 144 刚好能发生全内反射时入瞳位置处光束的临界半径，且盖玻片 200 和油 300 的折射率大致相同，这样进入显微物镜 144 的环形光束在盖玻片 200 和待观察对象所在的组织溶液 400 的交界处发生全反射，不能透过盖玻片 200 进行传播，但会在盖玻片 200 和组织溶液 400 的交界处形成隐失场（图 3 中 C 区），隐失波能够激发界面附近的荧光分子，产生荧光。调整环形滤光片 1432 环带区域 B 的宽度可以改变穿出盖玻片 200 的隐失场的分布深度，从而可以改变荧光物质在 Z 轴方向的激发深度，可以实现 Z 轴方向不同分辨率的调节。隐失波的频率与入射光频率相同，其强度（单位面积和单位时间的能量）随离开界面的垂直距离呈指数衰减：

[0031] $I(z) = I(0)e^{-z/d}$

[0032] 可以看出，透射电磁场的振幅随进入样品的深度 z 减小得非常快，这种电磁场只存在于界面附近一薄层内。d 是理论渗透深度，等于从界面处到隐失波强度衰减到界面处数值 1/e 的距离，d 可表示为：

[0033] $d = (\lambda_0/4\pi)(n_1^2\sin^2\theta - n_2^2)^{-1/2}$

[0034] d 与入射角（θ）、波长 λ_0 以及组织溶液 400 折射率（n_2）和盖玻片 200 的折射率（n_1）有关。d 随入射角增大而减小，大小与入射光波长为同一数量级或更小。由于隐失场的独特特性，使荧光激发的区域非常靠近分界面（约 100nm）。这样不会激发距分界面更远区域的荧光，从而可实现背景噪声极小的荧光成像，使得生物荧光显微检测仪器 100 在轴向具有很高的分辨率。

[0035] 在该生物荧光显微检测仪器 100 中，通过照明针孔 114 及成像探测针孔 153 的联合使用，实现点对点的照明和点对点的成像。当不考虑噪声的情况下，光学上常用点扩散函数描述系统成像分辨率，对于激光扫描共焦系统来说，系统最终的点扩散函数由下式描述：

[0036] $PSF_{tot}(x, y, z) = PSF_{ill}(x, y, z) \cdot PSF_{det}(x, y, z)$

[0037] 其中 PSF_{ill} 对应照明激光点在物方的点扩散函数，PSF_{det} 对应成像探测光路的点扩散函数。由于照明针孔 114 的作用，入射激光光束通过各单元后在盖玻片 200 与待观察对象交界处形成一个很小的点状照明区域（照明方点扩散函数），成像探测针孔 153 的使用对成像探测方点扩散函数进一步整形，使得整个生物荧光显微检测仪器 100 的成像点扩散函数由照明方点扩散函数和探测方点扩散函数的乘积组成，由于乘积后的成像点扩散函数强度分布范围变窄，因而系统具有很高的横向分辨率。

[0038] 在该生物荧光显微检测仪器 100 中由于入射激光光束经显微成像单元 140 聚焦在区域面积约为艾利斑大小的点状区域内，即只有一个面积很小厚度很薄区域内的荧光物质能够被激发，而其它区域内的荧光物质不会被激发，即从源头上消除了杂散光的来源，因而系统具有很高的成像信噪比。

[0039] 当然本发明的生物荧光显微检测仪器还可具有多种变换及改型，并不局限于上述实施方式的具体结构。总之，本发明的保护范围应包括那些对于本领域普通技术人员来说显而易见的变换或替代以及改型。

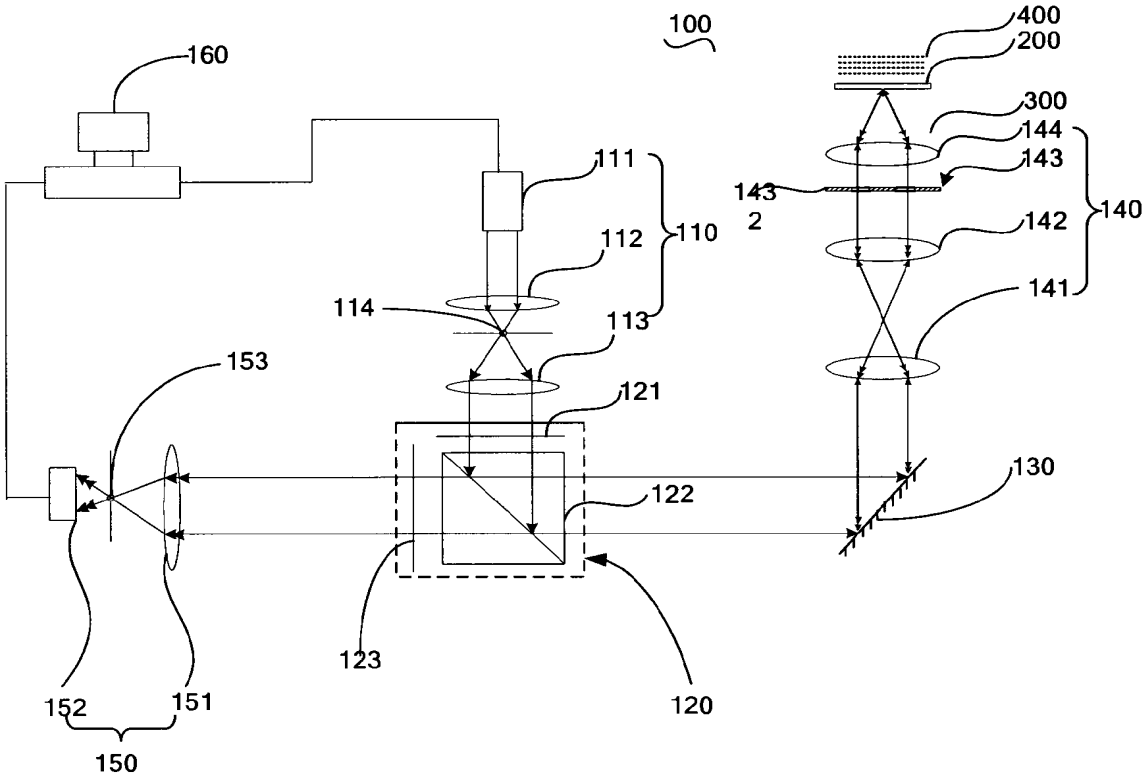

100
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图 1

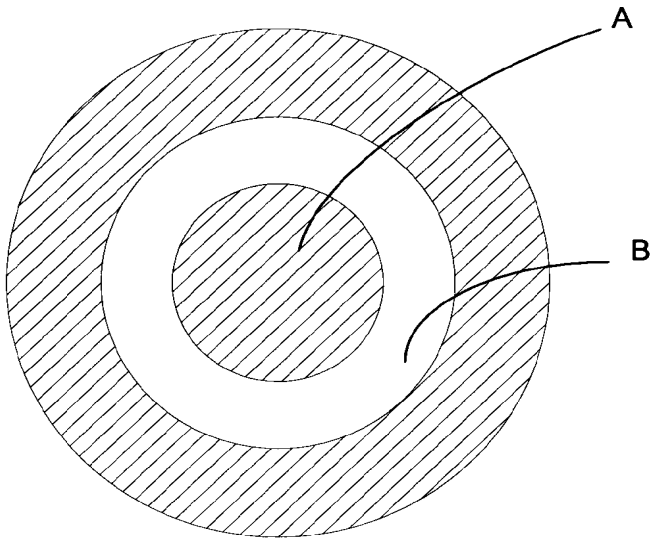

A
B

图 2

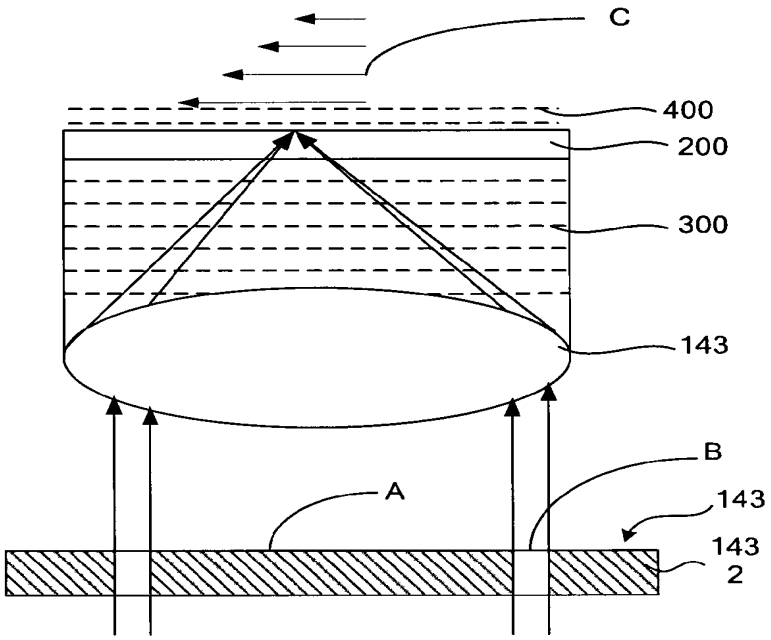


图 3

Espacenet

Bibliographic data: CN102818795 (A) — 2012-12-12

Biological fluorescence microscopic detection instrument

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- cooperative:

Application number: CN20121255748 20120723

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Abstract of CN102818795 (A)

The invention provides a biological fluorescence microscopic detection instrument which comprises an excitation light illumination unit, excitation light, a fluorescence isolation unit, a laser scanning unit, a microimaging unit, a fluorescence detection unit and a control unit. An illumination pinhole is arranged between a first beam expander and a second beam expander of the excitation light illumination unit and located at a focal point of the first beam expander, an imaging detection pinhole is arranged between an imaging lens and a photomultiplier of the fluorescence detection unit and located at a focal point of the imaging lens, and therefore, the transverse resolution of the instrument is effectively improved; meanwhile, an annular beam shaping assembly is arranged at an entrance pupil position of a microobjective of the microimaging unit, which enables the axial resolution of the biological fluorescence microscopic detection instrument to be improved.

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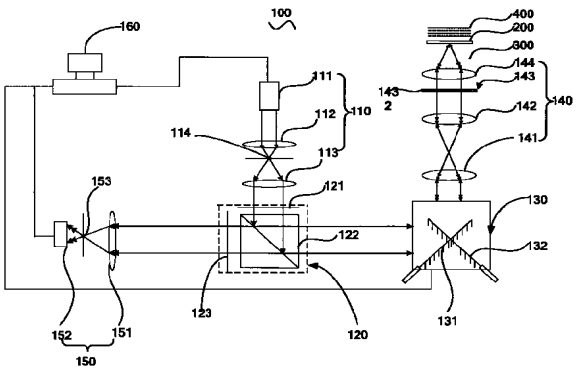
权利要求书 1 页 说明书 4 页 附图 2 页

(54)发明名称

生物荧光显微检测仪器

(57)摘要

本发明提供的生物荧光显微检测仪器包括激发光照明单元、激发光和荧光隔离单元、激光扫描单元、显微成像单元、荧光探测单元及控制单元。激发光照明单元的第一扩束镜及第二扩束镜之间且位于第一扩束镜的焦点处设有照明针孔，荧光探测单元的成像镜头和光电倍增管之间且位于成像镜头的焦点处设有成像探测针孔，有效提高了该仪器的横向分辨率；同时，在显微成像单元的显微物镜入瞳的位置设置环形光束整形组件，提高了该生物荧光显微检测仪器的轴向分辨率。



CN 102818795 A

1. 一种生物荧光显微检测仪器，其特征在于，包括激发光照明单元、激发光和荧光隔离单元、激光扫描单元、显微成像单元、荧光探测单元及控制单元；

所述激发光照明单元包括激光光源、第一扩束镜、第二扩束镜；第一扩束镜、第二扩束镜之间设有照明针孔，且所述照明针孔位于所述第一扩束镜的焦点处；所述激发光和荧光隔离单元包括激发光滤色片、二色镜、荧光滤色片；所述激光扫描单元包括可绕 X 轴做往返旋转运动的 X 扫描振镜组件、可绕 Y 轴做往返旋转运动的 Y 扫描振镜组件；所述显微成像单元包括扫描透镜、筒镜、环形光束整形组件、显微物镜，所述环形光束整形组件包括设于所述显微物镜的入瞳位置的环形滤光片，所述环形滤光片具有将激光截止的内环区域和透射激光的环带区域，该内环区域和环带区域均可透射荧光；所述内环区域的半径不小于刚好能发生全内反射时所述显微物镜入瞳位置的临界半径；所述显微物镜的入瞳位置与所述 X 扫描振镜组件的反射面和 Y 扫描振镜组件的反射面沿光轴线的中间位置相共轭；所述荧光探测单元包括成像镜头、光电倍增管，该成像镜头与所述光电倍增管之间设有成像探测针孔，所述成像探测针孔位于所述成像镜头的焦点处；待观察对象与所述照明针孔和成像探测针孔处于共轭位置上；所述光电倍增管可探测荧光，并将所述荧光转换成电信号；

所述第一扩束镜、照明针孔、第二扩束镜、激发光滤色片、二色镜、激光扫描单元、扫描透镜、筒镜、环形滤光片及显微物镜依次沿始于所述激光光源的光轴线设置，且所述显微物镜、环形滤光片、筒镜、扫描透镜、激光扫描单元、二色镜、荧光滤色片、成像镜头、成像探测针孔及光电倍增管依次沿始于由待观察对象激发的荧光光轴线设置；

所述控制单元与激光扫描单元、光电倍增管均电性连接，用于同步采集所述电信号与所述激光扫描单元的位置坐标并进行关联，以生成待观察对象区域图像。

2. 根据权利要求 1 所述的生物荧光显微检测仪器，其特征在于，所述环带区域的宽度可调节。

3. 根据权利要求 1 所述的生物荧光显微检测仪器，其特征在于，所述显微物镜为无穷远消像差型大数值孔径的浸油物镜。

4. 根据权利要求 1 所述的生物荧光显微检测仪器，其特征在于，所述控制单元还与所述激光光源电性连接，用于控制激光的波长和功率。

生物荧光显微检测仪器

技术领域

[0001] 本发明涉及显微检测仪器设计及制造领域，尤其是涉及一种生物荧光显微检测仪器。

背景技术

[0002] 全内反射显微成像技术和共聚焦显微成像技术各有优缺点：全内反射显微成像具有很高的轴向分辨率，但其横向分辨率较低；共聚焦显微成像具有很高的横向分辨率，但其轴向分辨率较差。如何在一台显微镜中同时利用全内反射显微成像高的轴向分辨率和共聚焦显微成像高的横向分辨率尤为重要。

[0003] 发明专利申请 CN201080024155.9 中提出了将全内反射荧光图像和共焦图像简单且正确地重合的图像处理装置、程序和显微镜，该发明中包含了两套显微镜，一套是全内反射显微镜，另一套是共聚焦显微镜，但两套显微镜是分开工作的，通过光路切换的方法先获得一套显微镜的图像，再获得另一套显微镜的图像，最后计算机再利用两套图像中的基准点将两套图像进行了重合。该方法虽然生成了全内反射荧光图像和共焦图像的重合图像，由于两种显微镜是先后工作的，在物理上并不能同时得到三维高分辨率图像；另外该发明要先后操作两个显微镜拍摄图像，并要对图像进行重合，显微镜结构比较复杂，控制系统的构成也比较复杂。

发明内容

[0004] 本发明的目的是：提供一种集成全内反射显微成像技术和共聚焦显微成像技术的生物荧光显微检测仪器，该生物荧光显微检测仪器在横向及轴向上具有很高的分辨率。

[0005] 本发明的技术方案是：生物荧光显微检测仪器包括激发光照明单元、激发光和荧光隔离单元、激光扫描单元、显微成像单元、荧光探测单元及控制单元；所述激发光照明单元包括激光光源、第一扩束镜及第二扩束镜；所述第一扩束镜及第二扩束镜之间设有照明针孔，且所述照明针孔位于所述第一扩束镜的焦点处；所述激发光和荧光隔离单元包括激发光滤色片、二色镜及荧光滤色片；所述激光扫描单元包括可绕 X 轴做往返旋转运动的 X 扫描振镜组件及可绕 Y 轴做往返旋转运动的 Y 扫描振镜组件；所述显微成像单元包括扫描透镜、筒镜、环形光束整形组件及显微物镜，所述环形光束整形组件包括设于所述显微物镜入瞳位置的环形滤光片，所述环形滤光片具有将激光截止的内环区域和透射激光的环带区域，且所述内环区域和环带区域均可透射荧光；所述内环区域的半径不小于刚好能发生全内反射时所述显微物镜入瞳位置的临界半径；所述显微物镜的入瞳位置与所述 X 扫描振镜组件的反射面和 Y 扫描振镜组件的反射面沿光轴线的中间位置相共轭；所述荧光探测单元包括成像镜头及光电倍增管，所述成像镜头与所述光电倍增管之间设有成像探测针孔，所述成像探测针孔位于所述成像镜头的焦点处；待观察对象与所述照明针孔和成像探测针孔处于共轭位置上；所述光电倍增管可探测荧光，并将所述荧光转换成电信号；

[0006] 所述第一扩束镜、照明针孔、第二扩束镜、激发光滤色片、二色镜、激光扫描单元、

扫描透镜、筒镜、环形滤光片及显微物镜依次沿始于所述激光光源的光轴线设置；且所述显微物镜、环形滤光片、筒镜、扫描透镜、激光扫描单元、二色镜、荧光滤色片、成像镜头、成像探测针孔及光电倍增管依次沿始于由待观察对象激发的荧光光轴线设置；

[0007] 所述控制单元，与所述激光扫描单元和所述光电倍增管均电性连接，用于同步采集所述电信号与所述激光扫描单元的位置坐标并进行关联，以生成待观察对象区域图像。

[0008] 下面对上述技术方案进一步解释：

[0009] 所述环带区域的宽度可调节。

[0010] 所述显微物镜为无穷远消像差型大数值孔径的浸油物镜。

[0011] 所述控制单元还与所述激光光源电性连接，用于控制激光的波长和功率。

[0012] 本发明的优点是：

[0013] 1. 本发明提供的生物荧光显微检测仪器在第一扩束镜及第二扩束镜之间且位于第一扩束镜的焦点处设有照明针孔，在成像镜头和光电倍增管之间且位于成像镜头的焦点处设有成像探测针孔，有效提高了该仪器的横向分辨率；同时，在位于显微物镜入瞳的位置设置环形光束整形组件，提高了该生物荧光显微检测仪器在轴向的分辨率。

[0014] 2. 本发明提供的生物荧光显微检测仪器由于入射激光光束经显微成像单元聚焦在区域面积约为艾利斑大小的点状区域内，即只有一个面积很小厚度很薄区域内的荧光物质能够被激发，而其它区域内的荧光物质不会被激发，即从源头上消除了杂散光的来源，因而具有很高的成像信噪比。

附图说明

[0015] 图 1 为本发明实施例提供的生物荧光显微检测仪器结构示意图。

[0016] 图 2 为本发明实施例提供的环形滤光片的结构示意图。

[0017] 图 3 为本发明实施例提供的环形光束通过显微物镜的光路传播示意图。

[0018] 图 4 为本发明实施例提供的激光扫描单元的结构示意图。

[0019] 其中：激发光照明单元 110、激光光源 111、第一扩束镜 112、第二扩束镜 113、照明针孔 114、激发光和荧光隔离单元 120、激发光滤色片 121、二色镜 122、荧光滤色片 123、激光扫描单元 130、X 扫描振镜组件 131、Y 扫描振镜组件 132、显微成像单元 140、扫描透镜 141、筒镜 142、环形光束整形组件 143、显微物镜 144、环形滤光片 1432、荧光探测单元 150、成像镜头 151、光电倍增管 152、成像探测针孔 153、控制单元 160。

具体实施方式

[0020] 请参考图 1 至图 4。图 1 中标有单向箭头的光路为激光传播光路；标有双向箭头的光路表示为荧光传播光路。

[0021] 实施例：生物荧光显微检测仪器 100 包括激发光照明单元 110、激发光和荧光隔离单元 120、激光扫描单元 130、显微成像单元 140、荧光探测单元 150 及控制单元 160。

[0022] 激发光照明单元 110 包括激光光源 111、第一扩束镜 112、第二扩束镜 113。在第一扩束镜 112 和第二扩束镜 113 之间且位于第一扩束镜 112 的焦点处设有照明针孔 114。

[0023] 激发光和荧光隔离单元 120 包括激发光滤色片 121、二色镜 122、荧光滤色片 123。激发光滤色 121 片用于接收激光，滤除激光中偏离中心波长的光束，并透射激光中中心波

长处的光束。二色镜 122 接收并反射经激发光滤色片 121 透射的激光，并透射荧光。荧光滤色片 123 接收并透射经二色镜 122 透射的荧光，并截止激光。

[0024] 激光扫描单元 130 包括 X 扫描振镜组件 131、Y 扫描振镜组件 132。X 扫描振镜组件 131 可绕 X 轴做往返旋转运动。Y 扫描振镜组件 132 可绕 Y 轴做往返旋转运动，随着 X 扫描振镜组件 131、Y 扫描振镜组件 132 的旋转运动，入射激光束反射后的角度也将随之改变。

[0025] 显微成像单元 140 包括扫描透镜 141、筒镜 142、环形光束整形组件 143、显微物镜 144。环形光束整形组件 143 包括环形滤光片 1432。该环形滤光片 1432 设置于显微物镜 144 的入瞳位置，具有将激光截止的内环区域 A 和透射激光的环带区域 B。内环区域 A 和环带区域 B 均可透射荧光。显微物镜 144 的入瞳位置与 X 扫描振镜组件 131 的反射面和 Y 扫描振镜组件 132 的反射面沿光轴线的中间位置相共轭，即 X 扫描振镜组件和 Y 扫描振镜组件置于零视场角位置时图 4 中过 M 点垂直光轴的位置与显微物镜 144 入瞳位置共轭。

[0026] 荧光探测单元 150 包括成像镜头 151、光电倍增管 152。在成像镜头 151 与光电倍增管 152 之间且位于成像镜头 151 的焦点处设有成像探测针孔 153。待观察对象与照明针孔 114 和成像探测针孔 153 处于共轭位置上。光电倍增管 152 可探测荧光，并将荧光转换成电信号。

[0027] 其中，第一扩束镜 112、照明针孔 114、第二扩束镜 113、激发光滤色片 121、二色镜 122、激光扫描单元 130、扫描透镜 141、筒镜 142、环形滤光片 1432、显微物镜 144 依次沿始于激光光源 111 的光轴线设置；显微物镜 144、环形滤光片 1432、筒镜 142、扫描透镜 141、激光扫描单元 130、二色镜 122、荧光滤色片 123、成像镜头 151、成像探测针孔 153、光电倍增管 152 依次沿始于待观察对象激发的荧光光轴线设置。

[0028] 激光光源 111 发射的准直激光依次经第一扩束镜 112、照明针孔 114、第二扩束镜 113 扩束后形成平行的激光束；该平行激光束依次经激发光滤色片 121、二色镜 122、激光扫描单元 130、扫描透镜 141、筒镜 142 后经环形光束整形组件 143 整形为环形光束，该环形光束经显微物镜 144 聚集于待观察对象处，并激发待观察对象产生荧光；该荧光光束依次经显微物镜 144、环形滤光片 1432、筒镜 142、扫描透镜 141、激光扫描单元 130、二色镜 122、荧光滤色片 123、成像镜头 151 后聚焦于成像探测针孔 153 处，光电倍增管 152 探测该荧光束并将其转换成电信号。

[0029] 控制单元 160 与激光扫描单元 130、光电倍增管 152 电性连接，控制单元 160 将光电倍增管 152 输出的微弱电信号进行放大，并对放大后的电信号进行实时采样，同时控制 X 扫描振镜组件 131、Y 扫描振镜组件 132 沿 X、Y 轴往返旋转，使得经显微物镜 144 形成的聚焦激光点在 X、Y 方向能够移动。控制单元 160 将采集到电信号和激光扫描单元 130X、Y 方向的位置坐标关联起来，生成了一个区域中荧光物质的图像。控制单元 160 还电性连接于激光光源 111，用于控制入射激光波长和功率。

[0030] 在该生物荧光显微检测仪器 100 中，显微物镜 144 为无穷远消像差型大数值孔径的浸油物镜，由于环形光束的内环半径（图 2 中 A 区域的半径）不小于显微物镜 144 刚好能发生全内反射时入瞳位置处光束的临界半径，且盖玻片 200 和油 300 的折射率大致相同，这样进入显微物镜 144 的环形光束在盖玻片 200 和待观察对象所在的组织溶液 400 的交界处发生全反射，不能透过盖玻片 200 进行传播，但会在盖玻片 200 和组织溶液 400 的交界处形成隐失场（图 3 中 C 区），隐失波能够激发界面附近的荧光分子，产生荧光。调整环形滤

光片 1432 环带区域 B 的宽度可以改变穿出盖玻片 200 的隐失场的分布深度，从而可以改变荧光物质在 Z 轴方向的激发深度，可以实现 Z 轴方向不同分辨率的调节。隐失波的频率与入射光频率相同，其强度（单位面积和单位时间的能量）随离开界面的垂直距离呈指数衰减：

[0031] $I(z) = I(0)e^{-z/d}$

[0032] 可以看出，透射电磁场的振幅随进入样品的深度 z 减小得非常快，这种电磁场只存在于界面附近一薄层内。d 是理论渗透深度，等于从界面处到隐失波强度衰减到界面处数值 1/e 的距离，d 可表示为：

[0033] $d = (\lambda_0/4\pi)(n_1^2\sin^2\theta - n_2^2)^{-1/2}$

[0034] d 与入射角（θ）、波长 λ_0 以及组织溶液 400 折射率（n_2）和盖玻片 200 的折射率（n_1）有关。d 随入射角增大而减小，大小与入射光波长为同一数量级或更小。由于隐失场的独特特性，使荧光激发的区域非常靠近分界面（约 100nm）。这样不会激发距分界面更远区域的荧光，从而可实现背景噪声极小的荧光成像，使得生物荧光显微检测仪器 100 在轴向具有很高的分辨率。

[0035] 在该生物荧光显微检测仪器 100 中，通过照明针孔 114 及成像探测针孔 153 的联合使用，实现点对点的照明和点对点的成像。当不考虑噪声的情况下，光学上常用点扩散函数描述系统成像分辨率，对于激光扫描共焦系统来说，系统最终的点扩散函数由下式描述：

[0036] $PSF_{tot}(x,y,z) = PSF_{ill}(x,y,z) \cdot PSF_{det}(x,y,z)$

[0037] 其中 PSF_{ill} 对应照明激光点在物方的点扩散函数，PSF_{det} 对应成像探测光路的点扩散函数。由于照明针孔 114 的作用，入射激光光束通过各单元后在盖玻片 200 与待观察对象交界处形成一个很小的点状照明区域（照明方点扩散函数），成像探测针孔 153 的使用对成像探测方点扩散函数进一步整形，使得整个生物荧光显微检测仪器 100 的成像点扩散函数由照明方点扩散函数和探测方点扩散函数的乘积组成，由于乘积后的成像点扩散函数强度分布范围变窄，因而系统具有很高的横向分辨率。

[0038] 在该生物荧光显微检测仪器 100 中由于入射激光光束经显微成像单元 140 聚焦在区域面积约为艾利斑大小的点状区域内，即只有一个面积很小厚度很薄区域内的荧光物质能够被激发，而其它区域内的荧光物质不会被激发，即从源头上消除了杂散光的来源，因而系统具有很高的成像信噪比。

[0039] 当然本发明的生物荧光显微检测仪器还可具有多种变换及改型，并不局限于上述实施方式的具体结构。总之，本发明的保护范围应包括那些对于本领域普通技术人员来说显而易见的变换或替代以及改型。

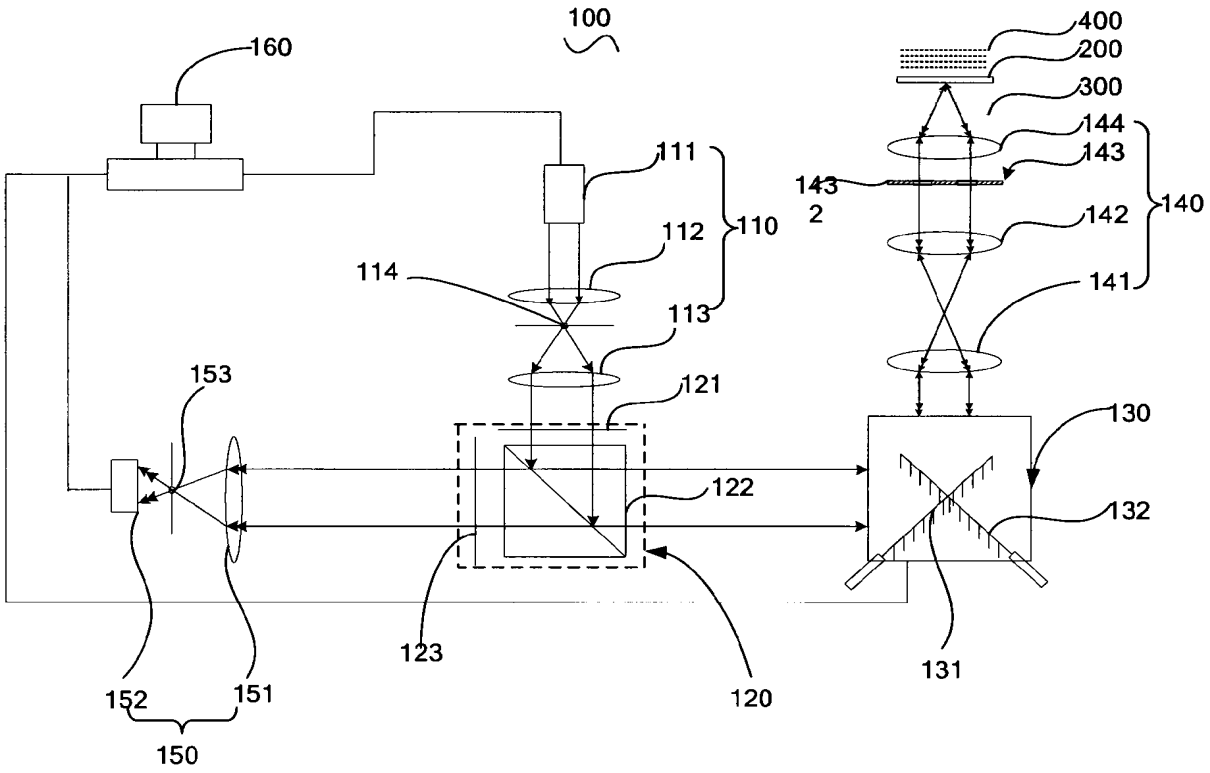

100
160
400
200
300
144
143
143
2
140
142
141
111
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153
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131
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151
150

图 1

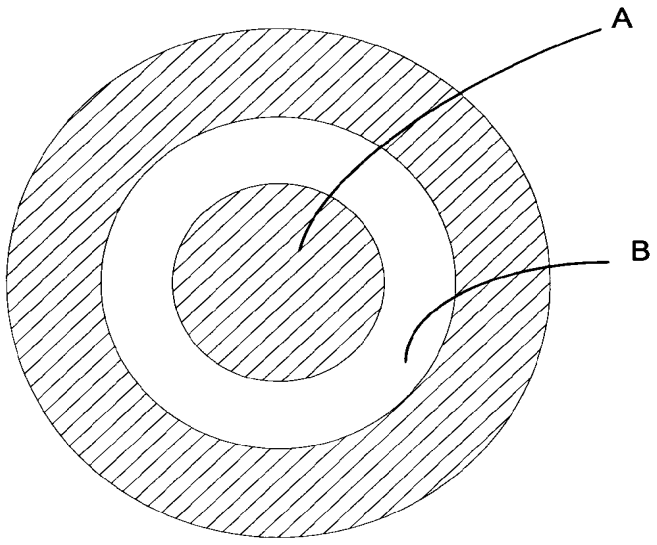

A
B

图 2

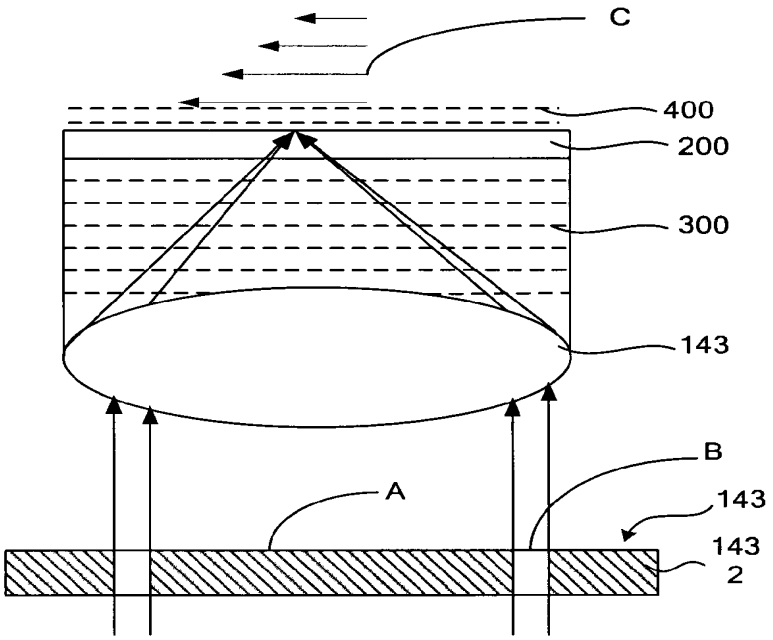


图 3

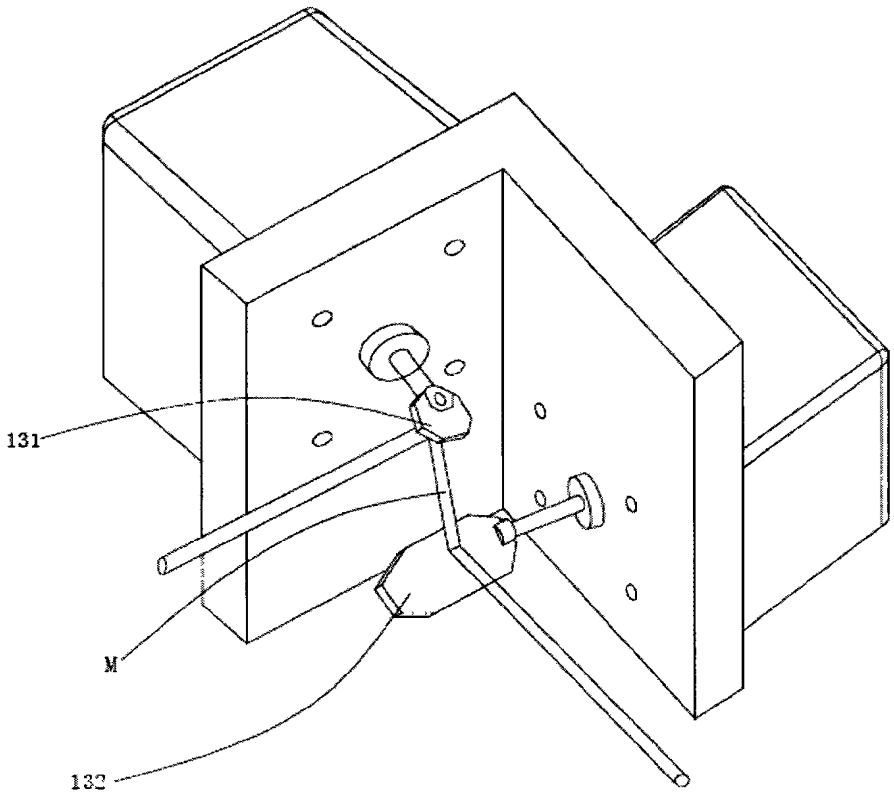


图 4

Espacenet

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Flow cytometry

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Classification: **- international:** ***G01N15/14***
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Application number: CN20102613651U 20101118

Priority number (s): CN20102613651U 20101118

Abstract of CN201917509 (U)

The utility model discloses a flow cytometry which comprises a light source illumination system, a liquid flow system, a light splitting system, a signal detection-analysis processing system, and a PC (personal computer) display control system. The liquid flow system comprises a flowing chamber; cells flowing linearly are arranged in the flowing chamber; a solid laser is used as an excitation light source of the light source illumination system; lasers are converged and irradiated on the flowing chamber through a reflector and a plane convex lens, and are further converged through an optical part integrated on the flowing chamber, and irradiated on the cells flowing linearly; the signal detection-analysis processing system comprises a lateral scattering detector, a yellow fluorescence channel detector, a near-infrared fluorescence channel detector, a forward scattering detector, and a signal processing module; and the detectors are all multi-pixel photon counters (MPPC). The flow cytometry has the characteristics of larger dynamic detection scope, high sensitivity, small and compact size, and convenience in maintenance.

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(12)实用新型专利

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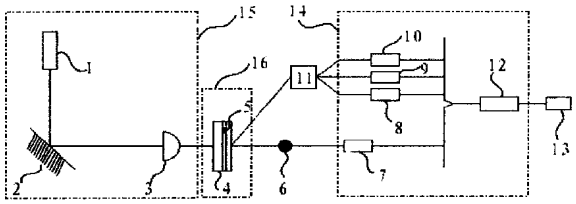
权利要求书 1 页 说明书 3 页 附图 1 页

(54)实用新型名称

一种流式细胞仪

(57)摘要

本实用新型公开了一种流式细胞仪，包括光源照射系统、液流系统、分光系统、信号探测分析处理系统和 PC 机显示控制系统，液流系统包括流动室，流动室内具有直线流动的细胞，一固体激光器作为光源照射系统的激发光源，激光经过反射镜、平凸透镜会聚照到流动室上，经过集成在流动室的光学部分进一步会聚，使激光照射在直线流动的细胞上，信号探测分析处理系统包括侧向散射探测器、黄色荧光通道探测器、近红外荧光通道探测器、前向散射探测器和信号处理模块，以上各探测器皆为多像素光子计数器 (MPPC)。本实用新型的流式细胞仪具有较大的动态探测范围，高的灵敏度，体积小而紧凑，便于维护的特点。



CN 201917509 U

1. 一种流式细胞仪，包括光源照射系统 (15)、液流系统 (16)、分光系统 (11)、信号探测分析处理系统 (14) 和 PC 机显示控制系统 (13)，所述液流系统 (16) 使细胞样品在鞘流的包裹下作直线流动，所述光源照射系统 (15) 对所述液流系统 (16) 中荧光染料标记的感兴趣的细胞流进行激光照射，激发得到所标记的荧光的发射信号，通过所述分光系统 (11) 进入所述信号探测分析处理系统 (14) 进行信号探测、分析和输出，所述 PC 机显示控制系统 (13) 对所述信号探测分析处理系统 (14) 的信息进行显示和存储并反馈控制所述光源照射系统 (15)、液流系统 (16) 以及信号探测分析处理系统 (14)，所述液流系统 (16) 包括流动室 (4)，所述流动室 (4) 内具有直线流动的细胞 (5)，一固体激光器 (1) 作为光源照射系统的激发光源，激光经过反射镜 (2)、平凸透镜 (3) 会聚照到流动室 (4) 上，经过集成在流动室 (4) 的光学部分进一步会聚，使激光照射在直线流动的细胞 (5) 上，其特征在于：所述信号探测分析处理系统包括分别设置在所述分光系统 (11) 后的一侧向散射探测器 (8)、一黄色荧光通道探测器 (9) 和一近红外荧光通道探测器 (10)，还包括一前向散射探测器 (7)，以及一连接所述前向散射探测器 (7)、侧向散射探测器 (8)、黄色荧光通道探测器 (9) 和近红外荧光通道探测器 (10) 的信号处理模块 (12)。

2. 根据权利要求 1 所述的流式细胞仪，其特征在于：所述前向散射探测器 (7) 与所述液流系统 (16) 之间还设置有一挡光板 (6)。

3. 根据权利要求 1 所述的流式细胞仪，其特征在于：所述侧向散射探测器 (8)、黄色荧光通道探测器 (9) 和近红外荧光通道探测器 (10) 组成探测器模块 (18)，所述流动室 (4)、分光系统 (11) 与探测器模块 (18) 组成一点对点成像系统 (17)。

4. 根据权利要求 1 或 2 或 3 所述的流式细胞仪，其特征在于：所述前向散射探测器 (7)、侧向散射探测器 (8)、黄色荧光通道探测器 (9) 和近红外荧光通道探测器 (10) 皆为多像素光子计数器。

一种流式细胞仪

技术领域

[0001] 本实用新型属于一种生物医学检测仪器，具体的涉及一种流式细胞仪。

背景技术

[0002] 流式细胞仪（flow cytometer，FCM）是集光学、电子学、流体力学、细胞化学、生物学、免疫学以及激光和计算机等多门学科和技术于一体的先进科学技术设备。它被广泛应用于临床医学、细胞学、生物学、微生物学、制药学、生殖学等领域，是现代科学研究中的先进仪器之一，被誉为实验室的“CT”。它对快速直线流动状态中的单列细胞或生物颗粒进行逐个、多参数、快速的定性、定量分析或分选的技术，具有检测速度快、测量参数多、采集数据量大、分析全面、分选纯度高、方法灵活等特点。

[0003] 目前商用的流式细胞仪采用气体激光器、固体激光器等，具有体积大，结构复杂，维护费用高的缺点，有些流式细胞仪采用单一的激发光源，限制了细胞荧光染料的选择。流式细胞仪的探测器一般采用光电倍增管（PMT），它具有大光敏面积，低噪声等效功率（NEP）的优点，但是体积庞大，量子效率低下，反向偏压过高，仅能够工作在 UV 和可见光谱范围内，对外部磁场抵抗力薄弱。

[0004] 商用流式细胞仪的液体控制系统价格昂贵，体积庞大，结构复杂。

实用新型内容

[0005] 为克服现有技术中的不足，本实用新型的目的在于提供一种具有较大的动态探测范围，高的灵敏度，体积小而紧凑，便于维护，真正实现便携式的流式细胞仪。

[0006] 为了解决上述技术问题，实现上述目的，本实用新型通过如下技术方案实现：

[0007] 一种流式细胞仪，包括光源照射系统、液流系统、分光系统、信号探测分析处理系统和 PC 机显示控制系统，所述液流系统使细胞样品在鞘流的包裹下作直线流动，所述光源照射系统对所述液流系统中荧光染料标记的感兴趣的细胞流进行激光照射，激发得到所标记的荧光的发射信号，通过所述分光系统进入所述信号探测分析处理系统进行信号探测、分析和输出，所述 PC 机显示控制系统对所述信号探测分析处理系统的信息进行显示和存储并反馈控制所述光源照射系统、液流系统以及信号探测分析处理系统，所述液流系统包括流动室，所述流动室内具有直线流动的细胞，一固体激光器作为光源照射系统的激发光源，激光经过反射镜、平凸透镜会聚照到流动室上，经过集成在流动室的光学部分进一步会聚，使激光照射在直线流动的细胞上。

[0008] 进一步的，所述信号探测分析处理系统包括分别设置在所述分光系统后的一侧向散射探测器、一黄色荧光通道探测器和一近红外荧光通道探测器，还包括一前向散射探测器，以及一连接所述前向散射探测器、侧向散射探测器、黄色荧光通道探测器和近红外荧光通道探测器的信号处理模块。

[0009] 优选的，所述侧向散射探测器、黄色荧光通道探测器和近红外荧光通道探测器组成探测器模块，所述括流动室、分光系统与探测器模块组成一点对点成像系统。

[0010] 进一步的，所述前向散射探测器与所述液流系统之间还设置有一挡光板。

[0011] 进一步的，所述前向散射探测器、侧向散射探测器、黄色荧光通道探测器和近红外荧光通道探测器皆为多像素光子计数器 (MPPC)。

[0012] 通过以上技术的运用，本实用新型具有以下优点 ：

[0013] 1、采用多像素光子计数器 (MPPC)，它有多个雪崩二极管 (APD) 组成的阵列，从每个 APD 的像素输出的总和构成了 MPPC 的输出，具有很好的光子计数性能，低的偏置电压，极好的时间分辨率，高增益、高灵敏度，体积小的优点，可以使仪器小而紧凑，灵敏度高。

[0014] 2、采用点对点成像系统，可以使流动室细胞的散射光和被激发产生的微弱荧光更好的被探测器响应。

[0015] 上述说明仅是本实用新型技术方案的概述，为了能够更清楚了解本实用新型的技术手段，并可依照说明书的内容予以实施，以下以本实用新型的较佳实施例并配合附图详细说明如后。本实用新型的具体实施方式由以下实施例及其附图详细给出。

附图说明

[0016] 下面结合附图和实施方式对本实用新型作进一步详细的说明。

[0017] 图 1 是本实用新型的流式细胞仪的一实施例的结构示意图。

[0018] 图 2 是本实用新型的流式细胞仪的一实施例的点对点成像系统结构示意图。

[0019] 图中标号说明 ：1、固体激光器，2、反射镜，3、平凸透镜，4、流动室，5、直线流动的细胞，6、挡光板，7、前向散射探测器，8、侧向散射探测器，9、黄色荧光通道探测器，10、近红外荧光通道探测器，11、分光系统，12、信号处理分析模块，13、PC 机显示控制系统，14、信号处理系统，15、光源照射系统，16、液流系统，17、点对点成像系统，18、探测器模块。

具体实施方式

[0020] 参见图 1 所示，一种流式细胞仪，包括光源照射系统 15、液流系统 16、分光系统 11、信号探测分析处理系统 14 和 PC 机显示控制系统 13，所述液流系统 16 使细胞样品在鞘流的包裹下作直线流动，所述光源照射系统 15 对所述液流系统 16 中荧光染料标记的感兴趣的细胞流进行激光照射，激发得到所标记的荧光的发射信号，通过所述分光系统 11 进入所述信号探测分析处理系统 14 进行信号探测、分析和输出，所述 PC 机显示控制系统 13 对所述信号探测分析处理系统 14 的信息进行显示和存储并反馈控制所述光源照射系统 15、液流系统 16 以及信号探测分析处理系统 14，所述液流系统 16 包括流动室 4，所述流动室 4 内具有直线流动的细胞 5。

[0021] 本实施例的流式细胞仪采用 532nm 的固体激光器 1 为激发光源，激光经过反射镜 2、平凸透镜 3 会聚照到流动室 4 上，经过集成在流动室的光学部分进一步会聚，使激光照射在直线流动的细胞 5 上。

[0022] 所述信号探测分析处理系统包括分别设置在所述分光系统 11 后的一侧向散射探测器 8、一黄色荧光通道探测器 9 和一近红外荧光通道探测器 10，还包括一前向散射探测器 7，以及一连接所述前向散射探测器 7、侧向散射探测器 8、黄色荧光通道探测器 9 和近红外荧光通道探测器 10 的信号处理模块 12。

[0023] 进一步的，所述前向散射探测器 7、侧向散射探测器 8、黄色荧光通道探测器 9 和近

红外荧光通道探测器 10 皆为多像素光子计数器。

[0024] 进一步的，前向散射光比较强，容易使前向散射探测器 7 饱和，故辅以小的挡光板 6 让少量前向散射光进入前向散射探测器 7 进行探测。

[0025] 进一步的，侧向散射光、激发产生的荧光经过分光系统 11 中的透镜进行会聚，然后经过滤光片使散射光，荧光进入侧向散射探测器 8、黄色荧光通道探测器 9 和近红外荧光通道探测器 10，再经过透镜会聚到探测器上，其中侧向散射探测器 8 为散射光 532nm 波长的探测通道、黄色荧光通道探测器 9 为黄色荧光 585nm 波长的探测通道、近红外荧光通道探测器 10 为近红外 780nm 波长的探测通道。

[0026] 优选的，参见图 2 所示，所述侧向散射探测器 8、黄色荧光通道探测器 9 和近红外荧光通道探测器 10 组成探测器模块 18，所述括流动室 4、分光系统 11 与探测器模块 18 组成一点对点成像系统 17，所述流动室 4 的被观测点可以经过分光系统 11 上的光学组件分别在侧向散射探测器 8、黄色荧光通道探测器 9、近红外荧光通道探测器 10 的感光面成像。被观测细胞的散射光，微弱荧光经过所述光学组件可以更好的会聚到探测器感光面上，完成微弱信号的探测。

[0027] 上述实施例只是为了说明本实用新型的技术构思及特点，其目的是在于让本领域内的普通技术人员能够了解本实用新型的内容并据以实施，并不能以此限制本实用新型的保护范围。凡是根据本实用新型内容的实质所作出的等效的变化或修饰，都应涵盖在本实用新型的保护范围内。

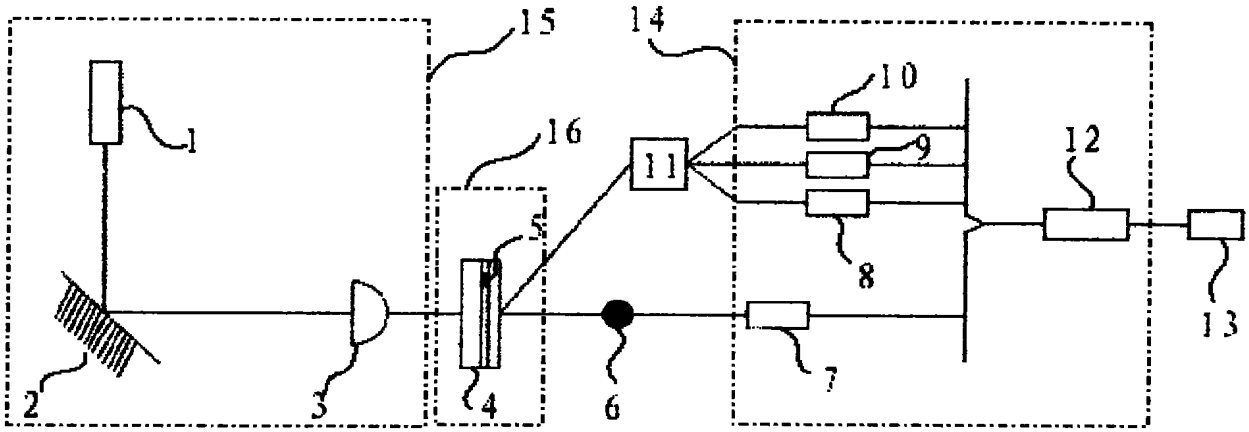


图 1

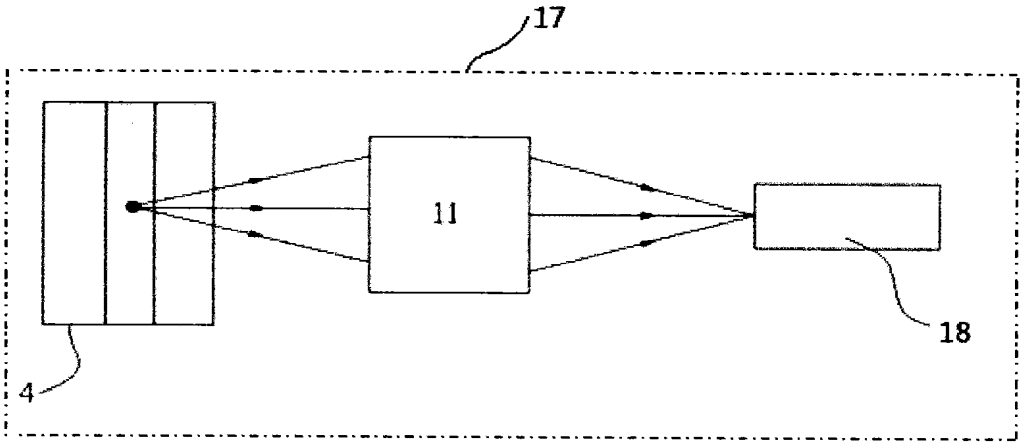


图 2

Espacenet

Bibliographic data: CN102818796 (A) — 2012-12-12

Biological fluorescence microscopic detection instrument

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Abstract of CN102818796 (A)

The invention provides a biological fluorescence microscopic detection instrument which comprises an excitation light illumination unit, excitation light, a fluorescence isolation unit, a reflecting unit, a microimaging unit, a fluorescence detection unit, a precision displacement platform and a control unit. An illumination pinhole is arranged between a first beam expander and a second beam expander of the excitation light illumination unit and located at a focal point of the first beam expander, an imaging detection pinhole is arranged between an imaging lens and a photomultiplier of the fluorescence detection unit and located at a focal point of the imaging lens, and therefore, the transverse resolution of the instrument is effectively improved; meanwhile, an annular beam shaping assembly is arranged between a tube lens and a microobjective of the microimaging unit, which enables the axial resolution of the biological fluorescence microscopic detection instrument to be improved.

(19)中华人民共和国国家知识产权局

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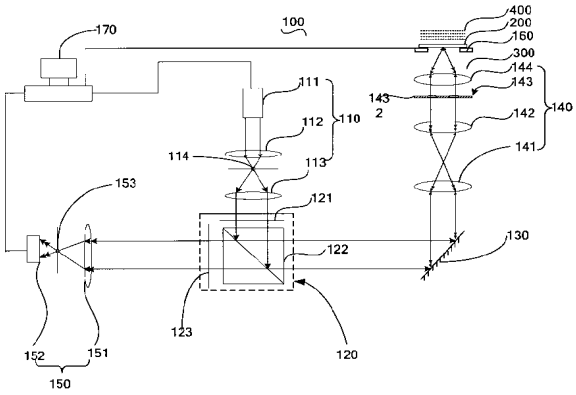
权利要求书 1 页 说明书 4 页 附图 2 页

(54)发明名称

生物荧光显微检测仪器

(57)摘要

本发明提供的生物荧光显微检测仪器包括激发光照明单元、激发光和荧光隔离单元、反射单元、显微成像单元、荧光探测单元、精密位移台及控制单元。激发光照明单元的第一扩束镜及第二扩束镜之间且位于第一扩束镜的焦点处设有照明针孔，荧光探测单元的成像镜头和光电倍增管之间且位于成像镜头的焦点处设有成像探测针孔，有效提高了该仪器的横向分辨率；同时，在显微成像单元的筒镜及显微物镜之间设置环形光束整形组件，提高了该生物荧光显微检测仪器的轴向分辨率。



CN 102818796 A

1. 一种生物荧光显微检测仪器，其特征在于，包括激发光照明单元、激发光和荧光隔离单元、反射单元、显微成像单元、荧光探测单元、精密位移台及控制单元；

所述激发光照明单元包括激光光源、第一扩束镜、第二扩束镜；所述第一扩束镜及第二扩束镜之间设有照明针孔，且所述照明针孔位于所述第一扩束镜的焦点处；所述激发光和荧光隔离单元包括激发光滤色片、二色镜、荧光滤色片；所述反射单元为平面反射镜；所述显微成像单元包括中继透镜、筒镜、环形光束整形组件、显微物镜，所述环形光束整形组件包括设于所述筒镜及显微物镜之间的环形滤光片，所述环形滤光片具有将激光截止的内环区域和透射激光的环带区域，该内环区域和环带区域均可透射荧光；所述内环区域的半径不小于刚好能发生全内反射时所述显微物镜入瞳位置的临界半径；所述荧光探测单元包括成像镜头、光电倍增管，该成像镜头与所述光电倍增管之间设有成像探测针孔，所述成像探测针孔位于所述成像镜头的焦点处；待观察对象与所述照明针孔和成像探测针孔处于共轭位置上；所述光电倍增管可探测荧光，并将所述荧光转换成电信号；

所述第一扩束镜、照明针孔、第二扩束镜、激发光滤色片、二色镜、反射单元、中继透镜、筒镜、环形滤光片及显微物镜依次沿始于所述激光光源的光轴线设置，且所述显微物镜、环形滤光片、筒镜、中继透镜、反射单元、二色镜、荧光滤色片、成像镜头、成像探测针孔及光电倍增管依次沿始于由待观察对象激发的荧光光轴线设置；

所述精密位移台为压电陶瓷移动台，用于承载待观察对象，且在 X、Y 方向可精密移动；

所述控制单元与所述光电倍增管和精密位移台均电性连接，用于同步采集所述电信号及精密位移台 X、Y 方向的位置坐标并进行关联生成待观察物质区域图像。

2. 根据权利要求 1 所述的生物荧光显微检测仪器，其特征在于，所述环带区域的宽度可调节。

3. 根据权利要求 1 所述的生物荧光显微检测仪器，其特征在于，所述显微物镜为无穷远消像差型大数值孔径的浸油物镜。

4. 根据权利要求 1 所述的生物荧光显微检测仪器，其特征在于，所述控制单元还与所述激光光源电性连接，用于控制激光的波长和功率。

生物荧光显微检测仪器

技术领域

[0001] 本发明涉及显微检测仪器设计及制造领域，尤其是涉及一种生物荧光显微检测仪器。

背景技术

[0002] 全内反射显微成像技术和共聚焦显微成像技术各有优缺点：全内反射显微成像具有很高的轴向分辨率，但其横向分辨率较低；共聚焦显微成像具有很高的横向分辨率，但其轴向分辨率较差。如何在一台显微镜中同时利用全内反射显微成像高的轴向分辨率和共聚焦显微成像高的横向分辨率尤为重要。

[0003] 发明专利申请 CN201080024155.9 中提出了将全内反射荧光图像和共焦图像简单且正确地重合的图像处理装置、程序和显微镜，该发明中包含了两套显微镜，一套是全内反射显微镜，另一套是共聚焦显微镜，但两套显微镜是分开工作的，通过光路切换的方法先获得一套显微镜的图像，再获得另一套显微镜的图像，最后计算机再利用两套图像中的基准点将两套图像进行了重合。该方法虽然生成了全内反射荧光图像和共焦图像的重合图像，由于两种显微镜是先后工作的，在物理上并不能同时得到三维高分辨率图像；另外该发明要先后操作两个显微镜拍摄图像，并要对图像进行重合，显微镜结构比较复杂，控制系统的构成也比较复杂。

发明内容

[0004] 本发明的目的是：提供一种集成全内反射显微成像技术和共聚焦显微成像技术的生物荧光显微检测仪器，该生物荧光显微检测仪器在横向及轴向上具有很高的分辨率。

[0005] 本发明的技术方案是：生物荧光显微检测仪器包括激发光照明单元、激发光和荧光隔离单元、反射单元、显微成像单元、荧光探测单元、精密位移台及控制单元；

[0006] 所述激发光照明单元包括激光光源、第一扩束镜、第二扩束镜；所述第一扩束镜及第二扩束镜之间设有照明针孔，且所述照明针孔位于所述第一扩束镜的焦点处；所述激发光和荧光隔离单元包括激发光滤色片、二色镜、荧光滤色片；所述反射单元为平面反射镜；所述显微成像单元包括中继透镜、筒镜、环形光束整形组件、显微物镜，所述环形光束整形组件包括设于所述筒镜及显微物镜之间的环形滤光片，所述环形滤光片具有将激光截止的内环区域和透射激光的环带区域，且所述内环区域和环带区域均可透射荧光；所述内环区域的半径不小于刚好能发生全内反射时所述显微物镜入瞳位置的临界半径；所述荧光探测单元包括成像镜头、光电倍增管，该成像镜头与所述光电倍增管之间设有成像探测针孔，所述成像探测针孔位于所述成像镜头的焦点处；待观察对象与所述照明针孔和成像探测针孔处于共轭位置上；所述光电倍增管可探测荧光，并将所述荧光转换成电信号；所述精密位移台为压电陶瓷移动台，用于承载待观察对象，且在 X、Y 方向可精密移动；

[0007] 所述第一扩束镜、照明针孔、第二扩束镜、激发光滤色片、二色镜、反射单元、中继透镜、筒镜、环形滤光片及显微物镜依次沿始于所述激光光源的光轴线设置，且所述显微物

镜、环形滤光片、筒镜、中继透镜、反射单元、二色镜、荧光滤色片、成像镜头、成像探测针孔及光电倍增管依次沿始于由待观察对象激发的荧光光轴线设置；

[0008] 所述控制单元与所述光电倍增管和精密位移台均电性连接，用于同步采集所述电信号与精密位移台 X、Y 方向的位置坐标并进行关联生成待观察物质区域图像。

[0009] 下面对上述技术方案进一步解释：

[0010] 所述环带区域的宽度可调节。

[0011] 所述显微物镜为无穷远消像差型大数值孔径的浸油物镜。

[0012] 所述控制单元还与所述激光光源电性连接，用于控制激光的波长和功率。

[0013] 本发明的优点是：

[0014] 1. 本发明提供的生物荧光显微检测仪器在第一扩束镜及第二扩束镜之间且位于第一扩束镜的焦点处设有照明针孔，在成像镜头和光电倍增管之间且位于成像镜头的焦点处设有成像探测针孔，有效提高了该仪器的横向分辨率；同时，在筒镜和显微物镜之间设置环形光束整形组件，提高了该生物荧光显微检测仪器在轴向的分辨率。

[0015] 2. 本发明提供的生物荧光显微检测仪器由于入射激光光束经显微成像单元聚焦在区域面积约为艾利斑大小的点状区域内，即只有一个面积很小厚度很薄区域内的荧光物质能够被激发，而其它区域内的荧光物质不会被激发，即从源头上消除了杂散光的来源，因而具有很高的成像信噪比。

附图说明

[0016] 图 1 为本发明实施例提供的生物荧光显微检测仪器结构示意图。

[0017] 图 2 为本发明实施例提供的环形滤光片的结构示意图。

[0018] 图 3 为本发明实施例提供的环形光束通过显微物镜的光路传播示意图。

[0019] 其中：激发光照明单元 110、激光光源 111、第一扩束镜 112、第二扩束镜 113、照明针孔 114、激发光和荧光隔离单元 120、激发光滤色片 121、二色镜 122、荧光滤色片 123、反射单元 130、显微成像单元 140、中继透镜 141、筒镜 142、环形光束整形组件 143、显微物镜 144、环形滤光片 1432、荧光探测单元 150、成像镜头 151、光电倍增管 152、成像探测针孔 153、精密位移台 160 及控制单元 170。

具体实施方式

[0020] 请参考图 1 至图 3。图 1 中标有单向箭头的光路为激光传播光路；标有双向箭头的光路表示为荧光传播光路。

[0021] 实施例：生物荧光显微检测仪器 100 包括激发光照明单元 110、激发光和荧光隔离单元 120、反射单元 130、显微成像单元 140、荧光探测单元 150、精密位移台 160 及控制单元 170。

[0022] 激发光照明单元 110 包括激光光源 111、第一扩束镜 112、第二扩束镜 113。在第一扩束镜 112 和第二扩束镜 113 之间且位于第一扩束镜 112 的焦点处设有照明针孔 114。

[0023] 激发光和荧光隔离单元 120 包括激发光滤色片 121、二色镜 122、荧光滤色片 123。激发光滤色 121 片用于接收激光，滤除激光中偏离中心波长的光束，并透射激光中中心波长处的光束。二色镜 122 接收并反射经激发光滤色片 121 透射的激光，并透射荧光。荧光

滤色片 123 接收并透射经二色镜 122 透射的荧光，并截止激光。

[0024] 反射单元 130 为平面反射镜，用于反射激光和荧光。

[0025] 显微成像单元 140 包括中继透镜 141、筒镜 142、环形光束整形组件 143、显微物镜 144。环形光束整形组件 143 包括环形滤光片 1432。该环形滤光片 1432 设置于筒镜 142 及显微物镜 144 之间，具有将激光截止的内环区域 A 和透射激光的环带区域 B。内环区域 A 和环带区域 B 均可透射荧光。

[0026] 荧光探测单元 150 包括成像镜头 151、光电倍增管 152。在成像镜头 151 与光电倍增管 152 之间且位于成像镜头 151 的焦点处设有成像探测针孔 153。待观察对象与照明针孔 114 和成像探测针孔 153 处于共轭位置上。光电倍增管 152 可探测荧光，并将荧光转换成电信号。

[0027] 其中，第一扩束镜 112、照明针孔 114、第二扩束镜 113、激发光滤色片 121、二色镜 122、反射单元 130、中继透镜 141、筒镜 142、环形滤光片 1432、显微物镜 144 依次沿始于激光光源 111 的光轴线设置；显微物镜 144、环形滤光片 1432、筒镜 142、中继透镜 141、反射单元 130、二色镜 122、荧光滤色片 123、成像镜头 151、成像探测针孔 153、光电倍增管 152 依次沿始于由待观察对象激发的荧光光轴线设置。

[0028] 激光光源 111 发射的准直激光依次经第一扩束镜 112、照明针孔 114、第二扩束镜 113 扩束后形成平行的激光束；该平行激光束依次经激发光滤色片 121、二色镜 122、反射单元 130、中继透镜 141、筒镜 142 后经环形光束整形组件 143 整形为环形光束，该环形光束经显微物镜 144 聚集于待观察对象处，并激发待观察对象产生荧光；该荧光光束依次经显微物镜 144、环形滤光片 1432、筒镜 142、中继透镜 141、反射单元 130、二色镜 122、荧光滤色片 123、成像镜头 151 后聚焦于成像探测针孔 153 处，光电倍增管 152 探测该荧光束并将其转换成电信号。

[0029] 精密位移台 160 为压电陶瓷移动台，用于承载待观察对象，且在 X、Y 方向可精密移动。

[0030] 控制单元 170 与光电倍增管 152、精密位移台 160 均电性连接，控制单元 170 将光电倍增管 152 输出的微弱电信号进行放大，并对放大后的电信号进行实时采样，同时控制精密位移台 160 在 X，Y 方向的移动，使得待观察对象的不同区域能够进入到光路中。控制单元 170 将采集到电信号和精密位移台 160 在 X，Y 方向的位置坐标关联起来，生成了一个区域中荧光物质的图像。同时，控制单元 170 还可以控制精密位移台 160 在 Z 方向的移动，这样使得显微物镜 143 能够快速聚焦到待观察对象上，此外控制单元 170 还电性连接于激光光源 111，用于控制入射激光光束的波长和功率。

[0031] 在该生物荧光显微检测仪器 100 中，显微物镜 144 为无穷远消像差型大数值孔径的浸油物镜，由于环形光束的内环半径（图 2 中 A 区域的半径）不小于显微物镜 144 刚好能发生全内反射时入瞳位置处光束临界半径，且盖玻片 200 和油 300 的折射率大致相同，这样进入显微物镜 144 的环形光束在盖玻片 200 和待观察对象所在的组织溶液 400 的交界处发生全反射，不能透过盖玻片 200 进行传播，但会在盖玻片 200 和组织溶液 400 的交界处形成隐失场（图 3 中 C 区），隐失波能够激发界面附近的荧光分子，产生荧光。调整环形滤光片 1432 环带区域 B 的宽度可以改变穿出盖玻片 200 的隐失场的分布深度，从而可以改变荧光物质在 Z 轴方向的激发深度，可以实现 Z 轴方向不同分辨率的调节。隐失波的频率与入射

光频率相同，其强度（单位面积和单位时间的能量）随离开界面的垂直距离呈指数衰减：

[0032] $I(z) = I(0)e^{-z/d}$

[0033] 可以看出，透射电磁场的振幅随进入样品的深度 z 减小得非常快，这种电磁场只存在于界面附近一薄层内。d 是理论渗透深度，等于从界面处到隐失波强度衰减到界面处数值 1/e 的距离，d 可表示为：

[0034] $d = (\lambda_0/4\pi)(n_1^2\sin^2\theta - n_2^2)^{-1/2}$

[0035] d 与入射角（θ）、波长 λ_0 以及组织溶液 400 折射率（n_2）和盖玻片 200 的折射率（n_1）有关。d 随入射角增大而减小，大小与入射光波长为同一数量级或更小。由于隐失场的独特特性，使荧光激发的区域非常靠近分界面（约 100nm）。这样不会激发距分界面更远区域的荧光，从而可实现背景噪声极小的荧光成像，使得生物荧光显微检测仪器 100 在轴向具有很高的分辨率。

[0036] 在该生物荧光显微检测仪器 100 中，通过照明针孔 114 及成像探测针孔 153 的联合使用，实现点对点的照明和点对点的成像。当不考虑噪声的情况下，光学上常用点扩散函数描述系统成像分辨率，对于激光扫描共焦系统来说，系统最终的点扩散函数由下式描述：

[0037] $PSF_{tot}(x,y,z) = PSF_{ill}(x,y,z) \cdot PSF_{det}(x,y,z)$

[0038] 其中 PSF_{ill} 对应照明激光点在物方的点扩散函数，PSF_{det} 对应成像探测光路的点扩散函数。由于照明针孔 114 的作用，入射激光光束通过各单元后在盖玻片 200 与待观察对象交界处形成一个很小的点状照明区域（照明方点扩散函数），成像探测针孔 153 的使用对成像探测方点扩散函数进一步整形，使得整个生物荧光显微检测仪器 100 的成像点扩散函数由照明方点扩散函数和探测方点扩散函数的乘积组成，由于乘积后的成像点扩散函数强度分布范围变窄，因而系统具有很高的横向分辨率。

[0039] 在该生物荧光显微检测仪器 100 中由于入射激光光束经显微成像单元 140 聚焦在区域面积约为艾利斑大小的点状区域内，即只有一个面积很小厚度很薄区域内的荧光物质能够被激发，而其它区域内的荧光物质不会被激发，即从源头上消除了杂散光的来源，因而系统具有很高的成像信噪比。

[0040] 当然本发明的生物荧光显微检测仪器还可具有多种变换及改型，并不局限于上述实施方式的具体结构。总之，本发明的保护范围应包括那些对于本领域普通技术人员来说显而易见的变换或替代以及改型。

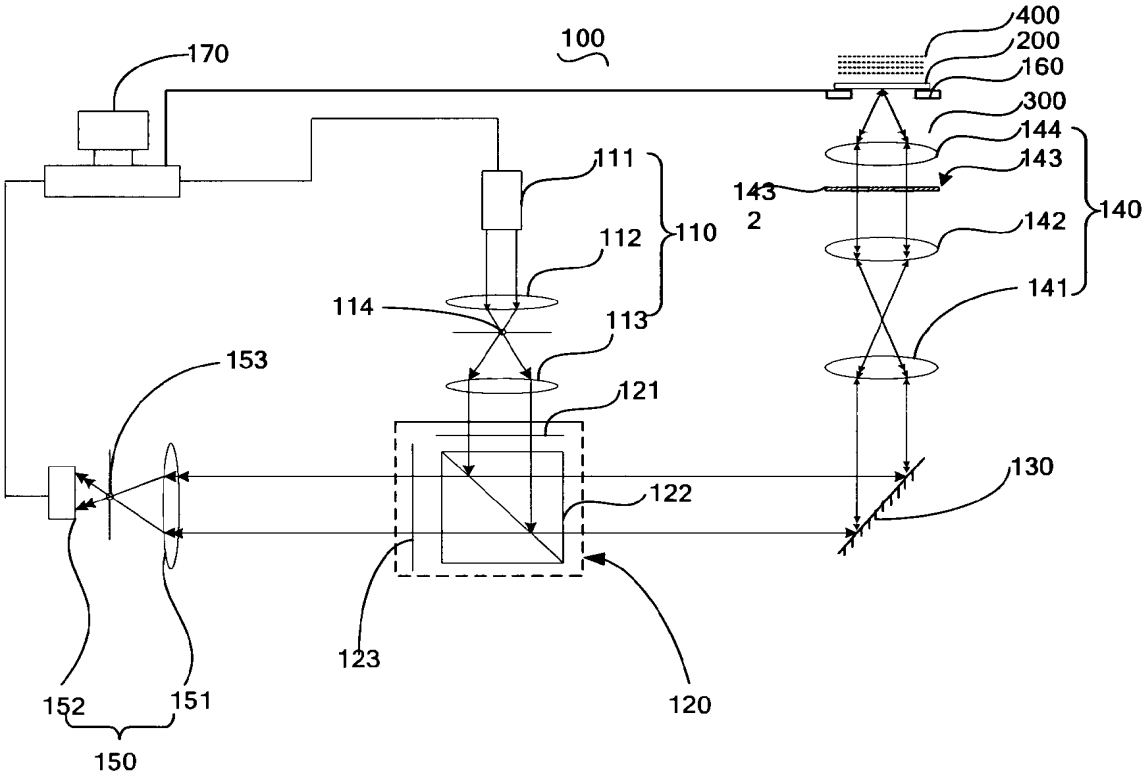

400
200
160
300
144
143
143
2
140
142
141
100
170
111
112
110
114
113
153
121
130
122
123
120
152
151
150

图 1

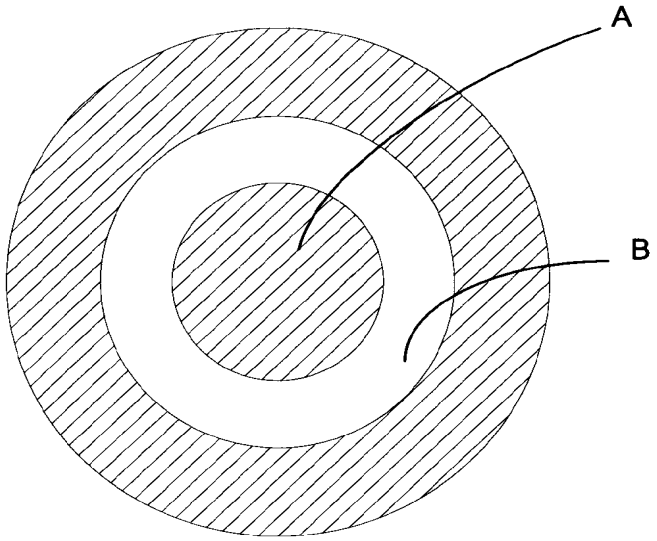

A
B

图 2

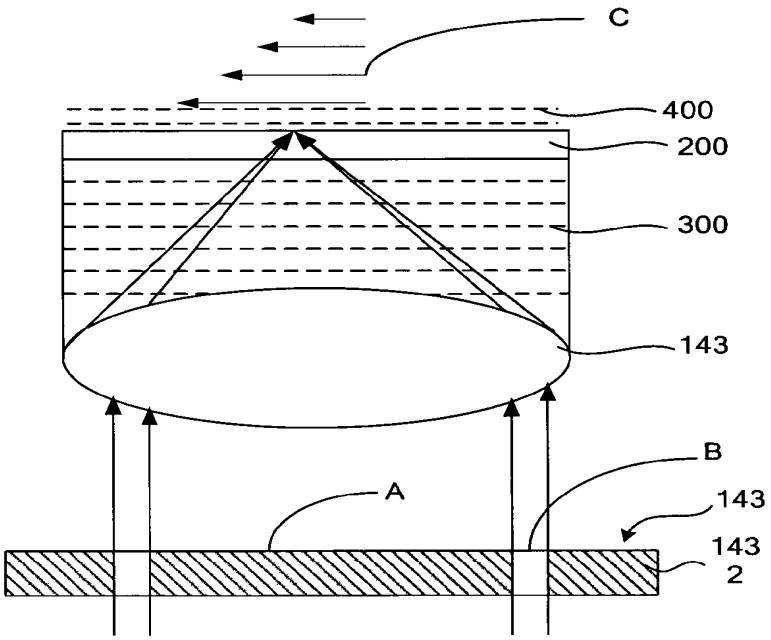


图 3

Espacenet

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Microscope for maintaining illuminance of viewing field consistent

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Classification: **- international:** ***G02B21/06; G02B21/36***
- cooperative:

Application number: CN20102650829U 20101209

Priority number(s): CN20102650829U 20101209

Abstract of CN201936066 (U)

The utility model relates to a microscope for maintaining illuminance of a viewing field consistent, which comprises an illumination part, a mechanism part and an optical part, wherein a viewing field optical signal pickup assembly is additionally arranged in the optical part, and a signal control processing system is also additionally arranged in the optical part; an optical signal acquired from the optical signal pickup assembly is converted into an electric signal for processing, and then, the electric signal is fed back to an illumination light source or an excitation light source or an illumination diaphragm to achieve the goal that the illuminance of the viewing field is kept consistent with an objective lens before being switched over to ensure that sample image results have comparability. The microscope disclosed by the utility model is capable of realizing the aim that the illuminance of the viewing field is kept to be a set value or the illuminance before the objective lens is switched over so as to facilitate people or an automatic imaging drawing system to receive and detect microscopic images through human eyes or photosensitive devices such as a charge coupled device (CCD), a complementary metal-oxide-semiconductor transistor (CMOS) and the like, so that the best observation effect is obtained and observation results have the comparability.

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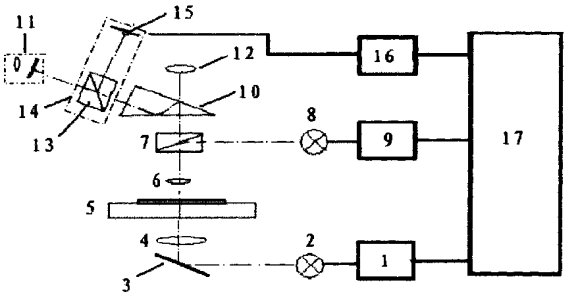
权利要求书 1 页 说明书 3 页 附图 2 页

(54)实用新型名称

一种保持视场光照度一致的显微镜

(57)摘要

本实用新型涉及一种保持视场光照度一致的显微镜，包括照明部分、机械部分和光学部分，其中，在光学部分中加入视场光信号采集装置，还加入信号控制处理系统，将从光信号采集装置采集到的光信号转变为电信号进行处理，然后将其反馈至照明光源、激发光源或照明光阑，达到保持视场光照度与物镜切换前一致，保证样本图像结果具备可比性的目的。本实用新型所述的显微镜可实现保持视场光光照度为设定值或物镜切换前光照度，方便人或自动成像绘图系统通过人眼或CCD、CMOS 等光敏器件接收、探测显微图像获得最佳观察效果和使观测结果具备可比性。



CN 201936066 U

1. 一种保持视场光照度一致的显微镜，

包括由照明光源 (2)、反射镜 (3)、照明光阑 (4) 和激发光源 (8) 构成的照明部分，包含有载物台 (5) 的机械部分，由物镜 (6)、荧光落射激发模块 (7)、分光棱镜 (10)、目镜 (11) 和图像传感器 (12) 构成的光学部分，

其特征在于，还包括信号控制处理系统 (17)，其中，信号控制处理系统 (17) 的 3 个端口分别连接有光信号采集模块 (16)、照明光源控制模块 (1) 和激发光源控制模块 (9)，所述光信号采集模块 (16) 接收从光学部分的光信号采集装置 (14) 中采集到的光信号，所述照明光源控制模块 (1) 与照明光源 (2) 连接，所述激发光源控制模块 (9) 与激发光源 (8) 连接。

2. 根据权利要求 1 所述的保持视场光照度一致的显微镜，其特征在于，所述光信号采集装置 (14) 包括光路分光模块 (13) 和光电探测敏感器件 (15)。

3. 根据权利要求 1 所述的保持视场光照度一致的显微镜，其特征在于，所述光路分光模块 (13) 置于光学部分的分光棱镜 (10) 和目镜 (11) 的光路之间。

4. 根据权利要求 1 所述的保持视场光照度一致的显微镜，其特征在于，所述光路分光模块 (13) 还可置于光学部分的分光棱镜 (10) 和图像传感器 (12) 的光路之间。

5. 根据权利要求 1 所述的保持视场光照度一致的显微镜，其特征在于，所述图像传感器 (12) 为 CDD 图像传感器或 CMOS 图像传感器。

6. 根据权利要求 1 所述的保持视场光照度一致的显微镜，其特征在于，所述光路分光模块 (13) 为分光棱镜。

7. 根据权利要求 1 所述的保持视场光照度一致的显微镜，其特征在于，所述光路分光模块 (13) 为比例透过反射镜。

8. 根据权利要求 6 或 7 所述的保持视场光照度一致的显微镜，其特征在于，所述光路分光模块 (13) 的迎光面上镀有比例反射分光光学薄膜。

9. 根据权利要求 8 所述的保持视场光照度一致的显微镜，其特征在于，所述光电探测敏感器件 (15) 为光电二极管、光电池或光敏电阻。

10. 根据权利要求 1 所述的保持视场光照度一致的显微镜，其特征在于，所述照明光阑 (4) 为孔径光阑，能够根据物面照度的不同改变光源的通光孔径。

一种保持视场光照度一致的显微镜

技术领域

[0001] 本实用新型涉及光学仪器领域，具体地涉及了一种保持视场光照度一致的显微镜。

背景技术

[0002] 现有的显微镜系统，因观察对象的需求或用途不同而需经常改变物镜倍率。当改变物镜倍率时，由于物镜数值孔径的不同而导致目视系统像面的光照度不同，从而导致视觉或成像传感器视场发生明暗变化，不利于所观察现象的一致性、可重复性和可比性。

[0003] 现有技术中，需要手动调节保持目视视场的光照度（或亮度），以便观察到更最佳的成像。操作繁琐、费时，效率不高。

[0004] 基于以上原因，实用新型一种保持视场光照度一致的显微镜，由电子学系统自动检测目视场的光照度及自动调节，以保持设定的光照度，是本技术领域内丞待解决的问题。

实用新型内容

[0005] 为克服现有技术中的不足，本实用新型的目的在于提出一种保持视场光照度一致的显微镜，该实用新型解决了现有技术中手动调节目视场光照度的操作繁琐、效率不高的问题。

[0006] 为解决上述技术问题，达到上述技术目的，本实用新型采用如下技术方案：

[0007] 一种保持视场光照度一致的显微镜，

[0008] 包括由照明光源、反射镜、照明光阑和激发光源构成的照明部分，包含有载物台的机械部分，由物镜、荧光落射激发模块、分光棱镜、目镜和图像传感器构成的光学部分，

[0009] 其中，还包括信号控制处理系统，其中，信号控制处理系统的 3 个端口分别连接有光信号采集模块、照明光源控制模块和激发光源控制模块，所述光信号采集模块接收从光学部分的光信号采集装置中采集到的光信号，所述照明光源控制模块与照明光源连接，所述激发光源控制模块与激发光源连接。

[0010] 优选的，所述光信号采集装置包括光路分光模块和光电探测敏感器件。

[0011] 优选的，所光路分光模块置于光学部分的分光棱镜和目镜的光路之间。

[0012] 优选的，所光路分光模块还可置于置于光学部分的分光棱镜和图像传感器光路之间，所述图像传感器为 CDD 图像传感器或 CMOS 图像传感器。

[0013] 优选的，所述光路分光模块为分光棱镜或比例透过反射镜，且迎光面上镀有比例反射分光光学薄膜。

[0014] 优选的，所述光电探测敏感器件为光电二极管、光电池或光敏电阻。

[0015] 优选的，所述照明光阑为孔径光阑，能够根据物面照度的不同改变光源的通光孔径。

[0016] 有益效果：本实用新型的保持视场光照度一致的显微镜，是在探测观察装置部分视场面上设置有光照度探测器（如光电二极管、光电池、光敏电阻等探测器），并由光电信

号处理系统测量光电压、光电流值，将其反馈至照明光源、激发光源或照明光阑，从而保持视场与物镜切换前一致或使视场照度为设定值。从而实现保持视场光照度为设定值或物镜切换前光照度，方便人或自动成像绘图系统通过人眼或 CCD、CMOS 图像传感器等接收、探测显微图像获得最佳观察效果。

附图说明

[0017] 下面结合附图和具体实施方式对本实用新型作进一步详细的说明。

[0018] 图 1 是本实用新型的保持视场光照度一致的显微镜的结构示意图。

[0019] 图 2 是本实用新型的保持视场光照度一致的显微镜的控制流程图。

[0020] 图中标号说明：1. 照明光源控制模块，2. 照明光源，3. 反射镜，4. 照明光阑，5. 载物台，6. 物镜，7. 荧光落射激发模块，8. 激发光源，9. 激光光源控制模块，10. 分光棱镜，11. 目镜，12. 图像传感器，13. 光路分光模块，14. 光信号采集装置，15. 光电探测敏感器件，16. 光信号采集模块，17. 信号控制处理系统。

具体实施方式

[0021] 下面结合附图，对本实用新型的优选实施例进行详细的介绍。

[0022] 如图 1，一种保持视场光照度一致的显微镜，

[0023] 包括由照明光源 2、反射镜 3、照明光阑 4 和激发光源 9 构成的照明部分，包含有载物台 5 的机械部分，由物镜 6、荧光落射激发模块 7、分光棱镜 10、目镜 11 和图像传感器 12 构成的光学部分，

[0024] 其中，还包括信号控制处理系统 17，其中，信号控制处理系统 17 的 3 个端口分别连接有光信号采集模块 16、照明光源控制模块 1 和激发光源控制模块 9，光信号采集模块 16 接收从光学部分的光电探测敏感器件 15 中采集到的光信号，照明光源控制模块 1 与照明光源连接 2，激发光源控制模块 9 与激发光源 8 连接。

[0025] 优选的，所述光信号采集装置 14 包括光路分光模块 13 和光电探测敏感器件 15。

[0026] 优选的，所光路分光模块 13 置于光学部分的分光棱镜 10 和目镜 11 的光路之间。

[0027] 优选的，所光路分光模块 13 还可置于光学部分的第二分光棱镜 10 和图像传感器 12 的光路之间，所述图像传感器 12 为 CCD 或 CMOS 图像传感器。

[0028] 所述光路分光模块 13 用于将视场光照度按照比例分光至探测通道，传递给光电探测敏感器件，其中，光电探测敏感器件 15 将光信号传递给光信号采集模块 16，转变为电压或电流信号，再通过信号控制处理系统 17，对输入信号进行读取、存储、预设定值的比较及反馈，反馈给照明光源控制模块 1 和激发光源控制模块 9，改变光源亮度，或通过照明光阑 4 对通光孔径进行调节，改变物面照度。达到根据视场光照度的不同进行调节，保持显微镜视场的一致性和稳定性的目的。

[0029] 上述光电探测敏感器件特征为能够灵敏探测其光敏面上的光照度，如光电二极管、光电池、光敏电阻等。

[0030] 上述将视场光强度按比例分光的光路分光模块包含分光棱镜等比例分光元件并在迎光面上镀有比例反射分光光学薄膜。

[0031] 上述固定装置用于将光电探测敏感器件与按照比例分光至探测通道的光学元件

相对固定，并避免杂散光进入光学系统。

[0032] 上述照明光源 2 和激发光源 8 能根据视场光照度读取并反馈，以控制装置的输出（电流、电压等）信号改变光照度。所述照明光源 2 和激发光源 8 具备与显微镜配合的接口固定装置。

[0033] 结合图 2，具体的控制流程图，进行本实用新型的具体实施。

[0034] 本实用新型的保持视场光照度一致的显微镜，是在目镜装置部分视场面上设置有光照度（或亮度）探测器（如光电二极管、光电池、光敏电阻等探测器），并由光电信号处理系统测量光电压、光电流值，将其反馈至光源或照明光阑保持视场与物镜切换前一致或使视场照度为设定值。从而实现保持视场光照度（或亮度）为设定值或物镜切换前光照度（或亮度），方便人或自动成像绘图系统通过人眼或 CCD、COMS 等图像传感器接收、探测显微图像获得最佳观察效果。

[0035] 上述实施例只是为了说明本实用新型的技术构思及特点，其目的是在于让本领域内的普通技术人员能够了解本实用新型的内容并据以实施，并不能以此限制本实用新型的保护范围。凡是根据本实用新型内容的实质所作出的等效的变化或修饰，都应涵盖在本实用新型的保护范围内。

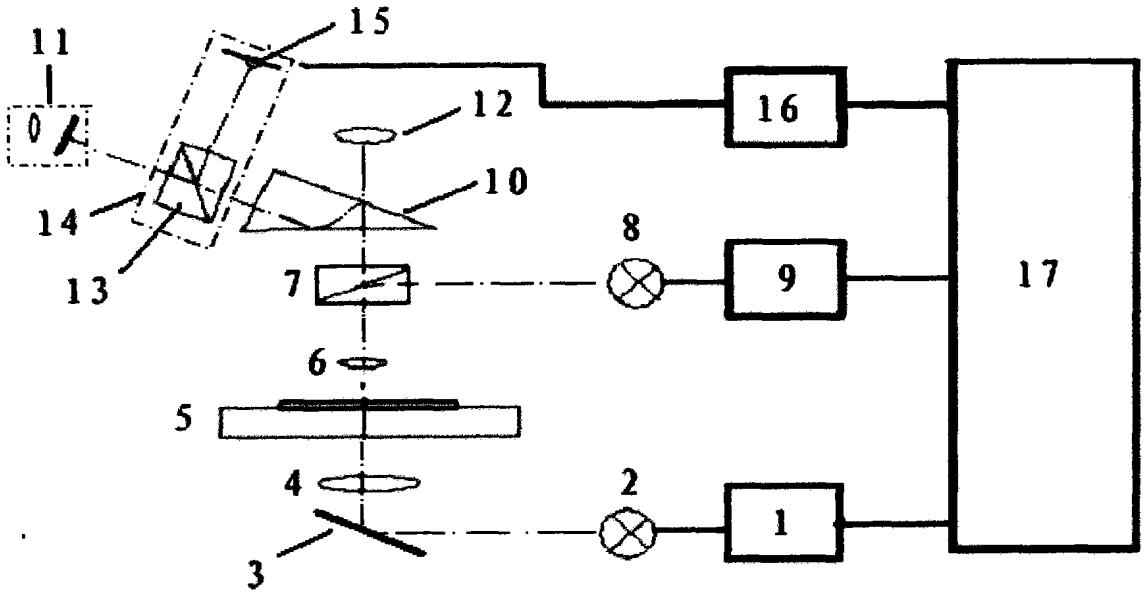


图 1

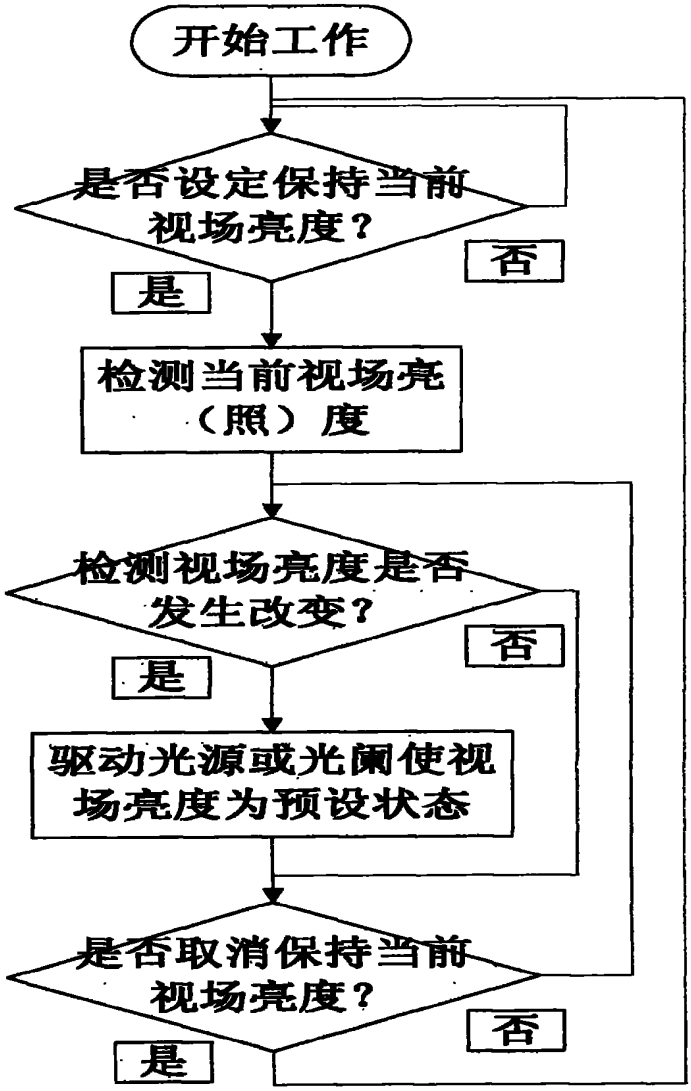

开始工作
是否设定保持当前视场亮度？
否
是
检测当前视场亮（照）度
检测视场亮度是否发生改变？
否
是
驱动光源或光阑使视场亮度为预设状态
是否取消保持当前视场亮度？
否
是

图 2

Espacenet

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Fluorescence microscope by utilizing touch screen system to control light source

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Priority number (s): CN20102650826U 20101209

Abstract of CN201984209 (U)

The utility model discloses a fluorescence microscope by utilizing a touch screen system to control light source, which comprises a main machine by utilizing an infinity multi-chromatic aberration optical system. The main machine is provided with a fluorescence objective glass utilizing a high resolution flat field infinite large numerical aperture, a trinocular eyepiece configured with a single lens reflex, a fluorescence filter group with an equal altitude sharpening character and with a transparence of 90 to 95%, an LED (light-emitting diode) white light source serving as back light of specimens and an LED fluorescence excitation light source used for emitting exciting light onto the specimens. The main machine is further assembled with a touch screen used for controlling the LED fluorescence excitation light source and luminance of the LED white light source. The touch screen is a regulating and controlling device of light source of the fluorescence microscope and the easiest, most convenient and most natural method of man-machine interaction at present.

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H05B 37/02(2006.01)

(ESM)同样的发明创造已同日申请发明专利

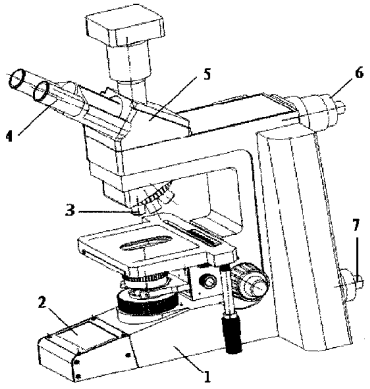
权利要求书 1 页 说明书 3 页 附图 2 页

(54)实用新型名称

一种使用触摸屏系统控制光源的荧光显微镜

(57)摘要

本实用新型公开了一种使用触摸屏系统控制光源的荧光显微镜，包括一采用了无限远多重色差校正光学系统的显微镜主机，所述显微镜主机上设置有一采用了高分辨率平场无限远大数值孔径的荧光物镜、一配置有单反数码相机的三目镜观察系统、一具有等高锐化特性，透过率为90％-95％的荧光滤色片组、一作为标本背景光来源的LED白光光源，以及一用于将激发光发射到标本上的LED荧光激发光源，所述显微镜主机还配置有一用于控制所述LED荧光激发光源和LED白光光源亮度的触摸屏。触摸屏作为本实用新型的荧光显微镜光源的调控设备，它是目前最简单、方便、自然的一种人机交互方式。



CN 201984209 U

1. 一种使用触摸屏系统控制光源的荧光显微镜，包括一采用了无限远多重色差校正光学系统的显微镜主机 (1)，所述显微镜主机 (1) 上设置有一采用了高分辨率平场无限远大数值孔径的荧光物镜 (3)、一配置有单反数码相机的三目镜观察系统 (4)、一具有等高锐化特性，透过率为 90％ -95％的荧光滤色片组 (5)、一作为标本背景光来源的 LED 白光光源 (7)，以及一用于将激发光发射到标本上的 LED 荧光激发光源 (6)，其特征在于：所述显微镜主机 (1) 还配置有一用于控制所述 LED 荧光激发光源 (6) 和 LED 白光光源 (7) 亮度的触摸屏 (2)。

2. 根据权利要求 1 所述的使用触摸屏系统控制光源的荧光显微镜，其特征在于：所述 LED 荧光激发光源 (6) 和 LED 白光光源 (7) 分别通过一 LED 光源控制模块 (8) 连接在所述触摸屏 (2) 上；所述 LED 光源控制模块 (8) 包括，一为 LED 荧光激发光源 (6) 和 LED 白光光源 (7) 提供可靠、恒定驱动电流的 LED 驱动电路模块 (801)、一用于检测用户触摸位置的触摸检测电路 (802)，一用于驱动触摸屏 (2) 的触摸屏驱动电路模块 (803)，一控制触摸屏 (2) 的触摸屏控制电路模块 (804)，以及一为所述 LED 驱动电路模块 (801)、触摸检测电路 (802) 和触摸屏驱动电路模块 (803) 提供稳定可靠电压电流的电源电路模块 (805)。

一种使用触摸屏系统控制光源的荧光显微镜

技术领域

[0001] 本实用新型涉及一种光学显微镜，具体涉及一种用于观察标本所发荧光的使用触摸屏系统控制光源的荧光显微镜。

背景技术

[0002] 观测其上的标本所发荧光的荧光显微镜是显微镜的一种类型。它是荧光检测的专用工具，属于高科技仪器。荧光显微镜是以紫外线为光源，用以照射被检物体，使之发出荧光，然后在显微镜下观察物体的形状及其所在位置。荧光显微镜用于研究细胞内物质的吸收、运输、化学物质的分布及定位等。细胞中有些物质，如叶绿素等，受紫外线照射后可发荧光；另有一些物质本身虽不能发荧光，但如果用荧光染料或荧光抗体染色后，经紫外线照射亦可发荧光，荧光显微镜就是对这类物质进行定性和定量研究的工具之一。荧光显微镜的基本结构包括把激发光照射到标本上的落射光照部分和观测由所述标本所发荧光行成的图像的荧光观测部分。来自落射光照部分的激发光通过滤光器照射在标本上。所述荧光观测部分包括目镜观测系统和 / 或诸如 CCD 之类的成像部分。

[0003] 荧光显微镜依照其独特的性能，应用广泛，是细菌学、病毒学、细胞学、肿瘤学、遗传学、免疫学、免疫病例学、寄生虫学等研究工作的理想仪器，可供科研、高效、医疗、防疫等部门使用，与社会发展息息相关。

[0004] 参见图 1 所示，一般的使用触摸屏系统控制光源的荧光显微镜的荧光激发原理如下：

[0005] 激发光源部分：提供激发荧光所需足够强的激发光（较短波长光线）。荧光滤色片组包括一激发滤光片和一遏止滤光片，其中所述激发滤光片：只通过相应标本需要的激发光，而不要的光线都被截止掉；所述遏止滤光片：遏止掉多余的激发光线，使通过标本中激发出来的荧光便于观察。荧光标本：被荧光色素染色过的标本。

[0006] 其中，荧光光源是荧光显微镜的关键零部件。它需要提供特定激发光波长范围及足够光效能量，以保证检测样品得到足够的激发而发出强的荧光。

[0007] 传统荧光光源也有使用卤素灯、氙灯等用来照明，但它们都有明显的缺点：寿命短，需经常更换灯泡；每次使用都需要等待预热一段时间；能耗大；含有铅、汞灯污染环境并对使用人员有害的物质。传统光源多采用机械式旋钮进行不同激发波长之间的切换，以及亮度的调节。但是机械式调节存在缺点：比如调节量不够细化、不精确，操作不够智能化，不方便等等。

实用新型内容

[0008] 为克服现有技术中的不足，本实用新型的目的在于提供一种使用触摸屏系统控制光源的荧光显微镜，该使用触摸屏系统控制光源的荧光显微镜可以有效避免现有技术中的缺点。

[0009] 为了解决上述技术问题，实现上述目的，本实用新型采用了如下技术方案：

[0010] 一种使用触摸屏系统控制光源的荧光显微镜，包括一采用了无限远多重色差校正光学系统的显微镜主机，所述显微镜主机上设置有一采用了高分辨率平场无限远大数值孔径的荧光物镜、一配置有单反数码相机的三目镜观察系统、一具有等高锐化特性，透过率为90％ -95％的荧光滤色片组、一作为标本背景光来源的LED白光光源，以及一用于将激发光发射到标本上的LED荧光激发光源，所述显微镜主机还配置有一用于控制所述LED荧光激发光源和LED白光光源亮度的触摸屏。

[0011] 进一步的，所述LED荧光激发光源和LED白光光源分别通过一LED光源控制模块连接在所述触摸屏上；所述LED光源控制模块包括，一为LED荧光激发光源和LED白光光源提供可靠、恒定驱动电流的LED驱动电路模块、一用于检测用户触摸位置的触摸检测电路，一用于驱动触摸屏的触摸屏驱动电路模块，一控制触摸屏的触摸屏控制电路模块，以及一为所述LED驱动电路模块、触摸检测电路和触摸屏驱动电路模块提供稳定可靠电压电流的电源电路模块。

[0012] 本实用新型的使用触摸屏系统控制光源的荧光显微镜的LED光源控制模块的工作过程如下：

[0013] 当电源电路模块通电后，LED驱动电路模块、触摸屏检测电路、触摸屏驱动电路模块、触摸屏控制电路模块获得电压。

[0014] 当触摸屏检测电路检测到用户触摸位置后送触摸屏控制电路，如触摸屏显示荧光激发光源亮度数据为0，LED荧光激发光源无输出，用触摸屏上的亮度调节模块对光源亮度进行调节，当LED驱动电路模块达到一定电压启动数值后，LED荧光激发光源输出；所述LED白光光源的工作模式如LED荧光激发光源的工作模式一样。触摸屏进行有效、实时的显示。

[0015] 当需要另外一种荧光光源时，可通过触摸屏检测电路进行控制，如当系统需要绿光时，可通过触摸屏检测电路切换到绿光工作状态，此时相应的LED荧光激发光源转换为绿色，相应的LED绿指示灯亮；同理，如当系统需要蓝光时，可通过触摸屏检测电路切换到蓝光工作状态，此时相应的LED荧光激发光源转换为蓝色，此时相应的LED蓝指示灯亮。在切换过程中，由于LED的响应及LED的驱动响应时间都是ns级，因此，这种延迟可以满足荧光光源的使用要求。

[0016] 与现有技术相比，本实用新型具有以下优点：

[0017] 触摸屏具有坚固耐用、反应速度快、节省空间、易于交流等很多优点。触摸屏作为本实用新型的荧光显微镜光源的调控设备，它是目前最简单、方便、自然的一种人机交互方式。

[0018] 上述说明仅是本实用新型技术方案的概述，为了能够更清楚了解本实用新型的技术手段，并可依照说明书的内容予以实施，以下以本实用新型的较佳实施例并配合附图详细说明如后。本实用新型的具体实施方式由以下实施例及其附图详细给出。

附图说明

[0019] 图1为荧光显微镜的荧光激发原理图。

[0020] 图2为本实用新型的使用触摸屏系统控制光源的荧光显微镜的结构示意图。

[0021] 图3为本实用新型的LED光源控制模块的框架示意图。

[0022] 图中标号说明：1、显微镜主机，2、触摸屏，3、荧光物镜，4、三目镜观察系统，5、荧光

滤色片组，6、LED 荧光激发光源，7、LED 白光光源，8、LED 光源控制模块，801、LED 驱动电路模块，802、触摸检测电路，803、触摸屏驱动电路模块，804、触摸屏控制电路模块，805、电源电路模块。

具体实施方式

[0023] 下面结合附图和实施例对本实用新型的技术实施过程做进一步说明。

[0024] 参见图 2 所示，一种使用触摸屏系统控制光源的荧光显微镜，包括一采用了无限远多重色差校正光学系统的显微镜主机 1，所述显微镜主机 1 上设置有一采用了高分辨率平场无限远大数值孔径的荧光物镜 3、一配置有单反数码相机的三目镜观察系统 4、一具有等高锐化特性，透过率为 90％ -95％的荧光滤色片组 5、一作为标本背景光来源的 LED 白光光源 7，以及一用于将激发光发射到标本上的 LED 荧光激发光源 6，所述显微镜主机 1 还配置有一用于控制所述 LED 荧光激发光源 6 和 LED 白光光源 7 亮度的触摸屏 2。

[0025] 进一步的，参见图 3 所示，所述 LED 荧光激发光源 6 和 LED 白光光源 7 分别通过一 LED 光源控制模块 8 连接在所述触摸屏 2 上；所述 LED 光源控制模块 8 包括，一为 LED 荧光激发光源 6 和 LED 白光光源 7 提供可靠、恒定驱动电流的 LED 驱动电路模块 801、一用于检测用户触摸位置的触摸检测电路 802，一用于驱动触摸屏 2 的触摸屏驱动电路模块 803，一控制触摸屏 2 的触摸屏控制电路模块 804，以及一为所述 LED 驱动电路模块 801、触摸检测电路 802 和触摸屏驱动电路模块 803 提供稳定可靠电压电流的电源电路模块 805。

[0026] 上述实施例只是为了说明本实用新型的技术构思及特点，其目的是在于让本领域内的普通技术人员能够了解本实用新型的内容并据以实施，并不能以此限制本实用新型的保护范围。凡是根据本实用新型内容的实质所作出的等效的变化或修饰，都应涵盖在本实用新型的保护范围内。

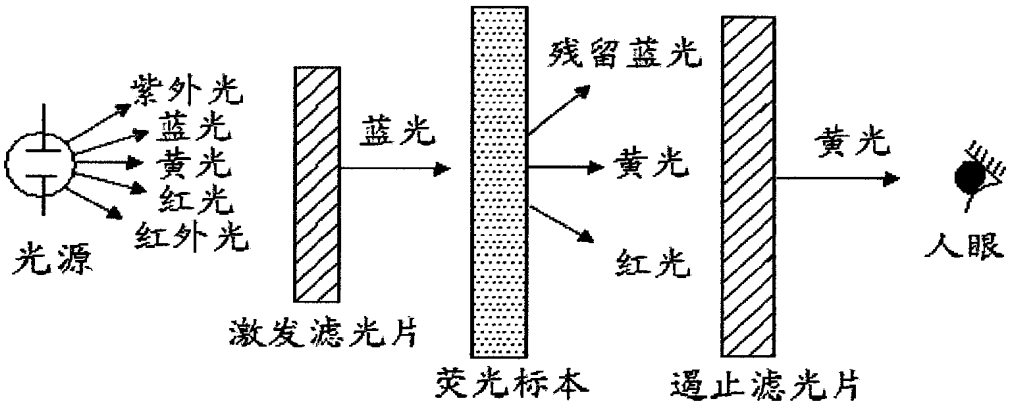


图 1

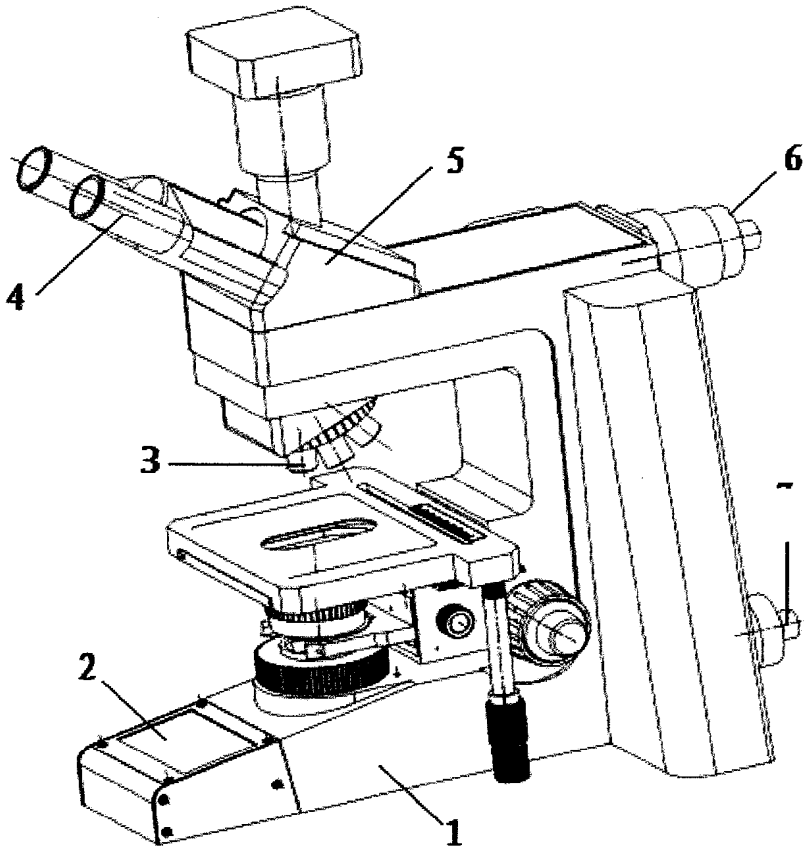


图 2

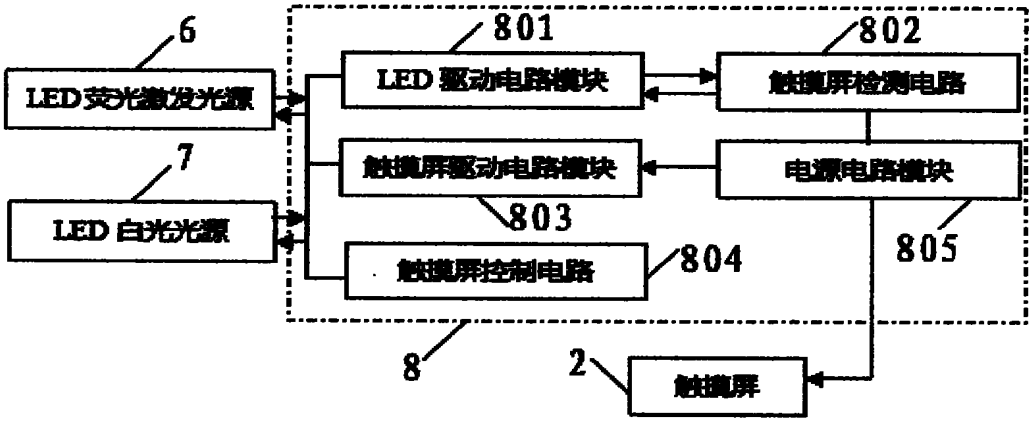

6
801
802
LED荧光激发光源
LED 驱动电路模块
触摸屏检测电路
7
触摸屏驱动电路模块
电源电路模块
LED 白光光源
803
805
触摸屏控制电路
804
8
2
触摸屏

图 3

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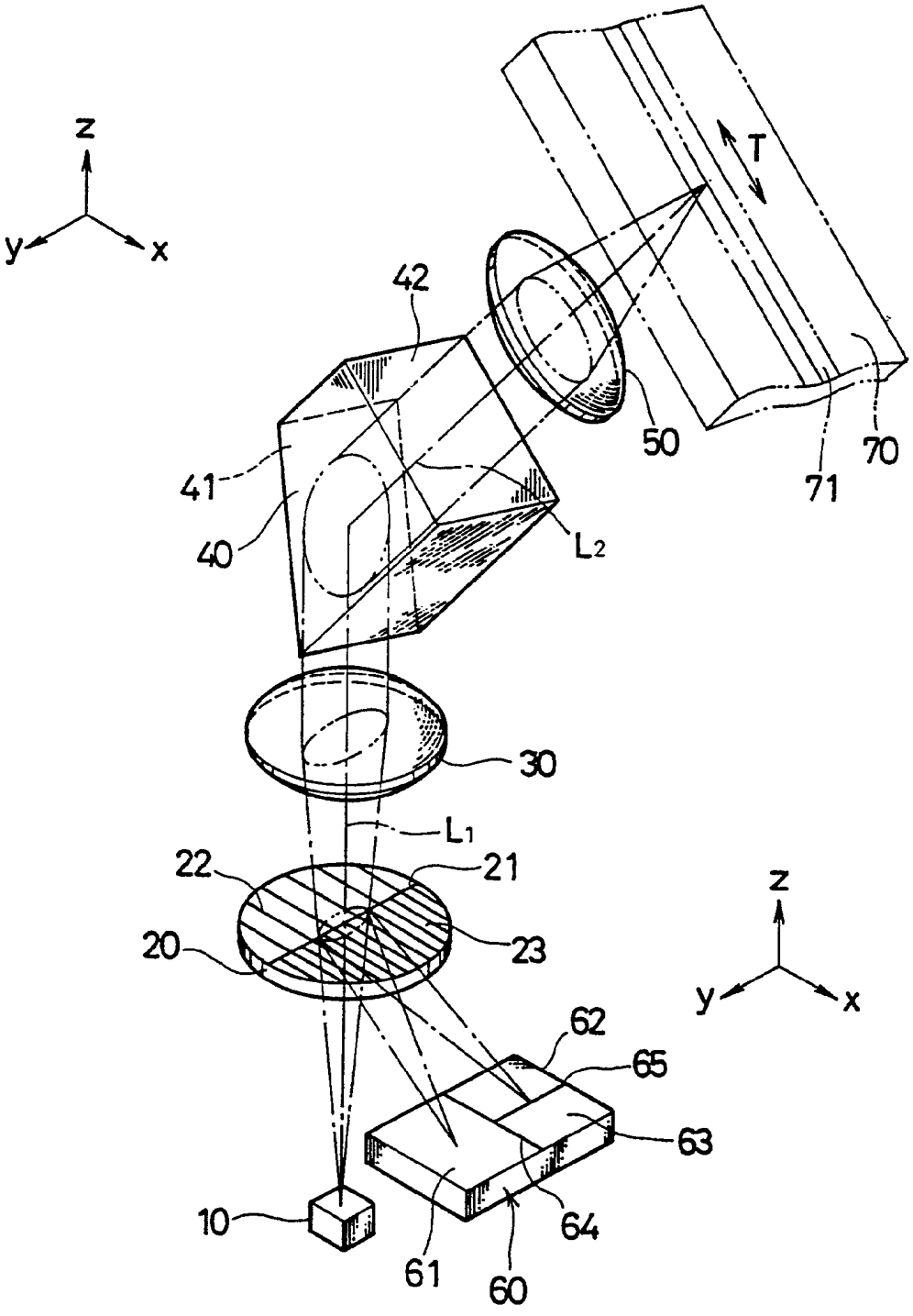
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(54) **Optical head device.**

(57) An optical head device having an optical system provided with at least a light source, a shaping prism, an diffracting element and a photodetector. The light source emits light whose intensity distribution has an elliptic shape in its cross section orthogonal to the optical axis. The shaping prism directs the light to a recording medium after shaping the intensity distribution thereof into a circular shape. The diffracting element has diffracting regions formed by at least one division line substantially parallel to a reference direction. The light reflected from the recording medium forms an elliptic bright portion on the diffracting element. The photodetector is divided by a plurality of division lines, at least one of which is parallel to the reference direction. When there is no focus error, the diffracting element diffracts the reflected light so as to converge it on the division line substantially parallel to the reference direction in the photodetector. For example, when the shaping prism is adapted to shape the elliptic intensity distribution with respect to its minor axis direction, the optical system is arranged so that the major axis of the bright portion is parallel to the division line of the diffracting element. Thus, it is possible to suppress offsets in focusing control due to wave-length variations of light.

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FIG. 1



FIELD OF THE INVENTION

The present invention relates to an optical head device to be provided in optical recording-reproduction apparatuses for recording/reproducing information on or from a recording medium such as an optical disk.

BACKGROUND OF THE INVENTION

Optical head devices are well known to the art, which record and reproduce information by applying a light beam to a recording medium such as an optical disk. In a commonly used optical head device, a light beam emitted from a semiconductor laser as a light source passes through a diffracting element, and forms a parallel beam of light through a collimating lens. An intensity distribution of the parallel beam of light shows an elliptic shape in its cross section orthogonal to the optical axis, according to the intensity distribution of the light beam emitted from the semiconductor laser. Here, such an intensity distribution of light related to a cross section orthogonal to the optical axis, is hereinafter referred to simply as a cross-sectional intensity distribution. The elliptic cross-sectional intensity distribution is shaped into a substantially circular shape by a shaping prism in order to improve the efficiency of light utilization in the optical head device. The light beam having its cross-sectional intensity distribution shaped into the substantially circular shape, is converged on the recording medium by an object lens.

The light beam reflected from the recording medium (hereinafter referred to simply as a reflected beam) follows a light path in the reverse order to the above-mentioned, and has its cross-sectional intensity distribution restored to be an elliptic shape by the shaping prism. Thereafter, the reflected beam is directed to a diffracting element via the collimating lens, and diffracted by the diffracting element so as to be converged on a photodetector.

Normally, each of the diffracting element and the photodetector is divided into a plurality of regions by a plurality of division lines so as to obtain a focus error signal or a tracking error signal by adopting the knife-edge method or push-pull method. For example, in accordance with the knife-edge method, a focus error signal can be obtained by finding a difference of detecting signals released from two detecting regions adjacent to each other in the photodetector.

Fig. 13 shows one example of the FES (Focus Error Signal) curve showing the relationship between the intensity of the focus error signal thus obtained and the amount of the displacement of the optical disk based on the focal point of the object lens taken as a reference. In Fig. 13, a reference point A represents a case without a focus error. The reflected beam hits the diffracting element, and a converging point of the resulting beam diffracted toward the photodetector by the diffracting element varies in its position in front of or behind the photodetector in response to fluctuations of the recording medium from the position causing no focus error. As a result, since a shape of a light spot formed on the photodetector by the diffracted beam is reversed, the value of the focus error signal is reversed from positive to negative in accordance with the amount of the displacement of the recording medium.

In this case, as shown in Fig. 13, when the recording medium moves away from the focal point of the object lens to a certain extent, an undesired zero-cross point B appears on the FES curve at a position other than the reference point A. Especially in an optical pickup device using a shaping prism in its optical system, an undesired zero-cross point may appear within a dynamic range of focusing control, depending on what arrangement is selected with respect to the optical system. This is related to the fact that the fluctuation of the recording medium causes the shaping prism to have astigmatism.

Meanwhile, the zero-cross point in the FES curve provides a drive target for the object lens in focus servo control, and if a zero-cross point B appears at a position other than the reference point A as described above, the following problems arise. When focusing control is performed so as to move the object lens from a farther position toward a closer position with respect to the optical disk, for example, in the case of occurrence of an excessive focus error due to an external cause or an initial state of the device, the object lens might be moved to focus on the incorrect drive target derived from the undesired zero-cross point B. As a result, normal information recording or reproduction might not be performed.

In order to solve the above problems, the inventors of the present invention have devised an optical head device shown in Fig. 10. The optical head device is provided with a laser light source 81 which emits in the shape of a cone a light beam whose cross-sectional intensity distribution has an elliptic shape. The light beam passes through a diffracting element 82, and is directed to the collimating lens 83. The light beam formed into a parallel beam of light by a collimating lens 83 is directed to a shaping prism 84. The shaping prism 84 shapes the parallel beam of light in its cross-sectional intensity distribution from the elliptic shape into a round shape. Through an object lens 85, the light beam having been subject to the shaping process is converged onto a belt-like track 71 formed on an optical disk 70.

In Fig. 10, an arrow T′ represents a microscopic rotation direction of the optical disk 70 at a converging point of the light beam converged thereon through the object lens 85. The object lens 85 is permitted to move closer to or farther from the optical disk 70 so as to always position the focal point of the object lens 85

just on the track 71 (this state is referred to as a just-in-focus state). Further, the object lens 85 is moved in a radial direction of the optical disk 70. The radial direction is orthogonal to the rotation direction indicated by the arrow T'. The light beam reflected from the optical disk 70 (hereinafter referred to simply as a reflected beam) returns to the disc-shaped diffracting element 82 following the path to the optical disk 70 in the reverse order.

The following description will discuss in detail an arrangement of optical members employed in the above optical head device. For example, a z-axis is determined to be parallel to an optical axis of a light beam emitted from a laser light source 81. Optical axes of the diffracting element 82 and collimating lens 83 coincide with the optical axis of the light beam emitted from the laser light source 81. The diffracting element 82 is divided equally into two diffracting regions by a division line 82a. The diffracting regions are respectively provided with a first diffraction grating 82b and a second diffraction grating 82c for diffracting the reflected beam. A y-axis is determined to be parallel to the division line 82a, while an x-axis is set to be orthogonal to both the x and z-axes. Then, the laser light source 81 is arranged such that the elliptic cross-sectional intensity distribution of the light beam emitted from the laser light source 81 has its major axis set to be parallel to the x-axis. In other words, concerning the elliptic bright portion formed on the diffracting element 82 by the light beam emitted from the laser light source 81 or by the reflected beam from the optical disk 70, the major axis of the bright portion is parallel to the x-axis, and orthogonal to the division line 82a. Further, the minor axis of the bright portion is parallel to the y-axis, and substantially coincides with the division line 82a. Additionally, directions indicated by respective arrows of the x, y and z-axes are supposed to be the positive directions.

Grating lines of each of diffraction gratings 82b and 82c are substantially parallel to the x-axis and disposed with a predetermined spacing. Thus, parts of the reflected beam directed to the respective diffraction gratings 82b and 82c are diffracted in directions different from the direction toward the laser light source 81 and substantially parallel to the y-z plane containing the division line 82a. Here, the grating pitch of the first diffraction grating 82b is greater than that of the second diffraction grating 82c. That is, the second diffraction grating 82c has its grating lines more closely pitched than those of the first diffraction grating 82b, thereby making an diffraction angle of the light beam diffracted by the second diffraction grating 82c greater than that of the light beam diffracted by the first diffraction grating 82b.

A photodetector 86 is disposed alongside of the laser light source 81 in the negative direction of the y-axis. Portions of the reflected beam are diffracted by the diffraction gratings 82b and 82c toward the photodetector 86, and converged on a detecting face of the photodetector 86. The detecting face of the photodetector 86 is divided equally into two detecting regions by a first division line 86d extending in parallel with the x-axis. One of the detecting regions closer to the laser light source 81 is referred to as a first detecting region 86a. The other detecting region is divided equally into a second detecting region 86b and a third detecting region 86c by a second division line 86e extending along the y-axis.

In the above arrangement, the reflected beam derived from a light beam having a reference wavelength, converged on the optical disk 70 in the just-in-focus state, is divided equally into two beams by the respective diffraction gratings 82b and 82c of the diffracting element 82, and the two beams are diffracted at respective angles different from each other. One of the beams diffracted by the first diffraction grating 82b has a smaller diffraction angle, and is converged on a center of the first detecting region 86a. On the other hand, the other beam diffracted by the second diffraction grating 82c has a greater diffraction angle, and is converged on a center of the second division line 86e. The photodetector 86 respectively releases detection signals depending on intensities of the respective light beams converged on the detecting regions 86a, 86b and 86c.

The detection signals of the first, second and third detecting regions 86a, 86b and 86c are respectively denoted by Sa, Sb and Sc. Then, a focus error signal FES for use in focusing control of the light beam to be projected on the optical disk 70 is obtained by the difference of the detection signals of the second and third detecting regions 86b and 86c, that is, Sb - Sc. The object lens 85 is moved closer to or farther from the optical disk 70 so as to make the value of the focus error signal FES equal to "0". Thus, the light beam is projected on the optical disk 70 in the just-in-focus state.

Further, a radial error signal RES for use in tracking control of the light beam to be projected on the optical disk 70 is obtained by an equation; Sa - (Sb + Sc). The object lens 85 is moved in a radial direction of the optical disk 70 so as to make the value of the radial error signal RES equal to "0".

The optical head device which is arranged so that the minor axis of an elliptic bright portion formed on the diffracting element 82 by the reflected beam may coincide with the division line 82a, as described above, has the following advantages. As illustrated in Fig. 14, for example, in the case where the optical disk 70 is fluctuated to move away from a focal point of the object lens 85, there is formed a line-like bright portion on the diffracting element 82 due to the astigmatism of the shaping prism 84 (shown by a heavy line P in Fig. 14). The line-like bright portion P may be formed overlapping on the division line 82a depending on the alignment of optical members constituting the optical

head device, or it may be formed being biassed onto the first diffraction grating 82b due to an installation error of the diffracting element 82 or other reasons. In this case, outputs of the detecting regions 86b and 86c become "0". As a result, since the value of the focus error signal FES shows "0" in spite of the presence of a focus error, an offset occurs in focusing control. However, with the advantageous arrangement that the line-like bright portion P is formed so as to be orthogonal to the division line 82a, even if the division line 82a slightly tilts with respect to the x-axis, or slightly deviates from the optical axis, those factors have little adverse effects on the requirement that the bright portion P should be evenly allotted onto the regions 82b and 82c. Therefore, an appropriate focus error signal FES can be obtained.

Furthermore, it is avoidable to have the phenomenon that a semi-elliptic light spot is first formed either on the detecting regions 86b or on 86c, and then formed on the other region in an inverted shape as the optical disk 70 further moves away from the focal point of the object lens 85. (The value of the focus error signal FES undesirably shows "0" also in the case where a light spot first formed either on the detecting region 86b or 86c is then reversed in its position onto the other detecting region 86b or 86c). As described above, in the optical head device shown in Fig. 10, its focusing control is little adversely affected by fluctuations of the optical disk 70.

However, such a laser light source 81 employed in the above-mentioned optical head device, for example, a semiconductor laser, has a property that a wave-length of its projecting light beam varies depending on ambient temperature changes. Further, a diffraction angle of a light beam diffracted by a diffraction grating varies depending on its wave-length. In other words, the diffraction angle becomes greater as the wave-length of the light beam becomes longer.

For example, as shown in Fig. 11 (b), when a light beam with a reference wave-length λ_0 emitted from the laser light source 81 is converged on the optical disk 70 in the just-in-focus state and reflected therefrom, a part of the resulting reflected beam directed to the first diffraction grating 82b is diffracted to be converged onto a center O_1 of the first detecting region 86a of the photodetector 86 to form a light spot. On the other hand, another part of the reflected beam directed to the second diffraction grating 82c is diffracted to be converged onto a center O_2 of the second division line 86e of the photodetector 86 to form a light spot. In this case, the value of the focus error signal FES becomes "0".

Meanwhile, in the case where the optical disk 70 is fluctuated with respect to the object lens 85 and a light beam projected on the optical disk 70 is not in the just-in-focus state, the focus error signal FES has an intensity responsive to an amount of displacement of the optical disk 70 from a focal point of the object lens 85 as a reference as shown by a solid line f in Fig. 12.

Here, suppose that ambient temperature has risen with respect to the laser light source 81, a wave-length λ of a light beam emitted from the laser light source 81 becomes longer than the reference wave-length λ_0. In this case, even if the light beam is projected on the optical disk 70 in the just-in-focus state, the resulting reflected beam reflected from the optical disk 70 and directed to the diffracting element 82 is diffracted with a greater angle than that of the reflected beam having the reference wave-length λ_0 as described earlier. For this reason, parts of the reflected beams with the wave-length λ, after having been diffracted by the respective diffraction gratings 82b and 82c, are focused in front of the detecting regions of the photodetector 86 before reaching them. As a result, as shown in Fig. 11(a), one of the beams diffracted by the first diffraction grating 82b is projected on the first detecting region 86a to form a semi-elliptic bright portion P_1 lying in the positive direction of the x-axis. On the other hand, the other beam diffracted by the second diffraction grating 82c is projected on the second detecting region 86b to form a semi-elliptic bright portion P_2 lying in the negative direction of the x-axis. The bright portions P_1 and P_2 are respectively positioned away from the center O_1 of the first detecting region 86a and the center O_2 of the second division line 86e in the departing direction from the laser light source 81. Further, as described earlier, the bright portions P_1 and P_2 are opposite to each other in their shapes with their respective major axes being parallel to the first division line 86d, that is, the x-axis.

Next, suppose that ambient temperature has dropped with respect to the laser light source 81, a wave-length λ' of a light beam emitted from the laser light source 81 becomes shorter than the reference wave-length λ_0. In this case, the beams diffracted by the respective diffraction gratings 82b and 82c have smaller diffraction angles. For this reason, the beams with the wave-length λ', after having been diffracted by the respective diffraction gratings 82b and 82c, are focused behind the detecting regions of the photodetector 86. As a result, as shown in Fig. 11(c), one of the beams diffracted by the first diffraction grating 82b is projected on the first detecting region 86a to form a semi-elliptic bright portion P_1' lying in the negative direction of the x-axis. The shape of the bright portion P_1' has an opposite orientation to that of the bright portion P_1. On the other hand, the other beam diffracted by the second diffraction grating 82c is projected on the second detecting region 86c to form a semi-elliptic bright portion P_2' lying in the positive direction of the x-axis. Similarly, the shape of the bright portion P_2' has an opposite orientation to that of the bright portion P_2. The bright portions P_1 and P_2 are respectively positioned away from the center O_1 of the first detecting region 86a and the center O_2 of the second division line 86e in the approaching direction to the

laser light source 81.

As described earlier, the cross-sectional intensity distribution of the reflected beam directed to the diffracting element 82 shows an elliptic shape whose major axis is orthogonal to the division line 82a, that is, the y-axis, and further is equally allotted to the diffraction gratings 82b and 82c. Therefore, the reflected beam with the wave-length λ or λ' having a variation from the reference wave-length λ_0 is diffracted by the diffracting element 82 to form on the respective detecting regions of the photodetector 86 the bright portions P_1 and P_2 or P_1' and P_2' which show elliptic shapes with their respective major axes orthogonal to the y-axis.

Thus, for example, in the case where the wave-length λ of a light beam is greater than the reference wave-length λ_0, even if the light beam is projected on the optical disk 70 in the just-in-focus state, the second detecting region 86b releases the detection signal Sb based on the intensity of the bright portion P_2 shown in Fig. 11(a). As a result, despite the fact that the optical disk 70 is situated at an appropriate position with respect to the object lens 85, a focus error signal FES (= Sb - Sc) having a value of $S_2(> 0)$ is produced as is shown by a broken line f' in Fig. 12. The focus error signal FES having a positive value indicates that the optical disk 70 is fluctuated to approach the object lens 85. Therefore, in order to make the value of the focus error signal FES set to "0", the object lens 85 is moved so that the optical disk 70 may be located away from the focal point of the object lens 85 by an offset value σ_2. This causes the light beam to be focused before reaching the optical disk 70, thereby disturbing an accurate recording or reproducing operation for information.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an optical head device wherein focusing control for converging light on a recording medium according to a focus error signal is always performed accurately and whereby high accuracy in recording or reproducing information is obtainable.

It is another object of the present invention to provide an optical head device wherein offsets in focusing control due to wave-length variation of light to be converged on a recording medium can be suppressed.

In order to achieve the above objects, the optical head device of the present invention is characterized in comprising at least the following means:

(1) light generation means (for example, a laser light source) for generating light having the following features, (a) and (b):

(a) having an intensity distribution showing a substantially elliptic shape in the cross section orthogonal to the optical axis.

(b) the major axis of the above elliptic shape being parallel to a reference direction.

(2) photodetecting means (for example, a photodetector) having the following features, (c), (d), (e) and (f):

(c) releasing detection signals so as to obtain a focus error signal.

(d) including a plurality of photodetecting sections

formed by a plurality of division lines dividing a light-receiving face thereof.

(e) setting at least one of the plurality of division lines to be substantially parallel to the reference direction.

(f) setting a hypothetical line extending through a projecting point of the light generation means and the center of the light-receiving face to be parallel to the reference direction.

(3) diffraction means (for example, a diffracting element) having the following features, (g) and (h):

(g) including a plurality of diffracting regions formed by a plurality of division lines dividing a diffracting face thereof.

(h) setting at least one of the plurality of division lines for dividing the diffracting face to be parallel to the reference direction.

(4) optical means (such as a collimating lens, a shaping prism,and an object lens) having the following features, (i) and (j):

(i) converging the light emitted from the light generation means on a recording track formed on the recording medium.

(j) directing reflected light from the recording track to the diffraction means so that the major axis of an elliptic bright portion formed on the diffracting face by the reflected light may be positioned on at least one of the division lines in the diffraction means, the division line being parallel to the reference direction.

Here, the provisions are made such that, when the reflected light that has been diffracted by at least one of the diffracting regions of the diffraction means is converged on at least one of the division lines in the photodetecting means, which is parallel to the reference direction, a value of the focus error signal is allowed to become zero.

In the above arrangement, the light generation means projects light whose intensity distribution has an elliptic shape with its major axis parallel to the reference direction, and at least one of the division lines of the diffraction means is set to be parallel to the reference direction. Thus, the reflected light directed to the diffraction means forms an elliptic bright portion whose major axis is situated on the division line of the diffraction means. Provided that there is no focus error and further there is no wave-length variation with

respect to light generated by the light generation means, the diffraction means diffracts reflected light that has directed to at least one of the diffracting regions so that the reflected light may be converged on at least one of the division lines in the photodetecting means, which is substantially parallel to the reference direction. At this time, the value of the focus error signal obtained based on detection signals released by the respective photodetecting sections is permitted to become "0".

On the other hand, occurrence of a wave-length variation in the light generated by the light generation means causes a variation in the diffraction angle of the reflected light directed to the diffraction means. This causes the position of a converging point, which was on the light-receiving face of the photodetecting means in the case where there is no focus error and further there is no wave-length variation with respect to the light generated by the light generation means, to vary in a direction orthogonal to and parallel to the light-receiving face. As a result, in the vicinity of at least one of the division lines which is substantially parallel to the reference direction in the photodetecting means, is formed a thin semi-elliptic bright portion whose major axis of the elliptic shape is parallel to the division line. In other words, even in the case of no focus error, if there is a wave-length variation, the value of the focus error signal does not become "0" due to the thin semi-elliptic bright portion formed in the photodetecting means.

However, an offset value of the focus error signal caused by the wave-length variation is extremely suppressed because of the facts that detection sensitivity is low in the vicinity of the division lines in the photodetecting means; and the bright portion formed on the photodetecting means lies in a thin shape along the division line.

Moreover, the bright portion formed on the diffracting face of the diffraction means, derived from the reflected light, has an elliptic shape whose minor axis is orthogonal to the reference direction. In the case where the optical means include an intensity distribution shaping means for shaping the intensity distribution of the light emitted by the light generation means in the minor axis direction of the elliptic shape, the shape of the bright portion formed on the diffracting face changes depending on changes in amount of focus error. A rate of change in the minor axis direction becomes greater than that in the major axis direction. With the arrangement, in conjunction with the shape of the bright portion formed on the photodetecting means, a rate of change in the minor axis direction becomes greater than that in the major axis direction, resulting in abrupt changes in the focus error signal. Therefore, response sensitivity in the focusing control can be improved.

For a fuller understanding of the nature and advantages of the invention, reference should be made to the ensuing detailed description taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view showing a structural example of an optical head device according to the present invention.

Fig. 2 is a side view of the optical head device shown in Fig. 1.

Fig. 3 is a plan view showing shapes of bright portions formed on a photodetector caused by a fluctuation of an optical disk.

Fig. 4 is an explanatory drawing showing an optical function of a shaping prism.

Fig. 5 is a graph showing the relationship between the amount of displacement of the optical disk, shown in Fig. 1, based on a focal point of an object lens as a reference and the variation of intensity of a focus error signal FES.

Fig. 6 is a plan view showing shapes of bright portions formed in the photodetector caused by a wave-length variation of a light beam.

Fig. 7 is a side view showing another structural example of an optical head device according to the present invention.

Fig. 8 is an explanatory drawing showing an optical function of a shaping prism provided in the optical head device of Fig. 7.

Fig. 9 is a graph showing the relationship between the amount of displacement of the optical disk, shown in Fig. 7, based on a focal point of an object lens as a reference and the variation of intensity of a focus error signal FES.

Fig. 10 is a perspective view showing a structural example of an optical head device.

Fig. 11 is a plan view showing shapes of bright portions formed in the photodetector caused by a wave-length variation of a light beam in conjunction with the optical head device of Fig. 10.

Fig. 12 is a graph showing the relationship between the amount of displacement of the optical disk, shown in Fig. 10, based on a focal point of an object lens as a reference and the variation of intensity of a focus error signal FES.

Fig. 13 is a graph showing an example of the FES curve containing an undesired zero-cross point.

Fig. 14 is a schematic plan view of a diffracting element and the photodetector shown in Fig. 10.

Fig. 15 is a side view of an optical head device as one structural example, which is capable of obtaining a desirable tracking error signal.

Fig. 16 is a plan view showing a second diffracting element in the optical head device of Fig. 15.

Fig. 17 is a plan view showing a photodetector in the optical head device of Fig. 15.

Fig. 18 is a plan view showing another diffracting element adaptable to the optical head device of Fig.

1.

Fig. 19 is a plan view showing another photodetector adaptable to the optical head device of Fig. 1.

DESCRIPTION OF THE EMBODIMENT

Referring to Figs. 1 to 6, the following description will discuss one embodiment of the present invention.

First, as shown in Figs. 1 and 2, three axis rectangular coordinates x-y-z are determined as follows in conjunction with an optical head device of the present invention. The z-axis is determined to be parallel to an optical axis L_1 of a light beam emitted from a laser light source 10 as light generation means. The y-axis is determined to be orthogonal to the z-axis and further parallel to a hypothetical line extending through a projecting point of the laser light source 10 and the center of a light-receiving face of a photodetector 60 as photodetecting means (or a converging point on the photodetector 60 in a just-in-focus state), while the x-axis is determined to be orthogonal to both the y and z-axes. Additionally, the y-axis can be determined so as to be parallel to a division line 21 of a diffracting element 20, which are described later. The direction parallel to the y-axis is hereinafter referred to as a reference direction. Further, directions as indicated by arrows of the respective x, y and z-axes in Figs 1 are supposed to be the respective positive directions. Additionally, Fig. 2 is a side view of the optical head device of Fig. 1, seen from the positive side toward the negative side along the y-axis.

A light beam emitted by the oscillation of the laser light source 10 gradually spreads to have an intensity distribution of an elliptic shape in its cross section orthogonal to the optical axis L_1. (Additionally, such an intensity distribution of light, related to a cross section orthogonal to the optical axis, is hereinafter referred to simply as a cross-sectional intensity distribution.) Here, the laser light source 10 is arranged so that the major axis of the elliptic shape is parallel to the y-axis, that is, the reference direction.

Moreover, respective centers of the diffracting element 20, a collimating lens 30 and an end face 41 of a shaping prism 40 are aligned along the optical axis L_1. Here, the end face 41 is a surface to receive the light beam emitted by the laser light source 10. The diffracting element 20 for transmitting the light beam emitted by the laser light source 10 is of a disc-shape, square-shape, elliptic-shape, etc. whose surfaces are parallel to the x-y plane. The light beam transmitted through the diffracting element 20 passes through the collimating lens 30. At this time, the collimating lens 30 produces a parallel beam of light from the light beam spreading in the shape of cone, directed thereto. Similarly, a cross-sectional intensity distribution of the parallel beam of light projected from the collimating lens 30 shows an elliptic shape whose major axis is parallel to the y-axis, that is, the reference direction.

The parallel beam of light projected from the collimating lens 30 is directed to the end face 41 of the shaping prism 40. The end face 41 is inclined with respect to the y-z plane and x-y plane. Therefore, the intensity distribution of the parallel beam of light is diffused in the minor axis direction by the end face 41 to form a substantially round shape. The parallel beam of light having impinged upon the end face 41 proceeds along the optical axis L_2 in the shaping prism 40 and is projected from the other end face 42. As shown in Fig. 2, since the end face 42 is orthogonal to the optical axis L_2, the parallel beam of light is projected from the end face 42 in a direction parallel to the optical axis L_2 and directed to the object lens 50.

The object lens 50 is disposed so as to have its optical axis coincided with the optical axis L_2, and converges the parallel beam of light on a recording track 71 formed on an optical disk 70 as a recording medium. A recording surface of the optical disk 70 is placed perpendicular to the optical axis L_2. A tangent direction to the rotatable recording track 71 at a converging point is represented by an arrow T in the drawing. Additionally, the tangent direction T is parallel to the x-z plane.

The light beam reflected from the recording track 71 (hereinafter referred to simply as the reflected beam) successively passes through the object lens 50, shaping prism 40 and collimating lens 30 following in the reverse order the path whereby the light beam was directed to the recording track 71. The reflected beam having passed through the shaping prism 40 forms a parallel beam of light. The reflected beam projected from the end face 41 has its intensity distribution returned from the round shape to an elliptic shape. Further, the reflected beam is directed to the diffracting element 20 by the collimating lens 30 along the path converging on the projecting point of the laser light source 10.

The diffracting face of the diffracting element 20 is equally divided into two regions by a division line 21 parallel to the y-axis, that is, the reference direction. Therefore, an elliptic bright portion formed on the diffracting element 20 by the reflected beam directed thereto has its major axis positioned substantially on the division line 21. The diffracting regions separated by the division line 21 are provided with respective first and second diffraction gratings 22 and 23. Grating lines formed on each diffraction grating 22 or 23 have a predetermined pitch and are orthogonal to the division line 21, that is, form a number of parallel lines extending in the x-direction. Moreover, the pitch of the grating lines in the first diffraction grating 22 is set to be greater than that in the second diffraction grating 23. With the above arrangement, each diffracting grating 22 or 23 diffracts a part of the reflected beam directed thereto in a direction substantially parallel to the

y-z plane including the division line 21. Here, a diffraction angle with respect to the part of the reflected beam diffracted by the first diffraction grating 22 toward the photodetector 60 (hereinafter referred to simply as the first diffracted beam) is set to be smaller than that with respect to another part of the reflected beam diffracted by the second diffraction grating 23 toward the photodetector 60 (hereinafter referred to simply as the second diffracted beam).

The diffracted beams are directed to the photodetector 60. The photodetector 60 has a photodetecting face whereon these diffracted beams are projected. The photodetecting face is equally divided into two regions by a first division line 64 parallel to the x-axis. One of the photodetecting regions positioned by the side of the laser light source 10 is referred to as a first detecting region 61. The other photodetecting region is further equally divided into two detecting regions, that is, a second detecting region 62 and a third detecting region 63 by a second division line 65 which is substantially parallel to the y-axis. The detecting regions 61, 62 and 63 respectively release detection signals according to intensities of the respective diffracted beams directed thereto.

When a light beam having a reference wavelength λ_0, emitted from the laser light source 10 is converged on the recording track 71 in the just-in-focus state, the resulting reflected beam from the recording track 71 is diffracted by the diffraction grating 22 and 23 so as to be divided into two parts. Then, as illustrated in Figs. 3(a) and 3(b), the first diffracted beam is converged on a center O_{10} of the first detecting region 61, while the second diffracted beam is converged on a center O_{20} of the second division line 65.

In an optical head device having the above arrangement, the object lens 50 is controlled according to detection signals released by the respective detecting regions 61, 62 and 63. The following description will discuss how the control of the object lens 50 is performed.

First, as illustrated in Fig. 3(a), in a state where the object lens 50 is located close to the optical disk 70, the second diffracted beam derived from the second diffraction grating 23 having a relatively narrower pitch of grating, forms a bright portion P_{20} on the third detecting region 63 in the vicinity of the center O_{20}. The bright portion P_{20} has a semi-elliptic shape lying in the positive direction of the x-axis, and its major axis is parallel to the first division line 64, that is, the x-axis. On the other hand, the first diffracted beam derived from the first diffraction grating 22 having a relatively wider pitch of grating, forms a bright portion P_{10} in the vicinity of the center O_{10}. The bright portion P_{10} has a semi-elliptic shape lying in the negative direction of the x-axis, and its major axis is parallel to the x-axis as with the aforementioned case. Thus, the bright portions P_{10} and P_{20} are substantially opposite to each other in their shapes.

Meanwhile, as illustrated in Fig. 3(b), in a state where the object lens 50 is located far away from the optical disk 70, the second diffracted beam forms a bright portion P_{21} on the second detecting region 62 in the vicinity of the center O_{20}. The bright portion P_{21} has a semi-elliptic shape lying in the negative direction of the x-axis, which is opposite to the above-mentioned case having the object lens 50 located close to the optical disk 70, and its major axis is parallel to the x-axis as with the above-mentioned case. On the other hand, the first diffracted beam forms a bright portion P_{11} in the vicinity of the center O_{10} of the first detecting region 61. Similarly, the bright portion P_{11} has a semi-elliptic shape lying in the positive direction of the x-axis, which is opposite to the above-mentioned case having the object lens 50 located close to the optical disk 70, and its major axis is parallel to the x-axis as with the above-mentioned case. The bright portions P_{11} and P_{21} are substantially opposite to each other in their shapes.

As illustrated in Fig. 3(a), each shape of the bright portions P_{10} and P_{20} is deformed with its center remained the same, according to an amount of displacement of the optical disk 70 based on a focal point of the object lens 50 as a reference. (The same is true with respect to the shapes of the bright portion $P_{11}$and P_{21}.) Here it is noted that in the optical head device of the present invention shown in Fig. 1, rate of change of the shapes of the bright portions P_{10} and P_{20} with respect to the x-direction (that is, rate of enlargement or rate of reduction) is greater than rate of change of those in the optical head device shown in Fig. 10.

This is because of optical properties of the shaping prism 40. In other words, in the optical head device shown in Fig. 1, a reflected beam which is produced from the light beam focused on the optical disk 70 is transmitted through the shaping prism 40 and projected therefrom in a manner as shown by a solid line in Fig. 4. In Fig. 4, suppose the reflected beam b_1 from the optical disk 70 hits the end face 41 of the shaping prism 40 at an angle α from the collimating lens 30 side, and let us denote an angle of refraction of the reflected beam b_1 by α' and a refractive index of the shaping prism 40 by n. Then using Snell's Law, the following equation holds:

$$n.\sin\alpha = \sin\alpha'. \quad (1)$$

Suppose a light beam directed onto the optical disk 70 has a focus error, thereby causing a reflected beam b_2 from the optical disk 70 to tilt with respect to the reflected beam b_1. Further, a difference of an incident angle of the reflected beam b_2 and the incident angle α of the reflected beam b_1 with respect to the end face 41 is denoted by β, and a difference of an angle of refraction of the reflected beam b_2 and the angle of refraction α' of the reflected beam $b_1$is denoted by β'. Then, a relationship between β and β' is given by the following equation:

$$\beta' = \sin^{-1}(n.\sin\alpha) - \sin^{-1}\{n.\sin(\alpha-\beta)\}. \quad (2)$$

The equation (2) shows that the difference β' in the angles of refraction of the respective reflected beams at the end face 41 of the outgoing side is amplified in comparison with the difference β when there occurs a focus error in the light beam to be projected on the optical disk 70. In other words, in the case of the optical head device shown in Fig. 1, shaping function of the shaping prism 40 is amplified with respect to the direction parallel to the minor axis of the cross-sectional intensity distribution of the light beam, that is, the x direction. Therefore, since rate of change of the shapes of the bright portions P_{10} and P_{20} with respect to the x direction is greater than rate of change of those with respect to the y direction, each of the bright portions P_{10} and P_{20} has a prolonged semi-elliptic shape lying in the x direction, as shown in Fig. 3(a).

Suppose that detection signals released from the detecting regions 61, 62 and 63 are represented respectively as Sa, Sb and Sc. Then, a focus error signal FES corresponding to a deviation between the optical disk 70 and the focal point of the object lens 50 is obtained by calculating a difference of the detection signals Sb and Sc; (Sb - Sc). Fig. 5 shows an example of the FES curve with respect to the optical head device shown in Fig. 1. As described earlier, when the shaping prism 40 shapes the cross-sectional intensity distribution not in the major-axis direction but in the minor-axis direction, the shapes of the bright portions P_{10} and P_{20} formed on the photodetector 60 change greatly in the x direction. For this reason, as shown by a solid line in Fig. 5, its FES intensity shows an abrupt change in the vicinity of a zero-cross point on the FES curve.

On the other hand, in the case of the optical head device shown in Fig. 10, a shaping prism 84 shapes a cross-sectional intensity distribution in the minor-axis direction, and the minor axis is set to be parallel to the y direction. For this reason, unlike the case of the bright portions P_{10} and P_{20}, rate of shape change of bright portions P_1 and P_2 formed on a photodetector 86 shown in Fig. 11, is substantially small with respect to the x direction. Therefore, as shown by a solid line in Fig. 12, its FES intensity shows a gradual change in the vicinity of a zero-cross point on an FES curve f in comparison with the FES curve shown in Fig. 5.

Consequently, when a converging state of the light beam on the optical disk 70 slightly deviates from the just-in-focus state, rate of change in FES intensity is relatively great in the optical head device shown in Fig. 1. Therefore, a higher response in focusing control can be obtained.

Next, in the case where ambient temperature has risen with respect to the laser light source 10, causing a wave-length λ of a light beam emitted from the laser light source 10 to become longer than the reference wave-length λ_0, each of diffraction angles at the diffraction gratings 22 and 23 becomes greater. Then, each of the diffracted beams has an extended light path to the photodetector 60. That is, the first and second diffracted beams derived from the reflected beam with the wave-length λ are respectively focused before reaching the detecting face of the photodetector 60. As a result, as illustrated in Fig. 6(a), a bright portion P_{13} formed on the first detecting region 61 by the first diffracted beam is dislocated from the center O_{10} of the first detecting region 61 to move away from the laser light source 10 along the y direction, that is, the reference direction. Similarly, a bright portion P_{23} formed on the second detecting region 62 by the second diffracted beam is dislocated from the center O_{20} of the second division line 65 to move away from the laser light source 10 along the reference direction.

Each of the bright portions P_{13} and P_{23}, unlike those shown in Fig. 3, shows a semi-elliptic shape whose major axis is parallel to the second division line 65. These shapes are derived from the fact that the bright portion formed on the diffracting element 20 by the reflected beam from the optical disk 70 has an elliptic shape whose major axis is parallel to the division line 21. Further, since each focal point of the diffracted beams is formed in front of the detecting face of the photodetector 60, the orientations of the bright portions P_{13} and P_{23} lying in the x directions from the major axis are the same as those of the bright portions P_{11} and P_{21} (see Fig. 3(b)) which are obtained in the case of moving the optical disk 70 away from the object lens 50. Therefore, the bright portion P_{23} formed on the second detecting region 62 lies in the negative direction of the x-axis, while the bright portion P_{13} lies in the positive direction of the x-axis.

On the contrary, in the case where ambient temperature has dropped with respect to the laser light source 10, causing a wave-length λ' of a light beam emitted from the laser light source 10 to become shorter than the reference wave-length λ_0, each of diffraction angles at the diffraction gratings 22 and 23 becomes smaller. For this reason, the first and second diffracted beams derived from the reflected beam with the wave-length λ' are respectively focused behind the detecting face of the photodetector 60. As a result, as illustrated in Fig. 6(b), a bright portion P_{14} formed on the first detecting region 61 by the first diffracted beam is dislocated from the center O_{10} of the first detecting region 61 to be positioned closer to the laser light source 10 along the reference direction. Similarly, a bright portion P_{24} formed on the third detecting region 63 by the second diffracted beam is dislocated from the center O_{20} of the second division line 65 to be positioned closer to the laser light source 10 along the reference direction.

Each of the bright portions P_{14} and P_{24} also shows a semi-elliptic shape whose major axis is parallel to the second division line 65. Further, since each focal point of the diffracted beams is formed behind the detecting face of the photodetector 60, the bright portion P_{24} lies in the positive direction of the x-axis to be

located on the third detecting region 63 while the bright portion P_{14} lies in the negative direction of the x-axis, both having the opposite orientations to the respective bright portions P_{23} and P_{13}.

Also in these cases, the focus error signal FES is obtained by the aforementioned equation, (Sb - Sc). However, in proportion to variations of the wave-length, each shape of the bright portions P_{23} and P_{24} respectively formed on the second detecting region 62 and the third detecting region 63 becomes larger keeping a similar shape, and each position of those varies. For this reason, even if a light beam is projected on the optical disk 70 in the just-in-focus state, the focus error signal FES does not become "0". One example of the FES curve is shown by a broken line in Fig. 5, wherein the wave-length λ of the light beam is longer than the reference wave-length λ_0. The FES curve shown by the broken line possesses an undesired detection value S_1 in the case of the just-in-focus state. Accordingly, there is produced an offset value σ_1 that forms an improper target value for focusing control. However, since the bright portion P_{23} is formed in the vicinity of the second division line 65 of the second detecting region 62 in an semi-elliptic shape whose major axis is parallel to the second division line 65, and further, detection sensitivity is low in the vicinity of the second division line 65, the undesired detection value S_1 due to a longer wave-length of the light beam can be extremely suppressed. In addition, since responsibility of the FES has been improved as described earlier, the offset value σ_1 of the optical disk 70 is further extremely reduced. This offset value σ_1 is sufficiently smaller than the offset value σ_2 shown in Fig. 12.

Similarly, since detection sensitivity is low in the vicinity of the second division line 65 in the third detecting region 63 and responsibility of the focus error signal FES has been improved, an undesired value of the focus error signal FES in the just-in-focus state and an offset value of the optical disk 70, due to a shorter wave-length of the light beam, can be extremely suppressed, thereby making it possible to reduce an offset value from the focal point with respect to the optical disk 70 to a substantially small amount.

Additionally, in the above embodiment, the shaping prism 40 as an example is arranged such that the end face 41 whereon a light beam emitted from the laser light source 10 is directed is inclined with respect to the light beam; and the other end face 42 is set to be orthogonal to the light beam refracted at the end face 41. Alternatively, as shown in Fig. 7, another shaping prism 40′ can be adopted, which is arranged such that an end face 42′ whereon a light beam emitted from the laser light source 10 is directed is set to be orthogonal to the light beam; and the other end face 41′ facing the object lens 50 is inclined at a predetermined angle with respect to the x-y plane and x-z plane.

In this case, according to optical properties of the shaping prism 40′ as will be described below, a rate of change of the shapes of the bright portions formed on the photodetector 60 due to a focus error becomes smaller with respect to y direction than that of the bright portions P_{10} and P_{20} shown in Fig. 3. In the optical head device shown in Fig. 7, a reflected beam b_3 produced from the light beam focused on the optical disk 70 passes through the shaping prism 40′ and is projected therefrom following a light path shown by a solid line in Fig. 8. Now, suppose that the light beam directed on the optical disk 70 has a focus error, thereby causing a reflected beam b_4 from the optical disk 70 to tilt with respect to the reflected beam b_3. When a difference between incident angles of the reflected beam b_3 and the reflected beam b_4 with respect to the end face 41′ facing the object lens 50 is denoted by γ and a difference between angles of refraction of the reflected beam b_3 and the reflected beam b_4 is denoted by γ', γ' becomes smaller than γ. In other words, concerning rate of change in the bright portions formed on the photodetector 60 due to a focus error, a rate of change in y direction subjected to the shaping function of the shaping prism 40′ is smaller than a rate of change in x direction not subjected to the shaping function thereof. Therefore, as with the shapes of the bright portions P_{10} and P_{20} shown in Fig. 3, the bright portions formed on the photodetector 60 have semi-elliptic shapes stretching to a greater extent in x direction than in y direction.

Fig. 9 shows an example of FES curve g in the case of a presence of a wave-length variation in the optical head device shown in Fig. 7. Here, a rate of change in x direction not subjected to the shaping function of the shaping prism 40′ is identical to that of the optical head device shown in Fig. 10. Consequently, since the rate of change in the FES curve g is identical to that in the FES curve f′ shown in Fig. 12 (also drawn in Fig. 9 for reference), the responsibility for focusing control is identical to that of the optical head device shown in Fig. 10. However, in the case of the optical head device shown in Fig. 7, since the major axis of the elliptic cross-sectional intensity distribution is set to be parallel to the y direction, that is, the reference direction, the offset value of the optical disk 70 is further reduced. This is because, as described earlier, the bright portion is formed in the vicinity of the second division line 65 where detection sensitivity of the photodetector 60 is low, in a semi-elliptic shape whose major axis is parallel to the second division line 65 and prolonged in the reference direction. Therefore, even if responsibility in focusing control remains unchanged, the offset value σ_3 is reduced to less than the offset value σ_2 of the optical head device shown in Fig. 10.

Lastly, the following description will discuss structural examples of an optical head device for obtaining

a more desirable tracking error signal RES in conjunction with the structure of the optical head device shown in Fig. 1. Three cases are shown as those structural examples.

(1) With respect to relative positions between the optical head device and the recording track 71 on the optical disk 70, both shown in Fig. 1, a tangent direction at the converging point of the recording track 71 is set to be parallel to the y-axis. That is, the above tangent direction is orthogonal to the tangent direction T shown in Fig. 1.

(2) The three-beam method is adopted:

As shown in Fig. 15, in accordance with the three-beam method, a light beam is projected from a semiconductor laser 1, diffracted in a first diffracting element 2 and split into a zero-order diffracted light (main beam) and ±1 order diffracted lights (a pair of sub beams). In Fig. 15, the ±1 order diffracted lights are comprised in a plane orthogonal to the surface of the paper. The main beam and the sub beams are further diffracted in a second diffracting element 3. Zero-order diffracted lights respectively produced by the main beam and the sub beams are transmitted through a collimating lens 4 to be focused onto a recording medium 6 by an objective lens 5. Return lights reflected off the recording medium 6 pass through the objective lens 5 and the collimating lens 4, and are diffracted in the second diffracting element 3. First order diffracted lights are then directed onto a light receiving element 7 from which data signal, tracking error signal and focus error signal can be obtained.

When, for example, data is recorded in the form of physical pits on the disc-shaped recording medium 6, the data is read out by focusing the zero order diffracted light produced by the main beam in the second diffracting element 3 on the physical pits. The return light of the zero order diffracted light is diffracted again in the second diffracting element 3 to produce first order diffracted lights. The data signal is derived from the intensity of these first order diffracted lights.

The zero order diffracted lights produced by the two sub beams in the second diffracting element 3 are focused on positions symmetrical with respect to the zero order diffracted light produced by the main beam in the second diffracting element 3. These positions are offset greatly in a track direction and offset slightly in a radial direction from the position on the recording medium 6 where the zero order diffracted light of the main beam is focused. The return lights are respectively diffracted in the second diffracting element 3 to produce first order diffracted lights. The tracking error signal is derived from the intensities of these first order diffracted lights.

Fig. 16 shows the second diffracting element 3 as seen from the recording medium 6. As shown in Fig. 16, the second diffracting element 3 is divided into two diffracting regions 3a and 3b that are delineated by a division line 3e and whereon gratings 3c and 3d are respectively formed. The gratings 3c and 3d have mutually different pitches and the directions thereof are orthogonal to the division line 3e. Here, the direction of the division line 3e is set so as to coincide with the radial direction of the recording medium 6. As shown in Fig. 17, the photodetector 7 is divided into five light receiving regions 7a to 7e.

In the above arrangement, when the light beam projected from the semiconductor laser 1 is precisely focused on the recording medium 6, a portion of the return light corresponding to the zero order diffracted light produced by the main beam in the second diffracting element 3, is diffracted in the diffracting region 3a of the second diffracting element 3 to produce a first order diffracted light. This first order diffracted light is focused on a division line 7f separating the light receiving regions 7a and 7b of the photodetector 7, to form a spot-shaped diffracted image Q_1. Another portion of the return light corresponding to the zero order diffracted light of the main beam produced in the second diffracting element 3, is diffracted in the diffracting region 3b of the second diffracting element 3 to produce a first order diffracted light. This first order diffracted light is focused on the light receiving region 7c of the photodetector 7 to form a spot-shaped diffracted image Q_2. The return lights corresponding to the zero order diffracted lights produced by the two sub beams in the second diffracting element 3, respectively form two spot-shaped diffracted images Q_3 and Q_4 and two spot-shaped diffracted images Q_5 and Q_6 on the light receiving regions 7d and 7e of the photodetector 7.

Supposing that S_{1a} to S_{1e} respectively represent output signals released from the light receiving regions 7a to 7e, the focus error signal may be obtained by calculating $(S_{1a} - S_{1b})$. The tracking error signal may be obtained by calculating $(S_{1d} - S_{1e})$ and the data signal may be obtained by calculating $(S_{1a} + S_{1b} + S_{1c})$.

(3) The diffracting element 20 and photodetector 60 shown in Fig. 1 are respectively replaced with a diffracting element 12 shown in Fig. 18 and a light receiving element 17 shown in Fig. 19:

As shown in Fig. 18, the diffracting element 12 as seen from the recording medium, is divided by division lines 12g and 12h into three diffracting regions 12a to 12c. Gratings 12d to 12f are respectively formed on the diffracting regions 12a to 12c. The division line 12g extends in a y direction corresponding to a radial direction of the recording medium. The division line 12h starts from the center of the division line 12g and extends in the x direction orthogonal to the radial direction of the recording medium, i.e., in a track direction of the recording medium. The diffracting regions 12b and 12c (diffracting regions for tracking) are designed such as to have mutually equal areas. In addition, provision is made such that the area of the

diffracting region 12a (diffracting region for focusing) is equal to the sum of the areas of the diffracting regions 12b and 12c. A cross section 19 formed by the return light impinging upon the diffracting element 12 has a substantially elliptical shape. In the present embodiment, a major axis (y direction) of the cross section 19 coincides with the direction of the division line 12g. In other words, the major axis of the return light corresponds to the radial direction of the recording medium.

Here, the radial direction is defined as a direction extending from the center of rotation of the recording medium to a position where the light beam is irradiated on the recording medium. As for the track direction, it is defined as a direction on the recording medium orthogonal to the radial direction. The direction corresponding to the radial direction is defined as a projection of the radial direction upon the diffracting element 12 while the direction corresponding to the track direction is defined as a projection of the track direction upon the diffracting element 12.

The grating 12d formed on the diffracting region 12a has a grating direction orthogonal to the division line 12g. A grating direction of the grating 12e formed on the diffracting region 12b and a grating direction of the grating 12f of the diffracting region 12c are inclined in mutually opposite directions with respect to the division line 12h. Here, the pitches of the gratings 12d to 12f and the inclination of the gratings 12e and 12f are respectively determined according to relative positions of the diffracting regions 12a to 12c and diffracted images P_{31} to P_{33}, to be described later, formed on the photodetector 17. In order to correct aberrations, grating lines of the gratings 12d to 12f can be designed, when necessary, so as to describe gradual curves.

As shown in Fig. 19, the photodetector 17 is divided into four rectangular light receiving regions 17a to 17d. The light receiving regions 17a to 17d are aligned in the x direction corresponding to the track direction of the recording medium and extend in the y direction corresponding to the radial direction of the recording medium. The two central light receiving regions 17a and 17b (light receiving regions for focusing) are divided by a division line 17e. The division line 17e extends in the y direction corresponding to the radial direction of the recording medium. The light receiving regions 17c and 17d (light receiving regions for tracking) are respectively separated in the x direction from the light receiving regions 17a and 17b by a predetermined interval.

When the light beam projected from the semiconductor laser is precisely focused upon the recording medium, the diffracted light produced in the diffracting region 12a of the diffracting element 12 forms the spot-shaped diffracted image P_{31} on the division line 17e. Meanwhile, the diffracted light produced in the diffracting region 12b forms the spot-shaped diffracted image P_{32} on the light receiving region 17c and the diffracted light produced in the diffracting region 12c forms the spot-shaped diffracted image P_{33} on the light receiving region 17d.

When there is no focus error, the diffracted image P_{31} is equally distributed between the light receiving regions 17a and 17b, and ideally forms one spot on the division line 17e. On the other hand, when a focus error occurred, the diffracted image P_{31} spreads to a greater extent on either the light receiving region 17a or the light receiving region 17b. Therefore, supposing that Sa to Sd respectively represent output signals released from the light receiving regions 17a to 17d, the focus error signal may be obtained through a single knife edge method by calculating (Sa - Sb). The tracking error signal is obtained by comparing the respective light amounts of the diffracted lights from the diffracting regions 12b and 12c divided by the division line 12h extending in the x direction corresponding to the track direction of the recording medium, and calculating (Sc - Sd) through a push-pull method. In addition, the data signal is obtained by calculating (Sa + Sb + Sc + Sd).

As described above, in the optical head device according to the present invention, the light generation means projects light whose intensity distribution has an elliptic shape with its major axis parallel to the reference direction, and at least one of the division lines of the diffraction means is set to be parallel to the reference direction. Thus, the reflected light directed to the diffraction means forms an elliptic bright portion whose major axis is situated on the division line of the diffraction means. In the case of occurrence of a wave-length variation in the light emitted by the light generation means, a thin semi-elliptic bright portion is formed along the reference direction in the vicinity of the division line substantially parallel to the reference direction in the photodetector. Since detection sensitivity is low in the vicinity of the division lines in the photodetector, an offset in focusing control due to the wave-length variation is remarkably reduced.

Further, when the optical head device of the present invention includes an intensity distribution shaping means for shaping the intensity distribution of the light projected by the light generation means in the minor axis direction of the elliptic shape, in conjunction with rate of change on the shape of the bright portion formed on the photodetecting means, a rate of change with respect to the minor axis direction becomes greater than that with respect to the major axis direction. As a result, abrupt changes occur in the focus error signal, and therefore response sensitivity in the focusing control can be improved.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art

are intended to be included within the scope of the following claims.

There are described above novel features which the skilled man will appreciate give rise to advantages. These are each independent aspects of the invention to be covered by the present application, irrespective of whether or not they are included within the scope of the following claims.

Claims

1. An optical head device comprising:

light generation means for generating light having intensity distribution showing a substantially elliptic shape in a cross section orthogonal to an optical axis, a major axis of the elliptic shape being parallel to a reference direction;

photodetecting means for generating detection signals so as to obtain a focus error signal, the photodetecting means including a plurality of photodetecting sections formed by a plurality of division lines dividing a light-receiving face thereof, the detection signal being released by the respective photodetecting sections according to the intensity of the light entered thereto, at least one of the plurality of division lines being substantially parallel to the reference direction;

diffraction means including a plurality of diffracting regions formed by a plurality of division lines dividing a diffraction face, at least one of the plurality of division lines for dividing the diffraction face being parallel to the reference direction; and

optical means for converging the light emitted from the light generation means on a recording track formed on the recording medium and further directing reflected light from the recording track to the diffraction means so that the major axis of an elliptic bright portion formed on the diffracting face by the reflected light may be positioned on said one of the division lines in the diffraction means, the division line being parallel to the reference direction.

whereby a value of the focus error signal is allowed to become zero when a part of the reflected light that has been diffracted by said one of the diffracting regions of the diffraction means is converged on said one of the division lines in the photodetecting means, which is substantially parallel to the reference direction.

2. An optical head device as set forth in claim 1, wherein the optical means comprises:

intensity distribution shaping means for shaping an intensity distribution of the light to be directed on the recording track into a substantially circular shape by diffusing the elliptic intensity distribution of the light emitted by the light generation means with respect to the minor axis, and permitting the intensity distribution of the light reflected from the recording track to restore its substantially elliptic shape by a reversed function to the above function.

3. An optical head device as set forth in claim 1, wherein the optical means comprises:

intensity distribution shaping means for shaping an intensity distribution of the light to be directed on the recording track into a substantially circular shape by condensing the elliptic intensity distribution of the light emitted by the light generation means with respect to the major axis, and permitting the intensity distribution of the light reflected from the recording track to restore its substantially elliptic shape by a reversed function to the above function.

4. An optical head device as set forth in claim 2 or claim 3, wherein the optical means further comprises:

parallel light beam forming means for allowing the light emitted from the light generation means to form a parallel beam of light.

5. An optical head device as set forth in claim 2 or claim 3, wherein the optical means further comprises:

converging means for converging on the recording track of the recording medium the light whose intensity distribution is shaped into a circular shape by the intensity distribution shaping means.

6. An optical head device as set forth in claim 4, wherein the parallel light beam forming means comprises:

collimating lens means for allowing the light to form a parallel beam of light, the light being emitted from the light generation means and transmitted through the diffraction means to be directed to the intensity distribution shaping means.

7. An optical head device as set forth in claim 1, wherein the diffraction means includes at least two diffracting regions formed by at least one division line parallel to the reference direction, the diffracting regions being provided with respective gratings whose pitch is different from each other; and

the photodetecting means includes a first photodetecting section and a second photodetecting section formed by a first division line that are contained within the light-receiving face and orthogonal to the reference direction, the

second photodetecting section being provided with a third photodetecting section and a fourth photodetecting section formed by a second division line extending from a middle point of the first division line substantially in parallel with the reference direction,

whereby a value of the focus error signal is allowed to become zero when a part of the reflected light having been diffracted by said one of the diffracting regions of the diffraction means is converged on the second division line in the photodetecting means.

8. An optical pickup device as set forth in claim 7, wherein the diffraction means includes two diffracting regions formed by one division line substantially parallel to the reference direction,

whereby, when the value of the focus error signal becomes zero, a part of reflected light diffracted by one of the diffracting regions is converged on the second division line, while the other part of the reflected light diffracted by the other diffracting region is converged on a substantial center of the first photodetecting section, and

when a detection signal released from a first photodetecting section is represented as Sa; a detection signal released from a third photodetecting section is represented as Sb; and a detection signal released from a fourth photodetecting section is represented as Sc, a reproduction signal of information recorded on the recording medium is obtained by a calculation of Sa + Sb + Sc; the focus error signal is obtained by a calculation of Sc - Sb; and a tracking error signal is obtained by a calculation of Sa - (Sb + Sc).

9. An optical head for projecting a light beam onto a recording track on an optical recording medium, the optical head including a light source which produces a light beam having an elliptical cross-section, an optical system for focussing the beam into a beam spot on said recording medium, a light detector for detecting light reflected from the recording medium and having a division line which separates two light detecting sections, and diffraction means disposed in the path of the reflected light where it forms a reflected beam of elliptical cross-section and having a division line which separates two diffraction regions for deflecting respective portions of the reflected beam impinging thereon away from the optical axis of the diffraction means toward respective positions of focus on said light detector, one of said positions lying on said division line of said light detector, whereby a focus error signal is derivable from detection signals obtained from said two light detecting sections, characterised in that the diffraction means is arranged so that the major axis of the cross-section of said reflected beam is substantially coincident with said division line of said diffraction means and in that said respective positions of focus are spaced apart in a direction corresponding to the direction of said major axis.

FIG. 1

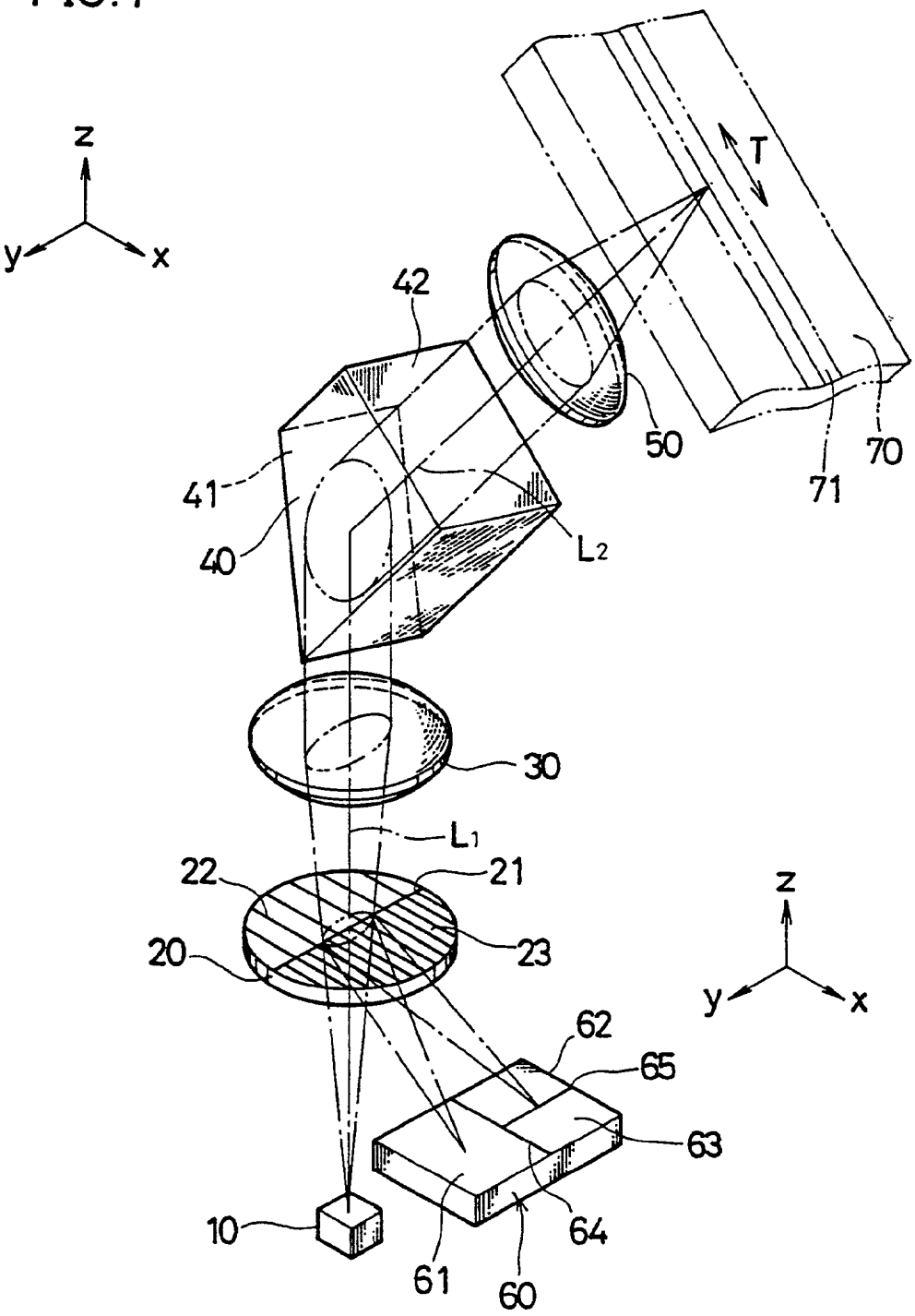


FIG. 2

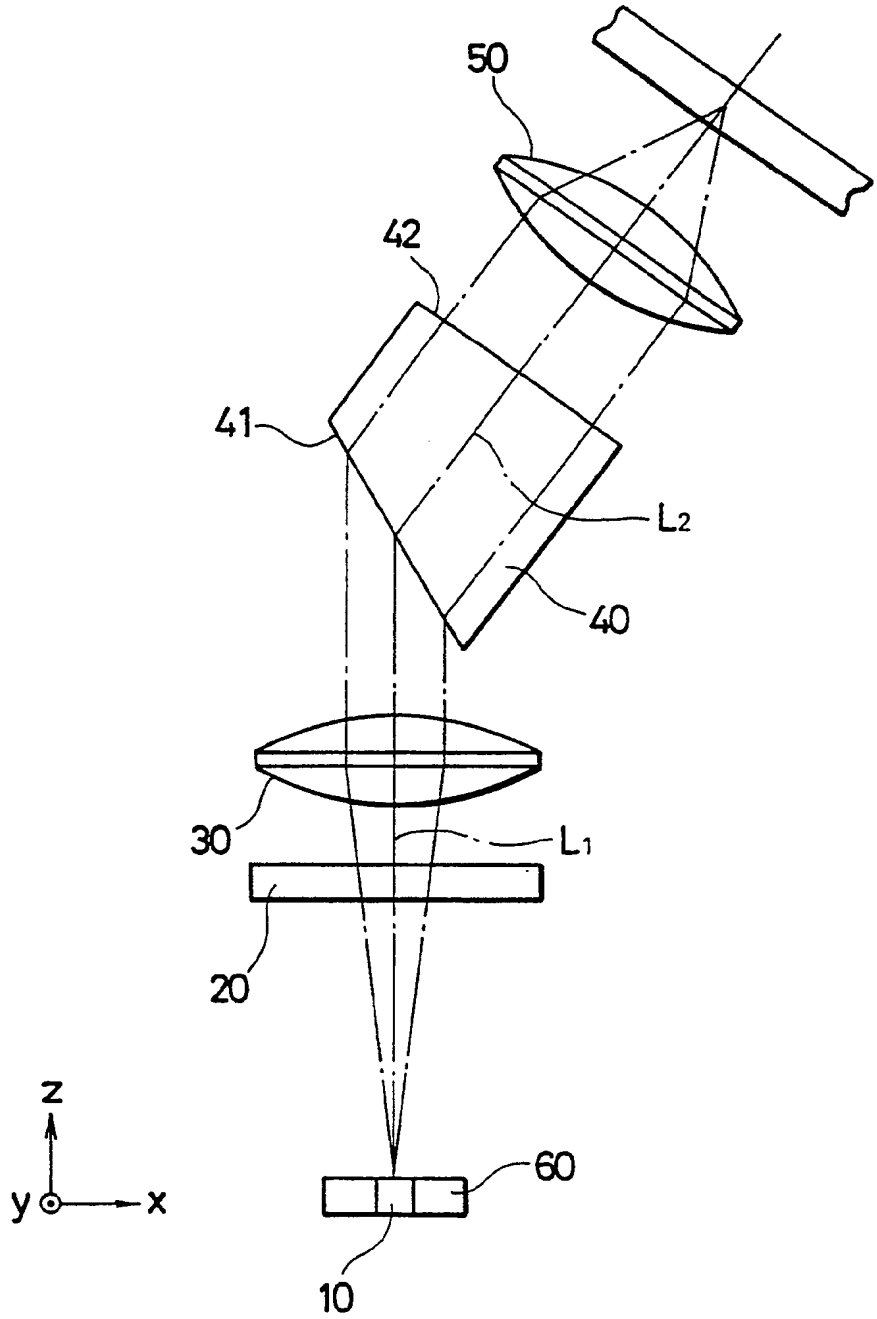


FIG. 3

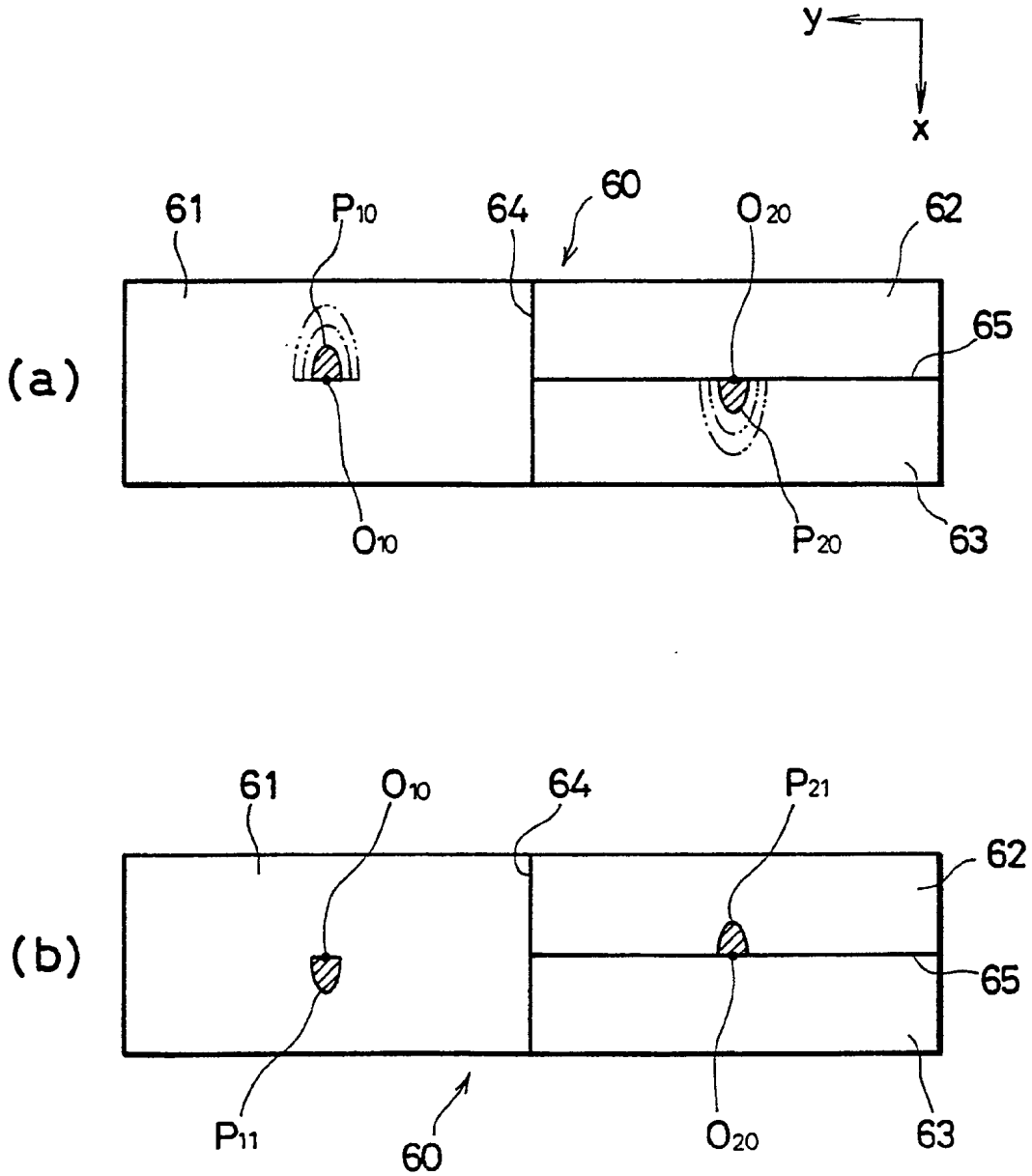

y
x
(a)
61
P10
64
60
O20
62
65
O10
P20
63
(b)
61
O10
64
P21
62
65
P11
60
O20
63

FIG. 4

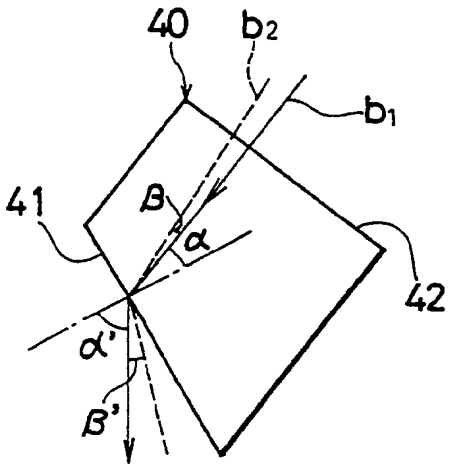


FIG. 5

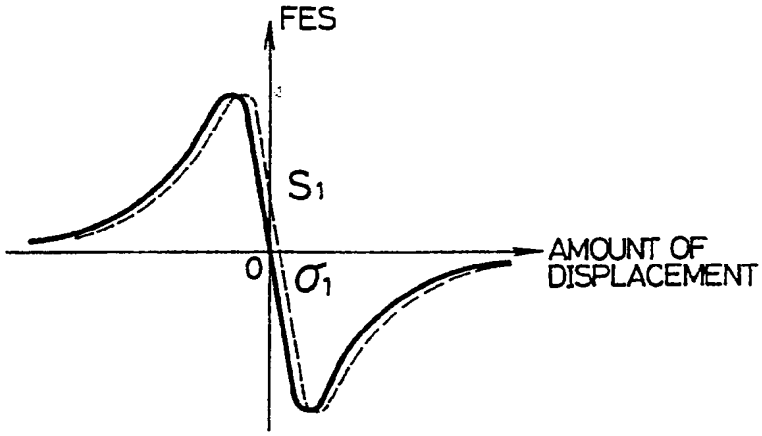


FIG. 6

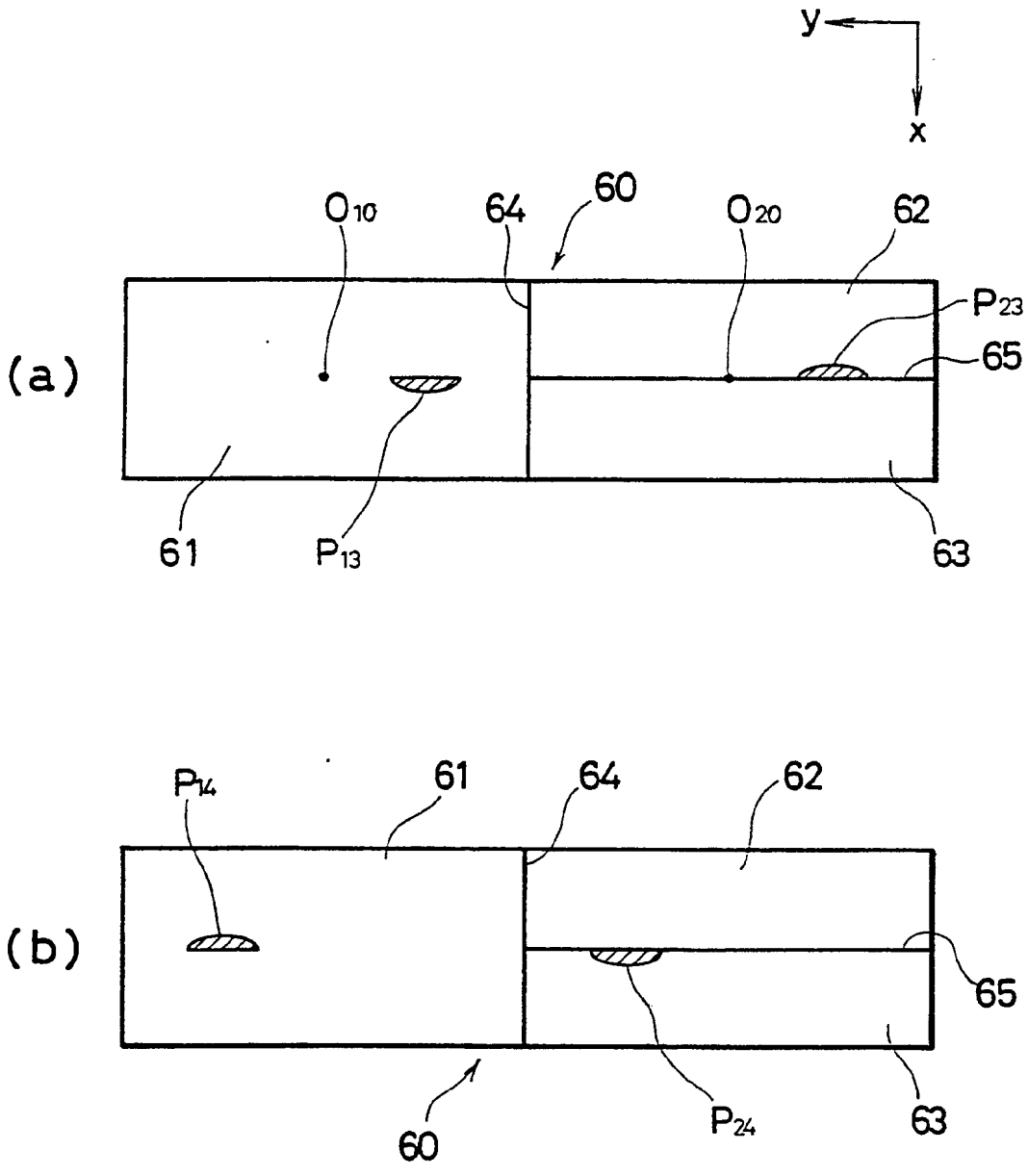


FIG.7

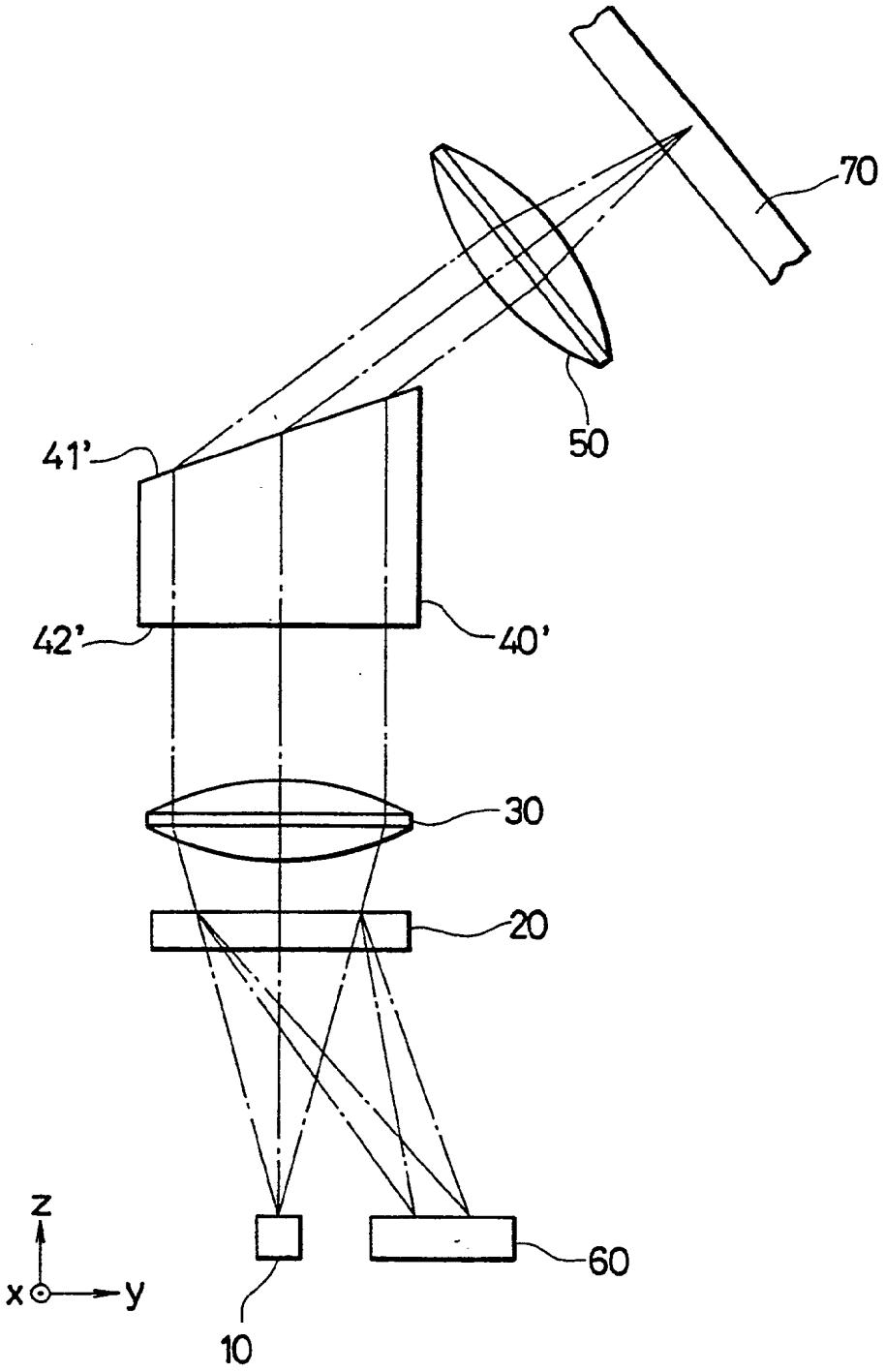


FIG. 8

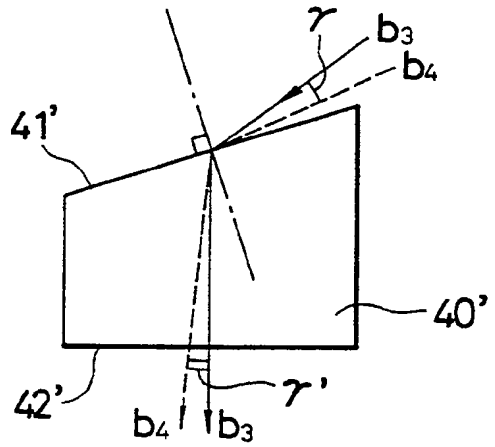


FIG. 9

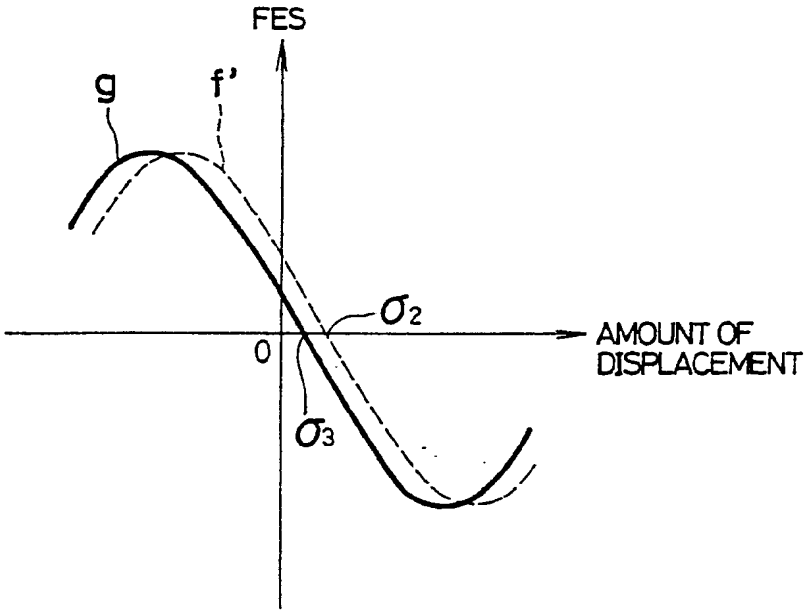


FIG. 10

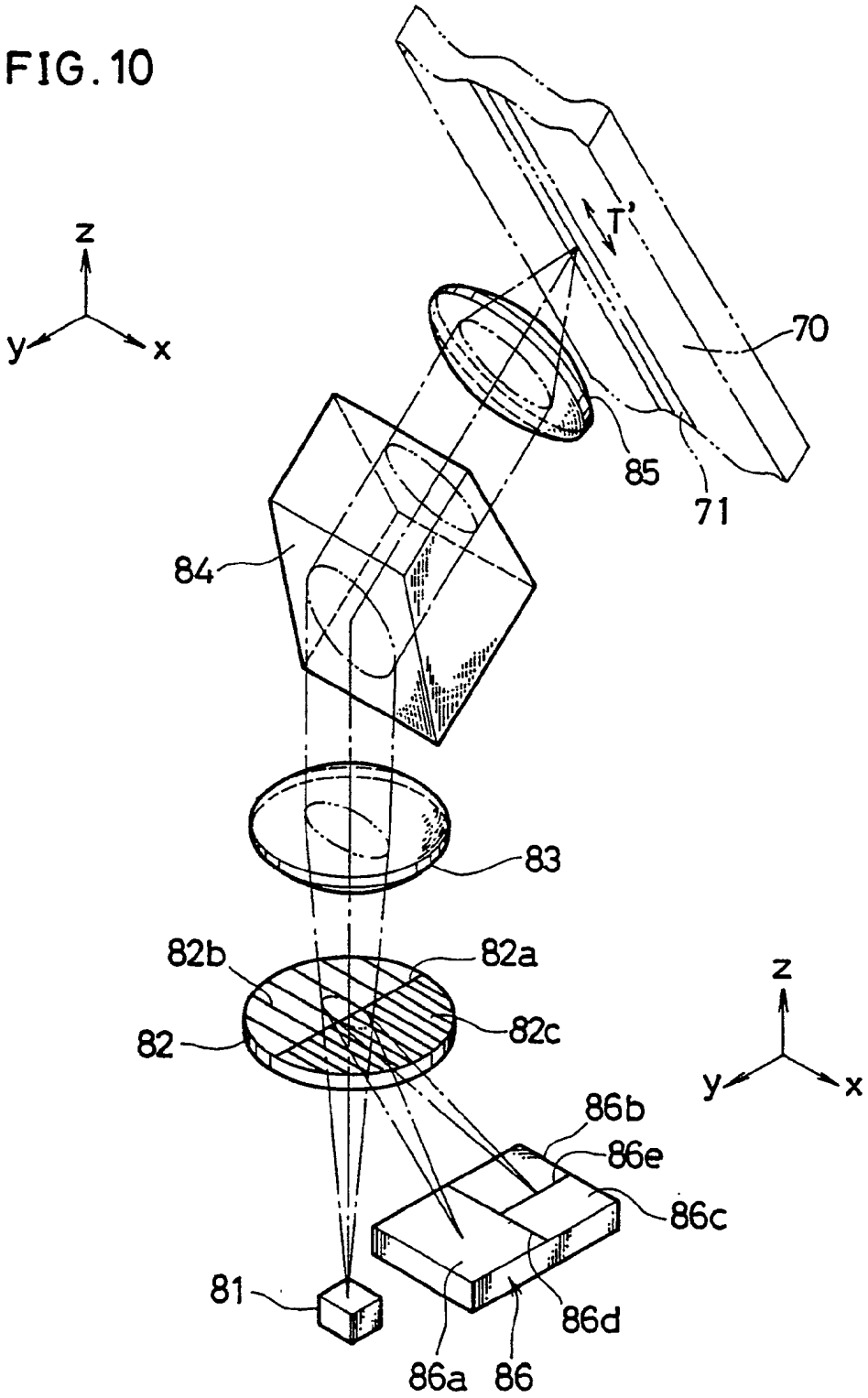


FIG.11

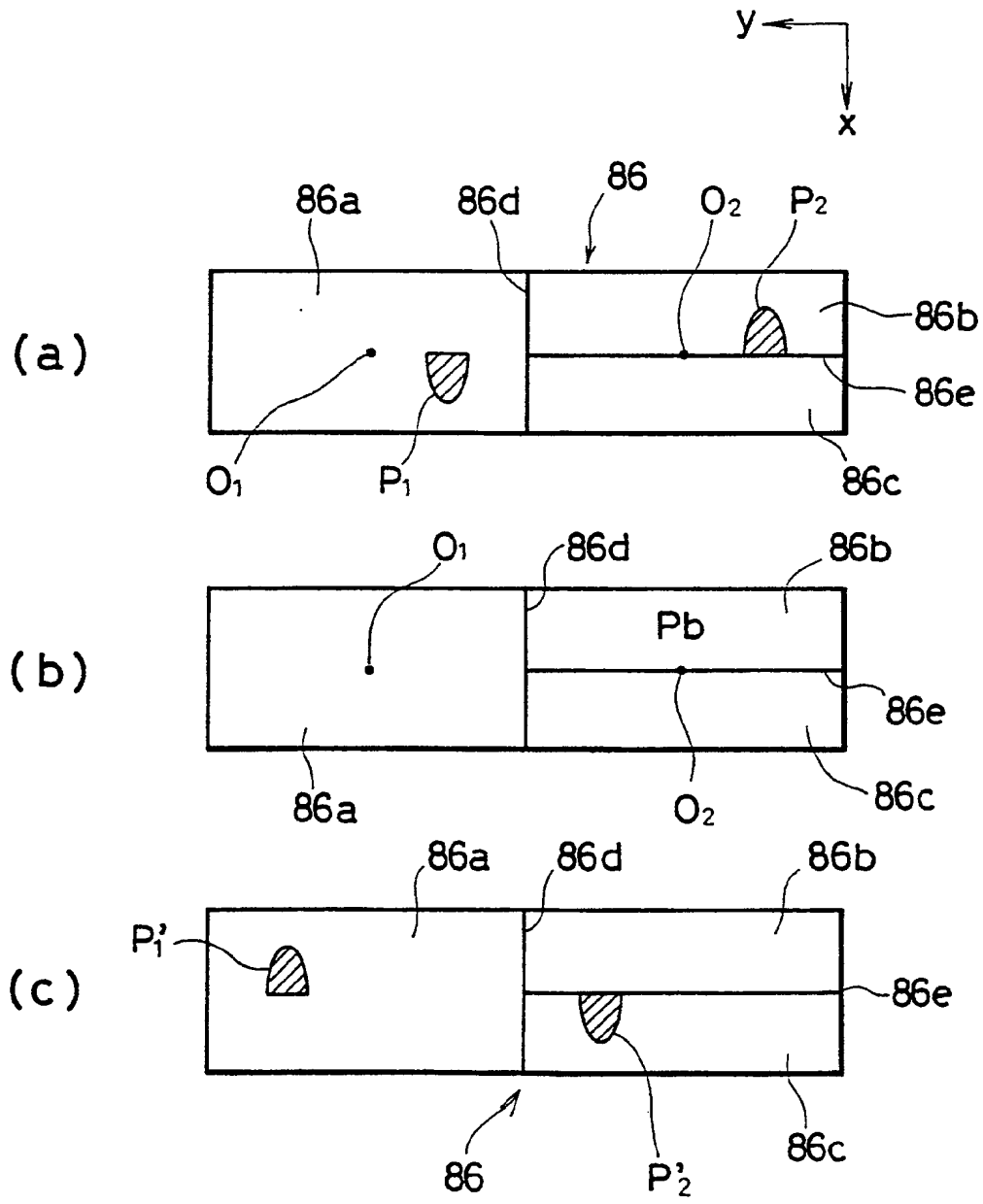

y
x
(a)
86a
86d
86
O2
P2
86b
86e
O1
P1
86c
(b)
O1
86d
86b
Pb
86e
86a
O2
86c
(c)
86a
86d
86b
P1'
86e
86
P'2
86c

FIG.12

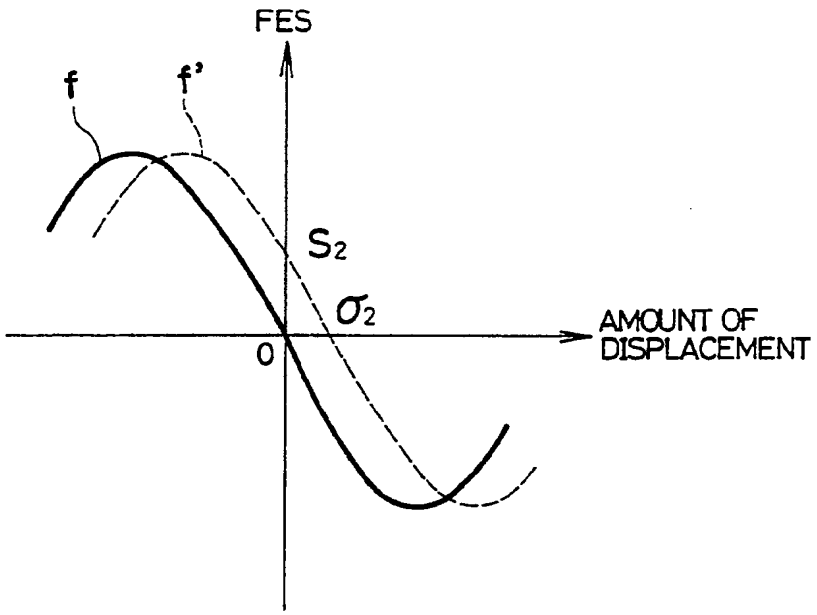


FIG. 13

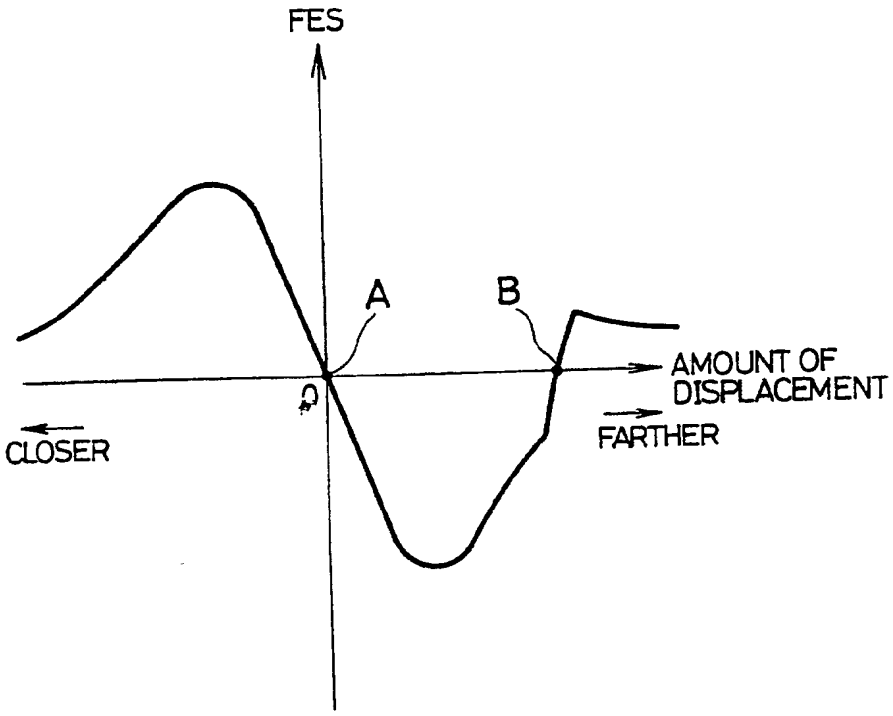

FES
A
B
AMOUNT OF DISPLACEMENT
0
CLOSER
FARTHER

FIG. 14

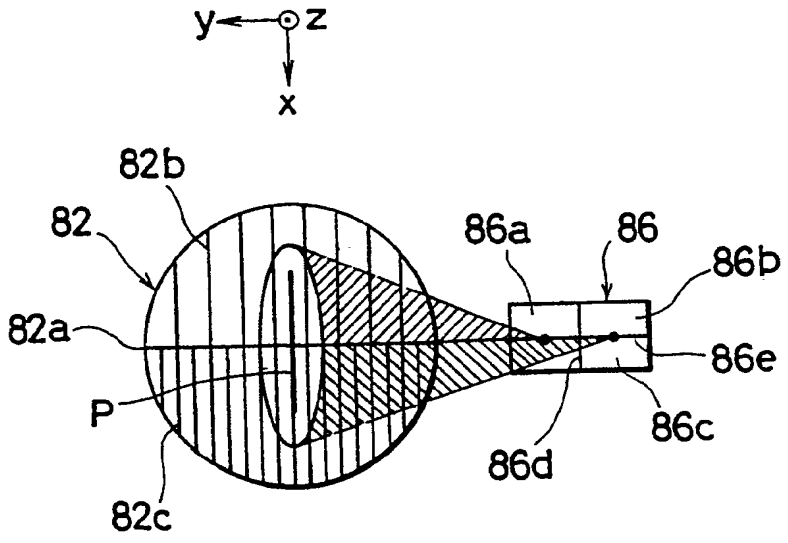

y
z
x
82b
82
86a
86
86b
82a
86e
P
86c
86d
82c

FIG. 15

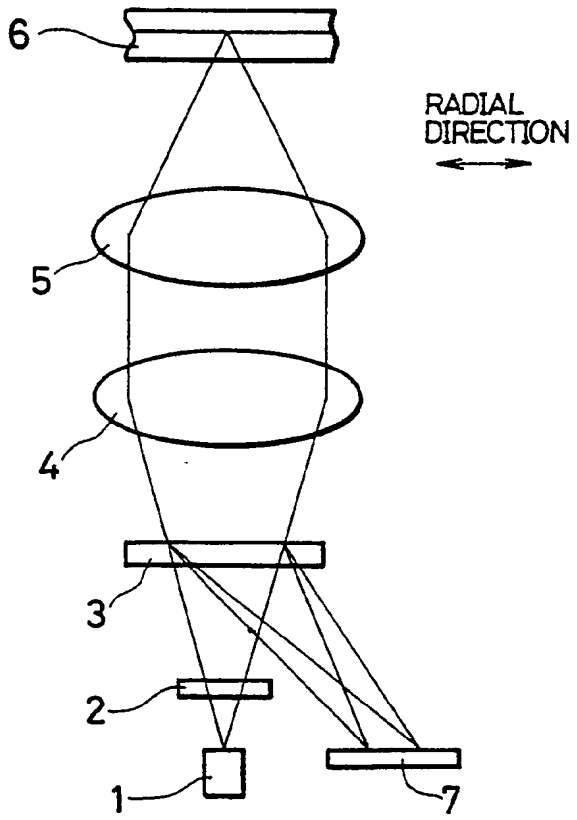


FIG. 16

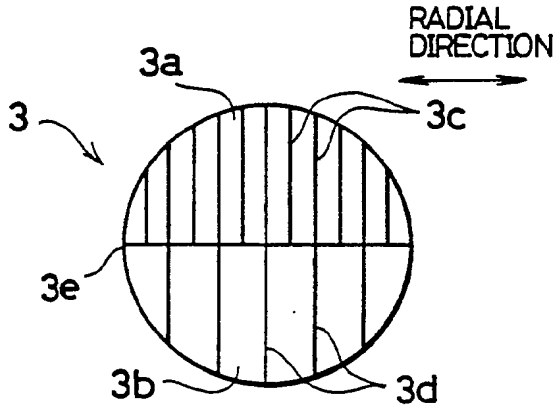

RADIAL
DIRECTION
3a
3
3c
3e
3b
3d

FIG. 17

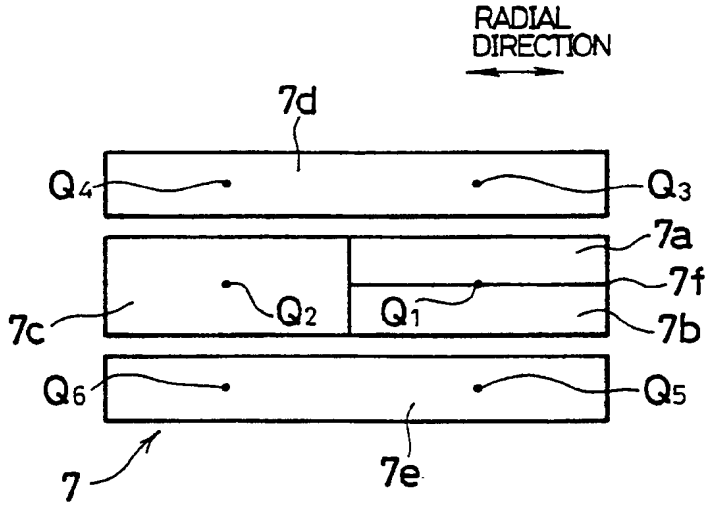

RADIAL
DIRECTION
7d
Q4
Q3
7a
7f
7c
Q2
Q1
7b
Q6
Q5
7
7e

FIG. 18

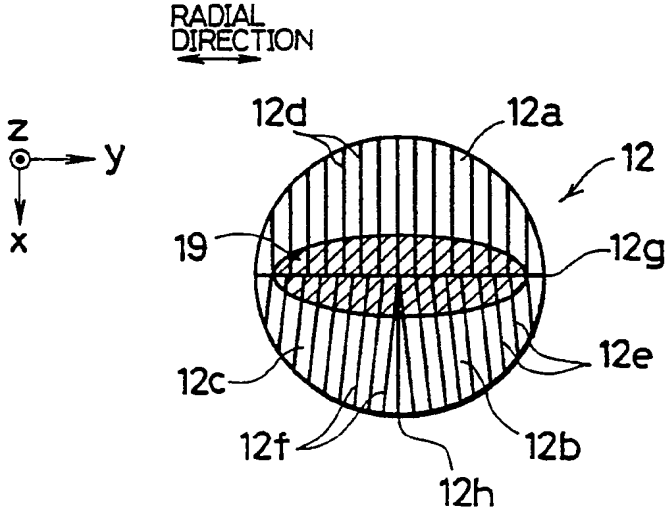
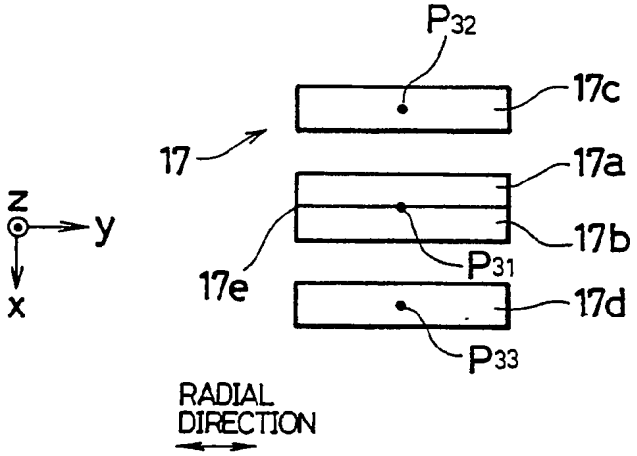

RADIAL
DIRECTION
z
y
x
12d
12a
12
19
12g
12e
12c
12f
12b
12h

FIG. 19


P32
17c
17
17a
z
y
x
17b
17e
P31
17d
P33
RADIAL
DIRECTION

(19) Europäisches Patentamt
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(22) Date of filing: **29.05.1991**

(54) **Optical head device**

Anordnung eines optischen Kopfes

Dispositif à tête optique

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- **PATENT ABSTRACTS OF JAPAN vol. 014, no. 050 (P-0998) 30 January 1990, & JP-A- 01 279433**

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Description

FIELD OF THE INVENTION

The present invention relates to an optical head device to be provided in optical recording-reproduction apparatuses for recording/reproducing information on or from a recording medium such as an optical disk.

BACKGROUND OF THE INVENTION

Optical head devices are well known to the art, which record and reproduce information by applying a light beam to a recording medium such as an optical disk. In a commonly used optical head device, a light beam emitted from a semiconductor laser as a light source passes through a diffracting element, and forms a parallel beam of light through a collimating lens. An intensity distribution of the parallel beam of light shows an elliptic shape in its cross section orthogonal to the optical axis, according to the intensity distribution of the light beam emitted from the semiconductor laser. Here, such an intensity distribution of light related to a cross section orthogonal to the optical axis, is hereinafter referred to simply as a cross-sectional intensity distribution. The elliptic cross-sectional intensity distribution is shaped into a substantially circular shape by a shaping prism in order to improve the efficiency of light utilization in the optical head device. The light beam having its cross-sectional intensity distribution shaped into the substantially circular shape, is converged on the recording medium by an object lens.

The light beam reflected from the recording medium (hereinafter referred to simply as a reflected beam) follows a light path in the reverse order to the above-mentioned, and has its cross-sectional intensity distribution restored to be an elliptic shape by the shaping prism. Thereafter, the reflected beam is directed to a diffracting element via the collimating lens, and diffracted by the diffracting element so as to be converged on a photodetector.

Normally, each of the diffracting element and the photodetector is divided into a plurality of regions by a plurality of division lines so as to obtain a focus error signal or a tracking error signal by adopting the knife-edge method or push-pull method. For example, in accordance with the knife-edge method, a focus error signal can be obtained by finding a difference of detecting signals released from two detecting regions adjacent to each other in the photodetector.

Fig. 10 shows one example of the FES (Focus Error Signal) curve showing the relationship between the intensity of the focus error signal thus obtained and the amount of the displacement of the optical disk based on the focal point of the object lens taken as a reference. In Fig. 10, a reference point A represents a case without a focus error. The reflected beam hits the diffracting element, and a converging point of the resulting beam diffracted toward the photodetector by the diffracting element varies in its position in front of or behind the photodetector in response to fluctuations of the recording medium from the position causing no focus error. As a result, since a shape of a light spot formed on the photodetector by the diffracted beam is reversed, the value of the focus error signal is reversed from positive to negative in accordance with the amount of the displacement of the recording medium.

In this case, as shown in Fig. 10, when the recording medium moves away from the focal point of the object lens to a certain extent, an undesired zero-cross point B appears on the FES curve at a position other than the reference point A. Especially in an optical pickup device using a shaping prism in its optical system, an undesired zero-cross point may appear within a dynamic range of focusing control, depending on what arrangement is selected with respect to the optical system. This is related to the fact that the fluctuation of the recording medium causes the shaping prism to have astigmatism.

Meanwhile, the zero-cross point in the FES curve provides a drive target for the object lens in focus servo control, and if a zero-cross point B appears at a position other than the reference point A as described above, the following problems arise. When focusing control is performed so as to move the object lens from a farther position toward a closer position with respect to the optical disk, for example, in the case of occurrence of an excessive focus error due to an external cause or an initial state of the device, the object lens might be moved to focus on the incorrect drive target derived from the undesired zero-cross point B. As a result, normal information recording or reproduction might not be performed.

In order to solve the above problems, the inventors of the present invention have devised an optical head device shown in Fig. 7 and disclosed in EP-0453323, relevant under Art.54(3) EPC. The optical head device is provided with a laser light source 81 which emits in the shape of a cone a light beam whose cross-sectional intensity distribution has an elliptic shape. The light beam passes through a diffracting element 82, and is directed to the collimating lens 83. The light beam formed into a parallel beam of light by a collimating lens 83 is directed to a shaping prism 84. The shaping prism 84 shapes the parallel beam of light in its cross-sectional intensity distribution from the elliptic shape into a round shape. Through an object lens 85, the light beam having been subject to the shaping process is converged onto a belt-like track 71 formed on an optical disk 70.

In Fig. 7, an arrow T' represents a microscopic rotation direction of the optical disk 70 at a converging point of the light beam converged thereon through the object lens 85. The object lens 85 is permitted to move closer to or farther from the optical disk 70 so as to always position the focal point of the object lens 85 just on the track 71 (this state is referred to as a just-in-focus state). Further, the object lens 85 is moved in a radial direction of the optical disk 70. The radial direction is

orthogonal to the rotation direction indicated by the arrow T'. The light beam reflected from the optical disk 70 (hereinafter referred to simply as a reflected beam) returns to the disc-shaped diffracting element 82 following the path to the optical disk 70 in the reverse order.

The following description will discuss in detail an arrangement of optical members employed in the above optical head device. For example, a z-axis is determined to be parallel to an optical axis of a light beam emitted from a laser light source 81. Optical axes of the diffracting element 82 and collimating lens 83 coincide with the optical axis of the light beam emitted from the laser light source 81. The diffracting element 82 is divided equally into two diffracting regions by a division line 82a. The diffracting regions are respectively provided with a first diffraction grating 82b and a second diffraction grating 82c for diffracting the reflected beam. A y-axis is determined to be parallel to the division line 82a, while an x-axis is set to be orthogonal to both the x and z-axes. Then, the laser light source 81 is arranged such that the elliptic cross-sectional intensity distribution of the light beam emitted from the laser light source 81 has its major axis set to be parallel to the x-axis. In other words, concerning the elliptic bright portion formed on the diffracting element 82 by the light beam emitted from the laser light source 81 or by the reflected beam from the optical disk 70, the major axis of the bright portion is parallel to the x-axis, and orthogonal to the division line 82a. Further, the minor axis of the bright portion is parallel to the y-axis, and substantially coincides with the division line 82a. Additionally, directions indicated by respective arrows of the x, y and z-axes are supposed to be the positive directions.

Grating lines of each of diffraction gratings 82b and 82c are substantially parallel to the x-axis and disposed with a predetermined spacing. Thus, parts of the reflected beam directed to the respective diffraction gratings 82b and 82c are diffracted in directions different from the direction toward the laser light source 81 and substantially parallel to the y-z plane containing the division line 82a. Here, the grating pitch of the first diffraction grating 82b is greater than that of the second diffraction grating 82c. That is, the second diffraction grating 82c has its grating lines more closely pitched than those of the first diffraction grating 82b, thereby making an diffraction angle of the light beam diffracted by the second diffraction grating 82c greater than that of the light beam diffracted by the first diffraction grating 82b.

A photodetector 86 is disposed alongside of the laser light source 81 in the negative direction of the y-axis. Portions of the reflected beam are diffracted by the diffraction gratings 82b and 82c toward the photodetector 86, and converged on a detecting face of the photodetector 86. The detecting face of the photodetector 86 is divided equally into two detecting regions by a first division line 86d extending in parallel with the x-axis. One of the detecting regions closer to the laser light source 81 is referred to as a first detecting region 86a. The other detecting region is divided equally into a second detecting region 86b and a third detecting region 86c by a second division line 86e extending along the y-axis.

In the above arrangement, the reflected beam derived from a light beam having a reference wave-length, converged on the optical disk 70 in the just-in-focus state, is divided equally into two beams by the respective diffraction gratings 82b and 82c of the diffracting element 82, and the two beams are diffracted at respective angles different from each other. One of the beams diffracted by the first diffraction grating 82b has a smaller diffraction angle, and is converged on a center of the first detecting region 86a. On the other hand, the other beam diffracted by the second diffraction grating 82c has a greater diffraction angle, and is converged on a center of the second division line 86e. The photodetector 86 respectively releases detection signals depending on intensities of the respective light beams converged on the detecting regions 86a, 86b and 86c.

The detection signals of the first, second and third detecting regions 86a, 86b and 86c are respectively denoted by Sa, Sb and Sc. Then, a focus error signal FES for use in focusing control of the light beam to be projected on the optical disk 70 is obtained by the difference of the detection signals of the second and third detecting regions 86b and 86c, that is, Sb - Sc. The object lens 85 is moved closer to or farther from the optical disk 70 so as to make the value of the focus error signal FES equal to "0". Thus, the light beam is projected on the optical disk 70 in the just-in-focus state.

Further, a radial error signal RES for use in tracking control of the light beam to be projected on the optical disk 70 is obtained by an equation; Sa - (Sb + Sc). The object lens 85 is moved in a radial direction of the optical disk 70 so as to make the value of the radial error signal RES equal to "0".

The optical head device which is arranged so that the minor axis of an elliptic bright portion formed on the diffracting element 82 by the reflected beam may coincide with the division line 82a, as described above, has the following advantages. As illustrated in Fig. 11, for example, in the case where the optical disk 70 is fluctuated to move away from a focal point of the object lens 85, there is formed a line-like bright portion on the diffracting element 82 due to the astigmatism of the shaping prism 84 (shown by a heavy line P in Fig. 11). The line-like bright portion P may be formed overlapping on the division line 82a depending on the alignment of optical members constituting the optical head device, or it may be formed being biassed onto the first diffraction grating 82b due to an installation error of the diffracting element 82 or other reasons. In this case, outputs of the detecting regions 86b and 86c become "0". As a result, since the value of the focus error signal FES shows "0" in spite of the presence of a focus error, an offset occurs in focusing control. However, with the advantageous arrangement that the line-like bright portion P is formed so as to be orthogonal to the division line 82a, even if

the division line 82a slightly tilts with respect to the x-axis, or slightly deviates from the optical axis, those factors have little adverse effects on the requirement that the bright portion P should be evenly allotted onto the regions 82b and 82c. Therefore, an appropriate focus error signal FES can be obtained.

Furthermore, it is avoidable to have the phenomenon that a semi-elliptic light spot is first formed either on the detecting regions 86b or on 86c, and then formed on the other region in an inverted shape as the optical disk 70 further moves away from the focal point of the object lens 85. (The value of the focus error signal FES undesirably shows "0" also in the case where a light spot first formed either on the detecting region 86b or 86c is then reversed in its position onto the other detecting region 86b or 86c). As described above, in the optical head device shown in Fig. 7, its focusing control is little adversely affected by fluctuations of the optical disk 70.

However, such a laser light source 81 employed in the above-mentioned optical head device, for example, a semiconductor laser, has a property that a wave-length of its projecting light beam varies depending on ambient temperature changes. Further, a diffraction angle of a light beam diffracted by a diffraction grating varies depending on its wave-length. In other words, the diffraction angle becomes greater as the wave-length of the light beam becomes longer.

For example, as shown in Fig. 8(b), when a light beam with a reference wave-length λ_0 emitted from the laser light source 81 is converged on the optical disk 70 in the just-in-focus state and reflected therefrom, a part of the resulting reflected beam directed to the first diffraction grating 82b is diffracted to be converged onto a center O_1 of the first detecting region 86a of the photodetector 86 to form a light spot. On the other hand, another part of the reflected beam directed to the second diffraction grating 82c is diffracted to be converged onto a center O_2 of the second division line 86e of the photodetector 86 to form a light spot. In this case, the value of the focus error signal FES becomes "0".

Meanwhile, in the case where the optical disk 70 is fluctuated with respect to the object lens 85 and a light beam projected on the optical disk 70 is not in the just-in-focus state, the focus error signal FES has an intensity responsive to an amount of displacement of the optical disk 70 from a focal point of the object lens 85 as a reference as shown by a solid line f in Fig. 9.

Here, suppose that ambient temperature has risen with respect to the laser light source 81, a wave-length λ of a light beam emitted from the laser light source 81 becomes longer than the reference wave-length λ_0. In this case, even if the light beam is projected on the optical disk 70 in the just-in-focus state, the resulting reflected beam reflected from the optical disk 70 and directed to the diffracting element 82 is diffracted with a greater angle than that of the reflected beam having the reference wave-length λ_0 as described earlier. For this reason, parts of the reflected beams with the wave-length λ, after having been diffracted by the respective diffraction gratings 82b and 82c, are focused in front of the detecting regions of the photodetector 86 before reaching them. As a result, as shown in Fig. 8(a), one of the beams diffracted by the first diffraction grating 82b is projected on the first detecting region 86a to form a semi-elliptic bright portion P_1 lying in the positive direction of the x-axis. On the other hand, the other beam diffracted by the second diffraction grating 82c is projected on the second detecting region 86b to form a semi-elliptic bright portion P_2 lying in the negative direction of the x-axis. The bright portions P_1 and P_2 are respectively positioned away from the center O_1 of the first detecting region 86a and the center O_2 of the second division line 86e in the departing direction from the laser light source 81. Further, as described earlier, the bright portions P_1 and P_2 are opposite to each other in their shapes with their respective major axes being parallel to the first division line 86d, that is, the x-axis.

Next, suppose that ambient temperature has dropped with respect to the laser light source 81, a wave-length λ' of a light beam emitted from the laser light source 81 becomes shorter than the reference wave-length λ_0. In this case, the beams diffracted by the respective diffraction gratings 82b and 82c have smaller diffraction angles. For this reason, the beams with the wave-length λ', after having been diffracted by the respective diffraction gratings 82b and 82c, are focused behind the detecting regions of the photodetector 86. As a result, as shown in Fig. 8(c), one of the beams diffracted by the first diffraction grating 82b is projected on the first detecting region 86a to form a semi-elliptic bright portion P_1' lying in the negative direction of the x-axis. The shape of the bright portion P_1' has an opposite orientation to that of the bright portion P_1. On the other hand, the other beam diffracted by the second diffraction grating 82c is projected on the second detecting region 86c to form a semi-elliptic bright portion P_2' lying in the positive direction of the x-axis. Similarly, the shape of the bright portion P_2' has an opposite orientation to that of the bright portion P_2. The bright portions P_1 and P_2 are respectively positioned away from the center O_1 of the first detecting region 86a and the center O_2 of the second division line 86e in the approaching direction to the laser light source 81.

As described earlier, the cross-sectional intensity distribution of the reflected beam directed to the diffracting element 82 shows an elliptic shape whose major axis is orthogonal to the division line 82a, that is, the y-axis, and further is equally allotted to the diffraction gratings 82b and 82c. Therefore, the reflected beam with the wave-length λ or λ' having a variation from the reference wave-length λ_0 is diffracted by the diffracting element 82 to form on the respective detecting regions of the photodetector 86 the bright portions P_1 and P_2 or P_1' and P_2' which show elliptic shapes with their respective major axes orthogonal to the y-axis.

Thus, for example, in the case where the wave-

length λ of a light beam is greater than the reference wave-length λ_0, even if the light beam is projected on the optical disk 70 in the just-in-focus state, the second detecting region 86b releases the detection signal Sb based on the intensity of the bright portion P_2 shown in Fig. 8(a). As a result, despite the fact that the optical disk 70 is situated at an appropriate position with respect to the object lens 85, a focus error signal FES (= Sb - Sc) having a value of $S_2(> 0)$ is produced as is shown by a broken line f' in Fig. 9. The focus error signal FES having a positive value indicates that the optical disk 70 is fluctuated to approach the object lens 85. Therefore, in order to make the value of the focus error signal FES set to "0", the object lens 85 is moved so that the optical disk 70 may be located away from the focal point of the object lens 85 by an offset value σ_2. This causes the light beam to be focused before reaching the optical disk 70, thereby disturbing an accurate recording or reproducing operation for information.

EP-A-0453323 also discloses an optical head device in which the major axis of the elliptical cross-section of the beam emitted by the laser light source, and the major axis of the elliptical light portion formed on the diffracting element by the reflected light beam are parallel to the division line dividing the diffraction face of the diffracting element. Nevertheless in this device, the beam shaping prism condenses the beam in relation to the major axis of the elliptical-shaped beam contrary to the present invention in which the beam is diffused in relation to the minor axis.

EP-A-0 457 573, also relevant under Art. 54(3) EPC, discloses another optical head device. A diffracting element which directs reflected light from the recording medium towards a light receiving element has two tracking diffracting regions separated by a division line extending in a direction corresponding to a track direction of a recording medium, and one focusing diffracting region separated from the tracking diffracting regions by a division line extending in a direction corresponding to a radial direction of the recording medium.

EP-A-0 338 840 discloses an optical pickup device in which a diffracting element in the light path between a lith source and a recording medium is divided into two sub-regions. The first causes diffraction in the track direction to form three spots on the medium spaced along the track. The other sub-region causes diffraction perpendicular to the track direction to deflect the reflected beams of the three spots onto respective portions of a photodetector. JP-A-1279433 discloses another optical pickup in which beam shaping and beam separation are implemented by a single diffracting rating.

SUMMARY OF THE INVENTION

It is an aim of the present invention to provide an optical head device wherein offsets in focusing control due to wave-length variation of light to be converged on a recording medium can be suppressed and response sensitivity in focusing control can be improved.

According to the present invention there is provided an optical head device comprising:

a light generation means for generating a light beam having a substantially elliptical intensity distribution in cross section orthogonal to the beam's optical axis, the major axis of the elliptic shape being parallel to a reference direction;

photodetecting means for generating detection signals so as to enable the derivation of a focus error signal, the photodetecting means including a plurality of photodetecting sections formed by a plurality of division lines dividing a light-receiving face thereof, the detection signals being released by respective said photodetecting sections according to the intensity of the light received thereby, at least one of the plurality of division lines being substantially parallel to the reference direction;

diffraction means having a diffraction face divided into two diffracting regions by a single division line which extends parallel to the reference direction; and

optical means for converging the light emitted from the light generation means on a recording track formed on a recording medium and further directing reflected light from the recording track to the diffraction means so that the major axis of an elliptic bright portion formed on the diffracting face by the reflected light may be positioned on said division line of the diffraction means, said optical means including intensity distribution shaping means for shaping an intensity distribution of the light to be directed on the recording track into a substantially circular shape by diffusing the elliptic intensity distribution of the light emitted by the light generation means with respect to the minor axis, and permitting the intensity distribution of the light reflected from the recording track to restore its substantially elliptic shape by a reversed function to the above diffusing function,

whereby a value of the focus error signal derived from the detection signals released by two photodetecting sections separated by said one of the division lines which is substantially parallel to the reference directions is allowed to become zero so as to indicate an in-focus state of said converged light beam on said recording medium when a part of the reflecting light that has been diffracted by one of the two diffracting regions of the diffraction means is converged on said one of the division lines of the photodetecting means.

In the above arrangement, the light generation means projects light whose intensity distribution has an elliptic shape with its major axis parallel to the reference direction, and the division line of the diffraction means is set to be parallel to the reference direction. Thus, the reflected light directed to the diffraction means forms an

elliptic bright portion whose major axis is situated on the division line of the diffraction means. Provided that there is no focus error and further there is no wave-length variation with respect to light generated by the light generation means, the diffraction means diffracts reflected light that has directed to one of the diffracting regions so that the reflected light may be converged on the division line in the photodetecting means which is substantially parallel to the reference direction. At this time, the value of the focus error signal obtained based on detection signals released by the respective photodetecting sections is permitted to become "0".

On the other hand, occurrence of a wave-length variation in the light generated by the light generation means causes a variation in the diffraction angle of the reflected light directed to the diffraction means. This causes the position of a converging point, which was on the light-receiving face of the photodetecting means in the case where there is no focus error and further there is no wave-length variation with respect to the light generated by the light generation means, to vary in a direction orthogonal to and parallel to the light-receiving face. As a result, in the vicinity of the division line which is substantially parallel to the reference direction in the photodetecting means, is formed a thin semi-elliptic bright portion whose major axis of the elliptic shape is parallel to the division line. In other words, even in the case of no focus error, if there is a wave-length variation, the value of the focus error signal does not become "0" due to the thin semi-elliptic bright portion formed in the photodetecting means.

However, an offset value of the focus error signal caused by the wave-length variation is extremely suppressed because of the facts that detection sensitivity is low in the vicinity of the division lines in the photodetecting means; and the bright portion formed on the photodetecting means lies in a thin shape along the division line.

Moreover, the bright portion formed on the diffracting face of the diffraction means, derived from the reflected light, has an elliptic shape whose minor axis is orthogonal to the reference direction. The effect of the intensity distribution shaping means which diffuses the elliptic intensity distribution of the light emitted by the light generation means in the minor axis direction of the elliptic shape, is to cause the shape of the bright portion formed on the diffracting face to change depending on changes in amount of focus error. A rate of change in the minor axis direction becomes greater than that in the major axis direction. With this arrangement, in conjunction with the shape of the bright portion formed on the photodetecting means, a rate of change in the minor axis direction becomes greater than that in the major axis direction, resulting in abrupt changes in the focus error signal. Therefore, response sensitivity in the focusing control can be improved.

Embodiments of the invention will now be described by way of example with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view showing a structural example of an optical head device according to the present invention.

Fig. 2 is a side view of the optical head device shown in Fig. 1.

Fig. 3 is a plan view showing shapes of bright portions formed on a photodetector caused by a fluctuation of an optical disk.

Fig. 4 is an explanatory drawing showing an optical function of a shaping prism.

Fig. 5 is a graph showing the relationship between the amount of displacement of the optical disk, shown in Fig. 1, based on a focal point of an object lens as a reference and the variation of intensity of a focus error signal FES.

Fig. 6 is a plan view showing shapes of bright portions formed in the photodetector caused by a wavelength variation of a light beam.

Fig. 7 is a perspective view showing a structural example of an optical head device.

Fig. 8 is a plan view showing shapes of bright portions formed in the photodetector caused by a wavelength variation of a light beam in conjunction with the optical head device of Fig. 7.

Fig. 9 is a graph showing the relationship between the amount of displacement of the optical disk, shown in Fig. 7, based on a focal point of an object lens as a reference and the variation of intensity of a focus error signal FES.

Fig. 10 is a graph showing an example of the FES curve containing an undesired zero-cross point.

Fig. 11 is a schematic plan view of a diffracting element and the photodetector shown in Fig. 7.

Fig. 12 is a side view of an optical head device as one structural example, which is capable of obtaining a desirable tracking error signal.

Fig. 13 is a plan view showing a second diffracting element in the optical head device of Fig. 12.

Fig. 14 is a plan view showing a photodetector in the optical head device of Fig. 12.

DESCRIPTION OF THE EMBODIMENT

Referring to Figs. 1 to 6, the following description will discuss one embodiment of the present invention.

First, as shown in Figs. 1 and 2, three axis rectangular coordinates x-y-z are determined as follows in conjunction with an optical head device of the present invention. The z-axis is determined to be parallel to an optical axis L_1 of a light beam emitted from a laser light source 10 as light generation means. The y-axis is determined to be orthogonal to the z-axis and further parallel to a hypothetical line extending through a projecting point of the laser light source 10 and the center of a light-

receiving face of a photodetector 60 as photodetecting means (or a converging point on the photodetector 60 in a just-in-focus state), while the x-axis is determined to be orthogonal to both the y and z-axes. Additionally, the y-axis can be determined so as to be parallel to a division line 21 of a diffracting element 20, which are described later. The direction parallel to the y-axis is hereinafter referred to as a reference direction. Further, directions as indicated by arrows of the respective x, y and z-axes in Figs 1 are supposed to be the respective positive directions. Additionally, Fig. 2 is a side view of the optical head device of Fig. 1, seen from the positive side toward the negative side along the y-axis.

A light beam emitted by the oscillation of the laser light source 10 gradually spreads to have an intensity distribution of an elliptic shape in its cross section orthogonal to the optical axis L_1. (Additionally, such an intensity distribution of light, related to a cross section orthogonal to the optical axis, is hereinafter referred to simply as a cross-sectional intensity distribution.) Here, the laser light source 10 is arranged so that the major axis of the elliptic shape is parallel to the y-axis, that is, the reference direction.

Moreover, respective centers of the diffracting element 20, a collimating lens 30 and an end face 41 of a shaping prism 40 are aligned along the optical axis L_1. Here, the end face 41 is a surface to receive the light beam emitted by the laser light source 10. The diffracting element 20 for transmitting the light beam emitted by the laser light source 10 is of a disc-shape, square-shape, elliptic-shape, etc. whose surfaces are parallel to the x-y plane. The light beam transmitted through the diffracting element 20 passes through the collimating lens 30. At this time, the collimating lens 30 produces a parallel beam of light from the light beam spreading in the shape of cone, directed thereto. Similarly, a cross-sectional intensity distribution of the parallel beam of light projected from the collimating lens 30 shows an elliptic shape whose major axis is parallel to the y-axis, that is, the reference direction.

The parallel beam of light projected from the collimating lens 30 is directed to the end face 41 of the shaping prism 40. The end face 41 is inclined with respect to the y-z plane and x-y plane. Therefore, the intensity distribution of the parallel beam of light is diffused in the minor axis direction by the end face 41 to form a substantially round shape. The parallel beam of light having impinged upon the end face 41 proceeds along the optical axis L_2 in the shaping prism 40 and is projected from the other end face 42. As shown in Fig. 2, since the end face 42 is orthogonal to the optical axis L_2, the parallel beam of light is projected from the end face 42 in a direction parallel to the optical axis L_2 and directed to the object lens 50.

The object lens 50 is disposed so as to have its optical axis coincided with the optical axis L_2, and converges the parallel beam of light on a recording track 71 formed on an optical disk 70 as a recording medium. A recording surface of the optical disk 70 is placed perpendicular to the optical axis L_2. A tangent direction to the rotatable recording track 71 at a converging point is represented by an arrow T in the drawing. Additionally, the tangent direction T is parallel to the x-z plane.

The light beam reflected from the recording track 71 (hereinafter referred to simply as the reflected beam) successively passes through the object lens 50, shaping prism 40 and collimating lens 30 following in the reverse order the path whereby the light beam was directed to the recording track 71. The reflected beam having passed through the shaping prism 40 forms a parallel beam of light. The reflected beam projected from the end face 41 has its intensity distribution returned from the round shape to an elliptic shape. Further, the reflected beam is directed to the diffracting element 20 by the collimating lens 30 along the path converging on the projecting point of the laser light source 10.

The diffracting face of the diffracting element 20 is equally divided into two regions by a division line 21 parallel to the y-axis, that is, the reference direction. Therefore, an elliptic bright portion formed on the diffracting element 20 by the reflected beam directed thereto has its major axis positioned substantially on the division line 21. The diffracting regions separated by the division line 21 are provided with respective first and second diffraction gratings 22 and 23. Grating lines formed on each diffraction grating 22 or 23 have a predetermined pitch and are orthogonal to the division line 21, that is, form a number of parallel lines extending in the x-direction. Moreover, the pitch of the grating lines in the first diffraction grating 22 is set to be greater than that in the second diffraction grating 23. With the above arrangement, each diffracting grating 22 or 23 diffracts a part of the reflected beam directed thereto in a direction substantially parallel to the y-z plane including the division line 21. Here, a diffraction angle with respect to the part of the reflected beam diffracted by the first diffraction grating 22 toward the photodetector 60 (hereinafter referred to simply as the first diffracted beam) is set to be smaller than that with respect to another part of the reflected beam diffracted by the second diffraction grating 23 toward the photodetector 60 (hereinafter referred to simply as the second diffracted beam).

The diffracted beams are directed to the photodetector 60. The photodetector 60 has a photodetecting face whereon these diffracted beams are projected. The photodetecting face is equally divided into two regions by a first division line 64 parallel to the x-axis. One of the photodetecting regions positioned by the side of the laser light source 10 is referred to as a first detecting region 61. The other photodetecting region is further equally divided into two detecting regions, that is, a second detecting region 62 and a third detecting region 63 by a second division line 65 which is substantially parallel to the y-axis. The detecting regions 61, 62 and 63 respectively release detection signals according to intensities of the respective diffracted beams directed

thereto.

When a light beam having a reference wave-length λ_0, emitted from the laser light source 10 is converged on the recording track 71 in the just-in-focus state, the resulting reflected beam from the recording track 71 is diffracted by the diffraction grating 22 and 23 so as to be divided into two parts. Then, as illustrated in Figs. 3 (a) and 3(b), the first diffracted beam is converged on a center O_{10} of the first detecting region 61, while the second diffracted beam is converged on a center O_{20} of the second division line 65.

In an optical head device having the above arrangement, the object lens 50 is controlled according to detection signals released by the respective detecting regions 61, 62 and 63. The following description will discuss how the control of the object lens 50 is performed.

First, as illustrated in Fig. 3(a), in a state where the object lens 50 is located close to the optical disk 70, the second diffracted beam derived from the second diffraction grating 23 having a relatively narrower pitch of grating, forms a bright portion P_{20} on the third detecting region 63 in the vicinity of the center O_{20}. The bright portion P_{20} has a semi-elliptic shape lying in the positive direction of the x-axis, and its major axis is parallel to the first division line 64, that is, the x-axis. On the other hand, the first diffracted beam derived from the first diffraction grating 22 having a relatively wider pitch of grating, forms a bright portion P_{10} in the vicinity of the center O_{10}. The bright portion P_{10} has a semi-elliptic shape lying in the negative direction of the x-axis, and its major axis is parallel to the x-axis as with the aforementioned case. Thus, the bright portions P_{10} and P_{20} are substantially opposite to each other in their shapes.

Meanwhile, as illustrated in Fig. 3(b), in a state where the object lens 50 is located far away from the optical disk 70, the second diffracted beam forms a bright portion P_{21} on the second detecting region 62 in the vicinity of the center O_{20}. The bright portion P_{21} has a semi-elliptic shape lying in the negative direction of the x-axis, which is opposite to the above-mentioned case having the object lens 50 located close to the optical disk 70, and its major axis is parallel to the x-axis as with the above-mentioned case. On the other hand, the first diffracted beam forms a bright portion P_{11} in the vicinity of the center O_{10} of the first detecting region 61. Similarly, the bright portion P_{11} has a semi-elliptic shape lying in the positive direction of the x-axis, which is opposite to the above-mentioned case having the object lens 50 located close to the optical disk 70, and its major axis is parallel to the x-axis as with the above-mentioned case. The bright portions P_{11} and P_{21} are substantially opposite to each other in their shapes.

As illustrated in Fig. 3(a), each shape of the bright portions P_{10} and P_{20} is deformed with its center remained the same, according to an amount of displacement of the optical disk 70 based on a focal point of the object lens 50 as a reference. (The same is true with respect to the shapes of the bright portion $P_{11}$and P_{21}.)

Here it is noted that in the optical head device of the present invention shown in Fig. 1, rate of change of the shapes of the bright portions P_{10} and P_{20} with respect to the x-direction (that is, rate of enlargement or rate of reduction) is greater than rate of change of those in the optical head device shown in Fig. 7.

This is because of optical properties of the shaping prism 40. In other words, in the optical head device shown in Fig. 1, a reflected beam which is produced from the light beam focused on the optical disk 70 is transmitted through the shaping prism 40 and projected therefrom in a manner as shown by a solid line in Fig. 4. In Fig. 4, suppose the reflected beam b_1 from the optical disk 70 hits the end face 41 of the shaping prism 40 at an angle α from the collimating lens 30 side, and let us denote an angle of refraction of the reflected beam b_1 by α' and a refractive index of the shaping prism 40 by n. Then using Snell's Law, the following equation holds:

$$n \cdot \sin \alpha = \sin \alpha'. \quad (1)$$

Suppose a light beam directed onto the optical disk 70 has a focus error, thereby causing a reflected beam b_2 from the optical disk 70 to tilt with respect to the reflected beam b_1. Further, a difference of an incident angle of the reflected beam b_2 and the incident angle α of the reflected beam b_1 with respect to the end face 41 is denoted by β, and a difference of an angle of refraction of the reflected beam b_2 and the angle of refraction α' of the reflected beam b_1 is denoted by β'. Then, a relationship between β and β' is given by the following equation:

$$\beta' = \sin^{-1}(n \cdot \sin \alpha) - \sin^{-1}\{n \cdot \sin(\alpha - \beta)\}. \quad (2)$$

The equation (2) shows that the difference β' in the angles of refraction of the respective reflected beams at the end face 41 of the outgoing side is amplified in comparison with the difference β when there occurs a focus error in the light beam to be projected on the optical disk 70. In other words, in the case of the optical head device shown in Fig. 1, shaping function of the shaping prism 40 is amplified with respect to the direction parallel to the minor axis of the cross-sectional intensity distribution of the light beam, that is, the x direction. Therefore, since rate of change of the shapes of the bright portions P_{10} and P_{20} with respect to the x direction is greater than rate of change of those with respect to the y direction, each of the bright portions P_{10} and P_{20} has a prolonged semi-elliptic shape lying in the x direction, as shown in Fig. 3(a).

Suppose that detection signals released from the detecting regions 61, 62 and 63 are represented respectively as Sa, Sb and Sc. Then, a focus error signal FES corresponding to a deviation between the optical disk

70 and the focal point of the object lens 50 is obtained by calculating a difference of the detection signals Sb and Sc; (Sb - Sc). Fig. 5 shows an example of the FES curve with respect to the optical head device shown in Fig. 1. As described earlier, when the shaping prism 40 shapes the cross-sectional intensity distribution not in the major-axis direction but in the minor-axis direction, the shapes of the bright portions P_{10} and P_{20} formed on the photodetector 60 change greatly in the x direction. For this reason, as shown by a solid line in Fig. 5, its FES intensity shows an abrupt change in the vicinity of a zero-cross point on the FES curve.

On the other hand, in the case of the optical head device shown in Fig. 7, a shaping prism 84 shapes a cross-sectional intensity distribution in the minor-axis direction, and the minor axis is set to be parallel to the y direction. For this reason, unlike the case of the bright portions P_{10} and P_{20}, rate of shape change of bright portions P_1 and P_2 formed on a photodetector 86 shown in Fig. 8, is substantially small with respect to the x direction. Therefore, as shown by a solid line in Fig. 9, its FES intensity shows a gradual change in the vicinity of a zero-cross point on an FES curve f in comparison with the FES curve shown in Fig. 5.

Consequently, when a converging state of the light beam on the optical disk 70 slightly deviates from the just-in-focus state, rate of change in FES intensity is relatively great in the optical head device shown in Fig. 1. Therefore, a higher response in focusing control can be obtained.

Next, in the case where ambient temperature has risen with respect to the laser light source 10, causing a wave-length λ of a light beam emitted from the laser light source 10 to become longer than the reference wave-length λ_0, each of diffraction angles at the diffraction gratings 22 and 23 becomes greater. Then, each of the diffracted beams has an extended light path to the photodetector 60. That is, the first and second diffracted beams derived from the reflected beam with the wave-length λ are respectively focused before reaching the detecting face of the photodetector 60. As a result, as illustrated in Fig. 6(a), a bright portion P_{13} formed on the first detecting region 61 by the first diffracted beam is dislocated from the center O_{10} of the first detecting region 61 to move away from the laser light source 10 along the y direction, that is, the reference direction. Similarly, a bright portion P_{23} formed on the second detecting region 62 by the second diffracted beam is dislocated from the center O_{20} of the second division line 65 to move away from the laser light source 10 along the reference direction.

Each of the bright portions P_{13} and P_{23}, unlike those shown in Fig. 3, shows a semi-elliptic shape whose major axis is parallel to the second division line 65. These shapes are derived from the fact that the bright portion formed on the diffracting element 20 by the reflected beam from the optical disk 70 has an elliptic shape whose major axis is parallel to the division line 21. Further, since each focal point of the diffracted beams is formed in front of the detecting face of the photodetector 60, the orientations of the bright portions P_{13} and P_{23} lying in the x directions from the major axis are the same as those of the bright portions P_{11} and P_{21} (see Fig. 3 (b)) which are obtained in the case of moving the optical disk 70 away from the object lens 50. Therefore, the bright portion P_{23} formed on the second detecting region 62 lies in the negative direction of the x-axis, while the bright portion P_{13} lies in the positive direction of the x-axis.

On the contrary, in the case where ambient temperature has dropped with respect to the laser light source 10, causing a wave-length λ' of a light beam emitted from the laser light source 10 to become shorter than the reference wave-length λ_0, each of diffraction angles at the diffraction gratings 22 and 23 becomes smaller. For this reason, the first and second diffracted beams derived from the reflected beam with the wave-length λ' are respectively focused behind the detecting face of the photodetector 60. As a result, as illustrated in Fig. 6(b), a bright portion P_{14} formed on the first detecting region 61 by the first diffracted beam is dislocated from the center O_{10} of the first detecting region 61 to be positioned closer to the laser light source 10 along the reference direction. Similarly, a bright portion P_{24} formed on the third detecting region 63 by the second diffracted beam is dislocated from the center O_{20} of the second division line 65 to be positioned closer to the laser light source 10 along the reference direction.

Each of the bright portions P_{14} and P_{24} also shows a semi-elliptic shape whose major axis is parallel to the second division line 65. Further, since each focal point of the diffracted beams is formed behind the detecting face of the photodetector 60, the bright portion P_{24} lies in the positive direction of the x-axis to be located on the third detecting region 63 while the bright portion P_{14} lies in the negative direction of the x-axis, both having the opposite orientations to the respective bright portions P_{23} and P_{13}.

Also in these cases, the focus error signal FES is obtained by the aforementioned equation, (Sb - Sc). However, in proportion to variations of the wave-length, each shape of the bright portions P_{23} and P_{24} respectively formed on the second detecting region 62 and the third detecting region 63 becomes larger keeping a similar shape, and each position of those varies. For this reason, even if a light beam is projected on the optical disk 70 in the just-in-focus state, the focus error signal FES does not become "0". One example of the FES curve is shown by a broken line in Fig. 5, wherein the wave-length λ of the light beam is longer than the reference wave-length λ_0. The FES curve shown by the broken line possesses an undesired detection value S_1 in the case of the just-in-focus state. Accordingly, there is produced an offset value σ_1 that forms an improper target value for focusing control. However, since the bright portion P_{23} is formed in the vicinity of the second division

line 65 of the second detecting region 62 in an semi-elliptic shape whose major axis is parallel to the second division line 65, and further, detection sensitivity is low in the vicinity of the second division line 65, the undesired detection value S_1 due to a longer wave-length of the light beam can be extremely suppressed. In addition, since responsivity of the FES has been improved as described earlier, the offset value σ_1 of the optical disk 70 is further extremely reduced. This offset value σ_1 is sufficiently smaller than the offset value σ_2 shown in Fig. 12.

Similarly, since detection sensitivity is low in the vicinity of the second division line 65 in the third detecting region 63 and responsivity of the focus error signal FES has been improved, an undesired value of the focus error signal FES in the just-in-focus state and an offset value of the optical disk 70, due to a shorter wave-length of the light beam, can be extremely suppressed, thereby making it possible to reduce an offset value from the focal point with respect to the optical disk 70 to a substantially small amount.

Lastly, the following description will discuss structural examples of an optical head device for obtaining a more desirable tracking error signal RES in conjunction with the structure of the optical head device shown in Fig. 1. Two cases are shown as those structural examples.

(1) With respect to relative positions between the optical head device and the recording track 71 on the optical disk 70, both shown in Fig. 1, a tangent direction at the converging point of the recording track 71 is set to be parallel to the y-axis. That is, the above tangent direction is orthogonal to the tangent direction T shown in Fig. 1.

(2) The three-beam method is adopted:

As shown in Fig. 12, in accordance with the three-beam method, a light beam is projected from a semiconductor laser 1, diffracted in a first diffracting element 2 and split into a zero-order diffracted light (main beam) and ±1 order diffracted lights (a pair of sub beams). In Fig. 12, the ±1 order diffracted lights are comprised in a plane orthogonal to the surface of the paper. The main beam and the sub beams are further diffracted in a second diffracting element 3. Zero-order diffracted lights respectively produced by the main beam and the sub beams are transmitted through a collimating lens 4 to be focused onto a recording medium 6 by an objective lens 5. Return lights reflected off the recording medium 6 pass through the objective lens 5 and the collimating lens 4, and are diffracted in the second diffracting element 3. First order diffracted lights are then directed onto a light receiving element 7 from which data signal, tracking error signal and focus error signal can be obtained.

When, for example, data is recorded in the form of physical pits on the disc-shaped recording medium 6, the data is read out by focusing the zero order diffracted light produced by the main beam in the second diffracting element 3 on the -physical pits. The return light of the zero order diffracted light is diffracted again in the second diffracting element 3 to produce first order diffracted lights. The data signal is derived from the intensity of these first order diffracted lights.

The zero order diffracted lights produced by the two sub beams in the second diffracting element 3 are focused on positions symmetrical with respect to the zero order diffracted light produced by the main beam in the second diffracting element 3. These positions are offset greatly in a track direction and offset slightly in a radial direction from the position on the recording medium 6 where the zero order diffracted light of the main beam is focused. The return lights are respectively diffracted in the second diffracting element 3 to produce first order diffracted lights. The tracking error signal is derived from the intensities of these first order diffracted lights.

Fig. 13 shows the second diffracting element 3 as seen from the recording medium 6. As shown in Fig. 13, the second diffracting element 3 is divided into two diffracting regions 3a and 3b that are delineated by a division line 3e and whereon gratings 3c and 3d are respectively formed. The gratings 3c and 3d have mutually different pitches and the directions thereof are orthogonal to the division line 3e. Here, the direction of the division line 3e is set so as to coincide with the radial direction of the recording medium 6. As shown in Fig. 14, the photodetector 7 is divided into five light receiving regions 7a to 7e.

In the above arrangement, when the light beam projected from the semiconductor laser 1 is precisely focused on the recording medium 6, a portion of the return light corresponding to the zero order diffracted light produced by the main beam in the second diffracting element 3, is diffracted in the diffracting region 3a of the second diffracting element 3 to produce a first order diffracted light. This first order diffracted light is focused on a division line 7f separating the light receiving regions 7a and 7b of the photodetector 7, to form a spot-shaped diffracted image Q_1. Another portion of the return light corresponding to the zero order diffracted light of the main beam produced in the second diffracting element 3, is diffracted in the diffracting region 3b of the second diffracting element 3 to produce a first order diffracted light. This first order diffracted light is focused on the light receiving region 7c of the photodetector 7 to form a spot-shaped diffracted image Q_2. The return lights corresponding to the zero order diffracted lights produced by the two sub beams in the second diffracting element 3, respectively form two spot-shaped diffracted images Q_3 and Q_4 and two spot-shaped diffracted images Q_5 and Q_6 on the light receiving regions 7d and 7e of the photodetector 7.

Supposing that S_{1_a} to S_{1_e} respectively represent output signals released from the light receiving regions

7a to 7e, the focus error signal may be obtained by calculating (S_{1_a} - S_{1_b}). The tracking error signal may be obtained by calculating (S_{1_d} - S_{1_e}) and the data signal may be obtained by calculating (S_{1_a} + S_{1_b} + S_{1_c}).

As described above, in the optical head device according to the present invention, the light generation means projects light whose intensity distribution has an elliptic shape with its major axis parallel to the reference direction, and the division line of the diffraction means is set to be parallel to the reference direction. Thus, the reflected light directed to the diffraction means forms an elliptic bright portion whose major axis is situated on the division line of the diffraction means. In the case of occurrence of a wave-length variation in the light emitted by the light generation means, a thin semi-elliptic bright portion is formed along the reference direction in the vicinity of the division line substantially parallel to the reference direction in the photodetector. Since detection sensitivity is low in the vicinity of the division lines in the photodetector, an offset in focusing control due to the wave-length variation is remarkably reduced.

Further, since the optical head device of the present invention includes an intensity distribution shaping means for shaping the intensity distribution of the light projected by the light generation means in the minor axis direction of the elliptic shape, the rate of change of the shape of the bright portion formed on the photodetecting means, is greater with respect to the minor axis direction than with respect to the major axis direction. As a result, abrupt changes occur in the focus error signal, and therefore response sensitivity in the focusing control can be improved.

The invention being thus described, it will be obvious that the same may be varied in many ways without departing from the scope of the following claims.

Claims

1. An optical head device comprising:

   a light generation means (10) for generating a light beam having a substantially elliptical intensity distribution in cross section orthogonal to the beam's optical axis (L_1), the major axis of the elliptic shape being parallel to a reference direction (y);
   photodetecting means (60) for generating detection signals so as to enable the derivation of a focus error signal, the photodetecting means including a plurality of photodetecting sections (61,62,63) formed by a plurality of division lines (64,65) dividing a light-receiving face thereof, the detection signals being released by respective said photodetecting sections according to the intensity of the light received thereby, at least one of the plurality of division lines (65) being substantially parallel to the reference direction;
   diffraction means (20) having a diffraction face divided into two diffracting regions (22,23) by a single division line (21) which extends parallel to the reference direction; and
   optical means (30,40,50) for converging the light emitted from the light generation means on a recording track (71) formed on a recording medium (70) and further directing reflected light from the recording track to the diffraction means (20) so that the major axis of an elliptic bright portion formed on the diffracting face by the reflected light may be positioned on said division line (21) of the diffraction means, said optical means including intensity distribution shaping means (40) for shaping an intensity distribution of the light to be directed on the recording track into a substantially circular shape by diffusing the elliptic intensity distribution of the light emitted by the light generation means with respect to the minor axis, and permitting the intensity distribution of the light reflected from the recording track to restore its substantially elliptic shape by a reversed function to the above diffusing function,
   whereby a value of the focus error signal derived from the detection signals released by two photodetecting sections (62,63) separated by said one of the division lines (65) which is substantially parallel to the reference direction is allowed to become zero so as to indicate an in-focus state of said converged light beam on said recording medium when a part of the reflected light that has been diffracted by one of the two diffracting regions (23) of the diffraction means is converged on said one of the division lines (65) of the photodetecting means.

2. An optical head device according to claim 1, wherein the optical means further comprises:

   parallel light beam forming means (30) for forming the light emitted from the light generation means into a parallel beam of light.

3. An optical head device according to claim 2, wherein the parallel light beam forming means comprises:

   collimating lens means for forming the light emitted from the light generation means and transmitted through the diffraction means into a parallel beam of light to be directed to the intensity distribution shaping means (40).

4. An optical head device according to any of claims 1 to 3, wherein the optical means further comprises:

   converging means (50) for converging on the recording track (71) of the recording medium (70) the light whose intensity distribution is shaped into a circular shape by the intensity distribution shaping

means (40).

5. An optical head device according to any preceding claim, wherein said two diffracting regions are provided with respective gratings of different pitch; and wherein the photodetecting means includes a first photodetecting section (61) and a second photodetecting section (62,63) formed by a first division line (64) extending in a direction orthogonal to the reference direction (y), the second photodetecting section (62,63) comprising a third photodetecting section (62) and a fourth photodetecting section (63) formed by a second division line (65) extending from a middle point of the first division line (64) in a direction substantially parallel to the reference direction (y),

whereby when a part of the reflected light having been diffracted by said one of the diffracting regions (23) of the diffraction means (20) is converged on the second division line (65) of the photodetecting means (60), a value of the focus error signal is allowed to become zero while the other part of the reflected light diffracted by the other diffracting region (22) is converged on a substantial center of the first photodetecting section (61), and
when a detection signal released from the first photodetecting section (61) is represented as Sa; a detection signal released for the third photodetecting section (62) is represented as Sb; and a detection signal released from the fourth photodetecting sections (63) is represented as Sc, a reproduction signal of information recorded on the recording medium (70) is obtained by a calculation so Sa + Sb + Sc; the focus error signal is obtained by a calculation of Sc - Sb; and a tracking error signal is obtained by a calculation of Sa - (Sb + Sc).

6. An optical head device according to any of claims 1 to 4 wherein said two diffracting regions (22,23) of said diffraction means (20) are arranged so that the respective positions (O_{10},O_{20}) of convergence on the photodetecting means (60) of the respective parts of the reflected light that have been diffracted by respective ones of said two diffracting regions (22,23) are spaced apart in said reference direction.

Patentansprüche

1. Optischer Kopf mit:

- einer Lichterzeugungseinrichtung (10) zum Erzeugen eines Lichtstrahls mit im wesentlichen elliptischer Intensitätsverteilung in einem Querschnitt rechtwinklig zur optischen Strahlachse (L_1), wobei die Hauptachse der elliptischen Form parallel zu einer Bezugsrichtung (Y) verläuft;
- einer Photodetektoreinrichtung (60) zum Erzeugen von Erfassungssignalen, um die Herleitung eines Fokusabweichungssignals zu ermöglichen, wobei die Photodetektoreinrichtung eine Anzahl Photodetektorabschnitte (61, 62, 63) aufweist, die durch eine Anzahl Unterteilungslinien (64, 65) gebildet werden, die die Lichtempfangsfläche derselben unterteilen, wobei die Erfassungssignale von jeweiligen Photodetektorabschnitten abhängig von der von diesen empfangenen Lichtintensität ausgegeben werden, wobei mindestens eine der Anzahl von Unterteilungslinien (65) im wesentlichen parallel zur Bezugsrichtung verläuft;
- einer Beugungseinrichtung (20) mit einer Beugungsfläche, die durch eine einzelne Unterteilungslinie (21), die sich parallel zur Bezugsrichtung erstreckt, in zwei Beugungsbereiche (22, 23) unterteilt ist; und
- einer optischen Einrichtung (30, 40, 50) zum Konvergieren des von der Lichterzeugungseinrichtung emittierten Lichts auf eine auf einem Aufzeichnungsträger (70) ausgebildete Aufzeichnungsspur (71) und ferner zum Lenken von Reflexionslicht von der Aufzeichnungsspur auf die Beugungseinrichtung (20) in solcher Weise, daß die Hauptachse eines auf der Beugungsfläche auf das reflektierte Licht erzeugten elliptischen, hellen Abschnitts auf der Unterteilungslinie (21) der Beugungseinrichtung liegt, wobei die optische Einrichtung eine Intensitätsverteilungs-Formungseinrichtung (40) aufweist, um die Intensitätsverteilung des auf die Aufzeichnungsspur zu lenkenden Lichts dadurch auf im wesentlichen Kreisform zu verteilen, daß die elliptische Intensitätsverteilung des von der Lichterzeugungseinrichtung emittierten Lichts in bezug auf die Nebenachse aufgeweitet wird, wobei sie es ermöglicht, daß die Intensitätsverteilung des von der Aufzeichnungsspur reflektierten Lichts ihre im wesentlichen elliptische Form durch eine Funktion umgekehrt zur obigen Aufweitungsfunktion wiedererlangt;
- wodurch der Wert des aus den Erfassungssignalen hergeleiteten Fokusabweichungssignals, wie sie von den zwei Photodetektorabschnitten (62, 63) ausgegeben werden, die durch die eine der Unterteilungslinien (65), die im wesentlichen parallel zur Bezugsrichtung verläuft, unterteilt sind, zu Null werden kann, um den fokussierten Zustand des auf den Aufzeichnungsträger konvergierten Lichtstrahls anzuzeigen, wenn ein Teil des reflektierten Lichts, wie von einem der zwei Beugungsberei-

che (23) der Beugungseinrichtung gebeugt, auf die eine der Unterteilungslinien (65) der Photodetektoreinrichtung konvergiert wird.

2. Optischer Kopf nach Anspruch 1, bei dem die optische Einrichtung ferner eine Parallellichtstrahl-Formungseinrichtung (30) aufweist, um das von der Lichterzeugungseinrichtung emittierte Licht zu einem parallelen Lichtstrahl zu formen.

3. Optischer Kopf nach Anspruch 2, bei dem die Parallellichtstrahl-Formungseinrichtung eine Kollimatorlinseneinrichtung aufweist, um das von der Lichterzeugungseinrichtung emittierte und durch die Beugungseinrichtung hindurchgestrahlte Licht zu einem parallelen Lichtstrahl zu formen, der auf die Intensitätsverteilungs-Formungseinrichtung (40) gelenkt wird.

4. Optischer Kopf nach einem der Ansprüche 1 bis 3, bei dem die optische Einrichtung ferner eine Konvergiereinrichtung (50) aufweist, um das Licht, dessen Intensitätsverteilung durch die Intensitätsverteilungs-Formungseinrichtung (40) zu Kreisform geformt wurde, auf die Aufzeichnungsspur (71) auf dem Aufzeichnungsträger (70) zu konvergieren.

5. Optischer Kopf nach einem der vorstehenden Ansprüche, bei dem die zwei Beugungsbereiche mit jeweiligen Gittern mit verschiedenen Strichweiten versehen sind und bei dem die Photodetektoreinrichtung einen ersten Photodetektorabschnitt (61) und einen zweiten Photodetektorabschnitt (62, 63) aufweist, die durch eine erste Unterteilungslinie (64) gebildet sind, die sich in einer Richtung rechtwinklig zur Bezugsrichtung (Y) erstreckt, wobei der zweite Photodetektorabschnitt (62, 63) einen dritten Photodetektorabschnitt (62) und einen vierten Photodetektorabschnitt (63) aufweist, die durch eine zweite Unterteilungslinie (65) gebildet sind, die sich vom Mittelpunkt der ersten Unterteilungslinie (64) in einer Richtung im wesentlichen parallel zur Bezugsrichtung (Y) erstreckt;

- wobei dann, wenn ein Teil des reflektierten Lichts, wie es durch den einen der Beugungsbereiche (23) der Beugungseinrichtung (20) gebeugt wurde, auf die zweite Unterteilungslinie (65) der Photodetektoreinrichtung (60) konvergiert wird, der Wert des Fokusabweichungssignals Null werden kann, während der andere Teil des reflektierten Lichts, wie durch den anderen Beugungsbereich (22) gebeugt, im wesentlichen auf das Zentrum des ersten Photodetektorabschnitts (61) konvergiert wird; und
- wobei dann, wenn das vom ersten Photodetektorabschnitt (61) ausgegebene Signal mit Sa repräsentiert wird, das vom dritten Photodetektorabschnitt (62) ausgegebene Erfassungssignal mit Sb repräsentiert wird und das vom vierten Photodetektorabschnitt (63) ausgegebene Erfassungssignal mit Sc repräsentiert wird, das Wiedergabesignal für auf dem Aufzeichnungsträger (70) aufgezeichnete Information durch die Berechnung Sa + Sb + Sc erhalten wird, das Fokusabweichungssignal durch die Berechnung Sc - Sb erhalten wird und ein Spurabweichungssignal durch die Berechnung Sa - (Sb + Sc) erhalten wird.

6. Optischer Kopf nach einem der Ansprüche 1 bis 4, bei dem die zwei Beugungsbereiche (22, 23) der Beugungseinrichtung (20) so angeordnet sind, daß die jeweiligen Konvergenzpositionen (O_{10}, O_{20}) auf der Photodetektoreinrichtung (60) hinsichtlich der jeweiligen Teile des reflektierten Lichts, die durch die jeweiligen der zwei Beugungsbereiche (22, 23) gebeugt wurden, in der Bezugsrichtung voneinander beabstandet sind.

Revendications

1. Dispositif de tête optique, comprenant :

un moyen générateur de lumière (10) pour émettre un faisceau lumineux ayant une répartition d'intensité sensiblement elliptique dans la section transversale orthogonale à l'axe optique (L_1) du faisceau, le grand axe de la forme elliptique étant parallèle à une direction de référence (y) ;
un moyen photodétecteur (60) pour émettre des signaux de détection de manière à permettre l'obtention d'un signal d'erreur de focalisation, le moyen photodétecteur comprenant une pluralité de parties photodétectrices (61, 62, 63) formées par une pluralité de lignes de séparation (64, 65) divisant une face réceptrice de lumière de celui-ci, les signaux de détection étant émis par lesdites parties photodétectrices respectives en fonction de l'intensité de la lumière reçue par elles, au moins une (65) de la pluralité de lignes de séparation étant sensiblement parallèle à la direction de référence ;
un moyen de diffraction (20) ayant une face de diffraction divisée en deux zones de diffraction (22, 23) par une seule ligne de séparation (21), qui s'étend parallèlement à la direction de référence ; et
des moyens optiques (30, 40, 50) pour faire converger la lumière, émise par le moyen générateur de lumière, sur une piste d'enregistrement (71) formée sur un support d'enregistrement (70) et pour en outre diriger vers le moyen de diffraction (20) la lumière réfléchie par la pis-

te d'enregistrement, de telle manière que le grand axe d'une partie elliptique brillante, formée sur la face de diffraction par la lumière réfléchie, puisse être situé sur ladite ligne de séparation (21) du moyen de diffraction, lesdits moyens optiques comprenant un moyen de mise en forme de répartition d'intensité (40) pour mettre en forme une répartition d'intensité de la lumière à diriger sur la piste d'enregistrement, pour lui conférer une forme sensiblement circulaire en diffusant la répartition elliptique de l'intensité de la lumière, émise par le moyen générateur de lumière, par rapport au petit axe, et pour permettre que la répartition d'intensité de la lumière, réfléchie par la piste d'enregistrement, reprenne sa forme sensiblement elliptique par un fonctionnement inverse du fonctionnement de diffusion précédent,
ce qui permet à une valeur du signal d'erreur de focalisation obtenu à partir des signaux de détection émis par deux parties photodétectrices (62, 63), séparées par ladite ligne de séparation (65), qui est sensiblement parallèle à la direction de référence, de devenir zéro de manière à indiquer un état focalisé dudit faisceau lumineux amené à converger sur ledit support d'enregistrement, lorsqu'une partie de la lumière réfléchie, qui a été diffractée par l'une (23) des deux zones de diffraction du moyen de diffraction, est amenée à converger sur ladite ligne (65) des lignes de séparation du moyen photodétecteur.

2. Dispositif de tête optique selon la revendication 1, dans lequel les moyens optiques comprennent en outre :

un moyen de formation de faisceau de rayons lumineux parallèles (30) pour former la lumière, émise par le moyen générateur de lumière, en un faisceau de rayons lumineux parallèles.

3. Dispositif de tête optique selon la revendication 2, dans lequel le moyen de formation de faisceau de rayons lumineux parallèles comprend :

un moyen formant lentille collimatrice pour former la lumière, émise à partir du moyen générateur de lumière et transmise par l'intermédiaire du moyen de diffraction, en un faisceau de rayons lumineux parallèles à diriger vers le moyen de mise en forme de répartition d'intensité (40).

4. Dispositif de tête optique selon l'une quelconque des revendications 1 à 3, dans lequel les moyens optiques comprennent en outre ;

un moyen de convergence (50) pour faire converger, sur la piste d'enregistrement (71) du support d'enregistrement (70), la lumière dont la répartition d'intensité est mise sous une forme circulaire par le moyen de mise en forme de répartition d'intensité (40).

5. Dispositif de tête optique selon l'une quelconque des revendications précédentes, dans lequel lesdites deux zones de diffraction sont pourvues de réseaux respectifs de pas différents ; et dans lequel le moyen photodétecteur comprend une première partie photodétectrice (61) et une deuxième partie photodétectrice (62, 63) formées par une première ligne de séparation (64), qui s'étend dans une direction orthogonale à la direction de référence (y), la deuxième partie photodétectrice (62, 63) comprenant une troisième partie photodétectrice (62) et une quatrième partie photodétectrice (63), formées par une deuxième ligne de séparation (65), qui s'étend à partir d'un point médian de la première ligne de séparation (64) dans une direction sensiblement parallèle à la direction de référence (y),

ce par quoi, lorsque qu'une partie de la lumière réfléchie, ayant été diffractée par ladite zone de diffraction (23) du moyen de diffraction (20), est amenée à converger sur la deuxième ligne de séparation (65) du moyen photodétecteur (60), une valeur du signal d'erreur de focalisation peut devenir zéro, tandis que l'autre partie de la lumière réfléchie, diffractée par l'autre zone de diffraction (22), est amenée à converger sur un centre sensible de la première partie photodétectrice (61), et
lorsqu'un signal de détection, émis par la première partie photodétectrice (61) est représenté par Sa ; un signal de détection, émis par la troisième partie photodétectrice (62) est représenté par Sb ; et un signal de détection, émis par la quatrième partie photodétectrice (63), est représenté pas Sc, on obtient alors un signal de reproduction d'informations, enregistrées sur le support d'enregistrement (70), par un calcul de Sa + Sb + Sc ; le signal d'erreur de focalisation est obtenu par un calcul de Sc - Sb ; et un signal d'erreur de piste est obtenu par un calcul de Sa - (Sb + Sc).

6. Dispositif de tête optique selon l'une quelconque des revendications 1 à 4, dans lequel lesdites deux zones de diffraction (22, 23) dudit moyen de diffraction (20) sont disposées de telle manière que les positions respectives (O_{10}, O_{20}) de convergence, sur le moyen photodétecteur (60), des parties respectives de la lumière réfléchie, qui ont été diffractées par lesdites deux zones de diffraction (22, 23) respectives, soient distantes l'une de l'autre dans ladite direction de référence.

FIG.1

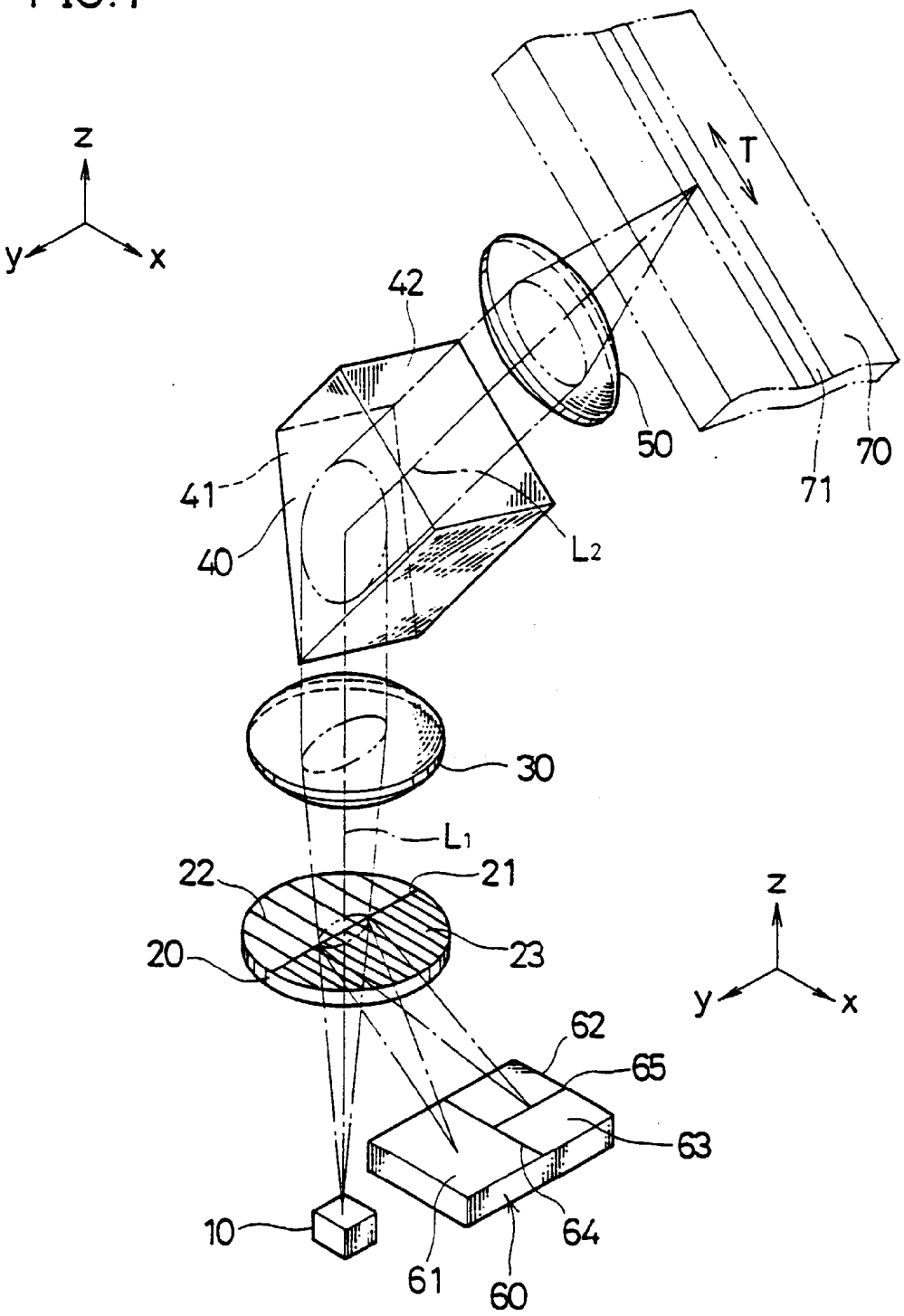


FIG. 2

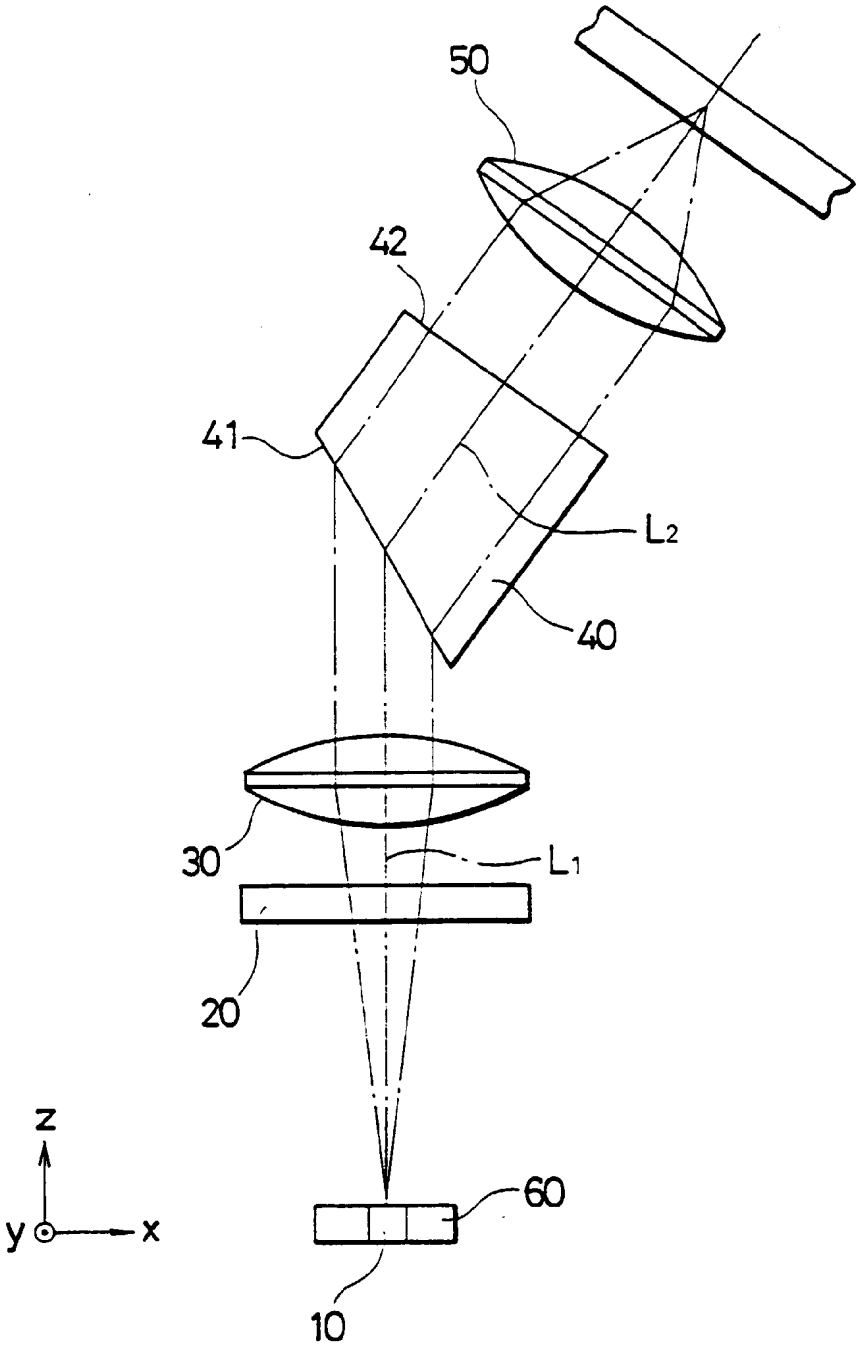


FIG. 3

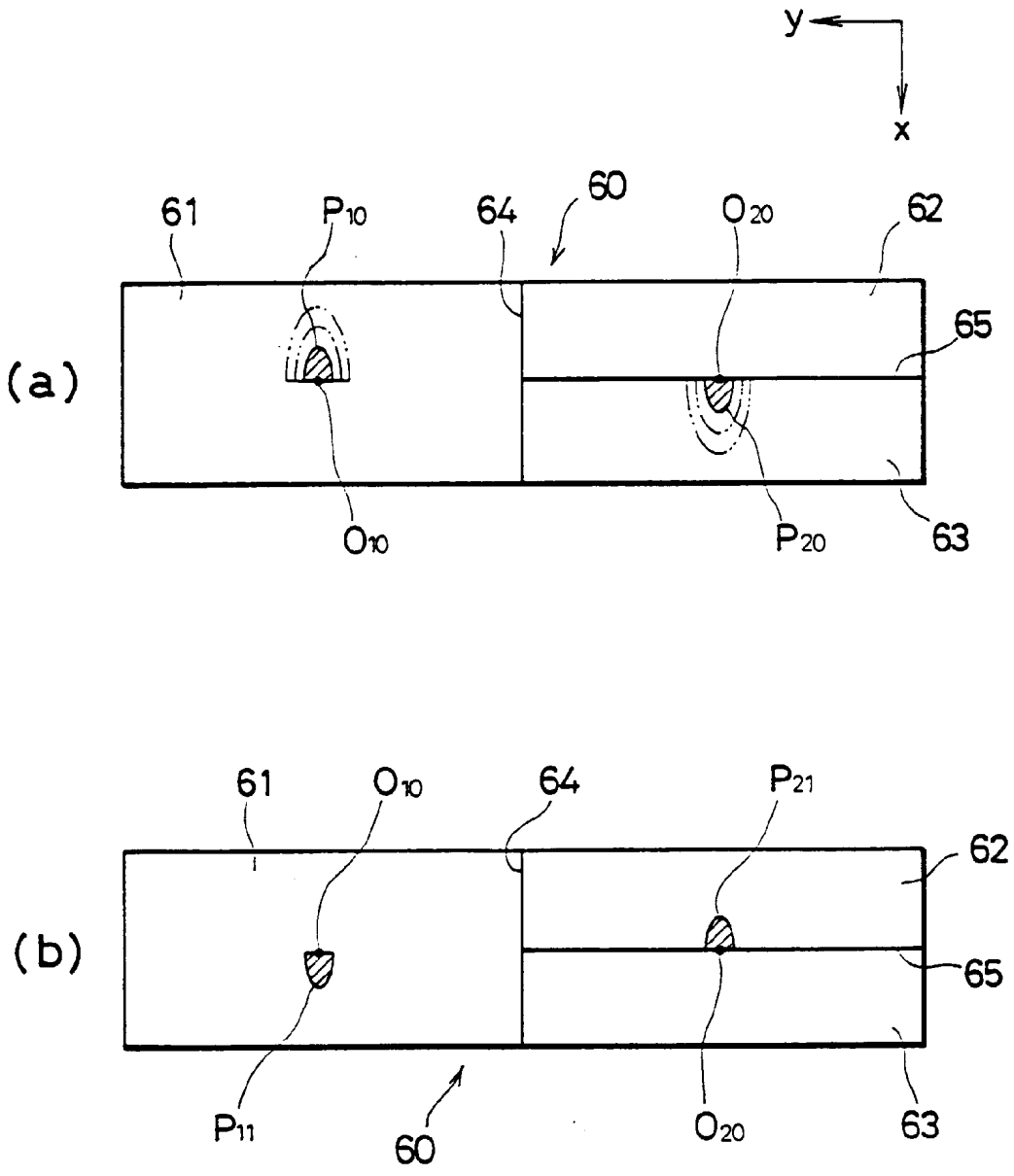

y
x
(a)
61
P10
64
60
O20
62
65
O10
P20
63
(b)
61
O10
64
P21
62
65
P11
60
O20
63

FIG. 4

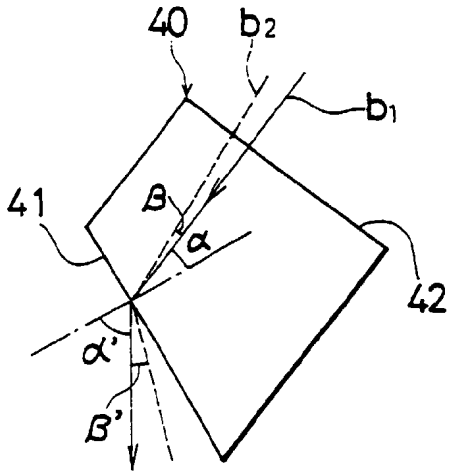


FIG. 5

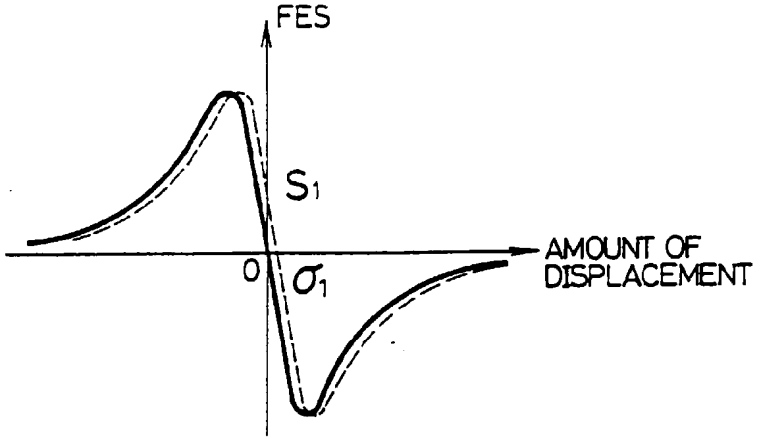


FIG. 6

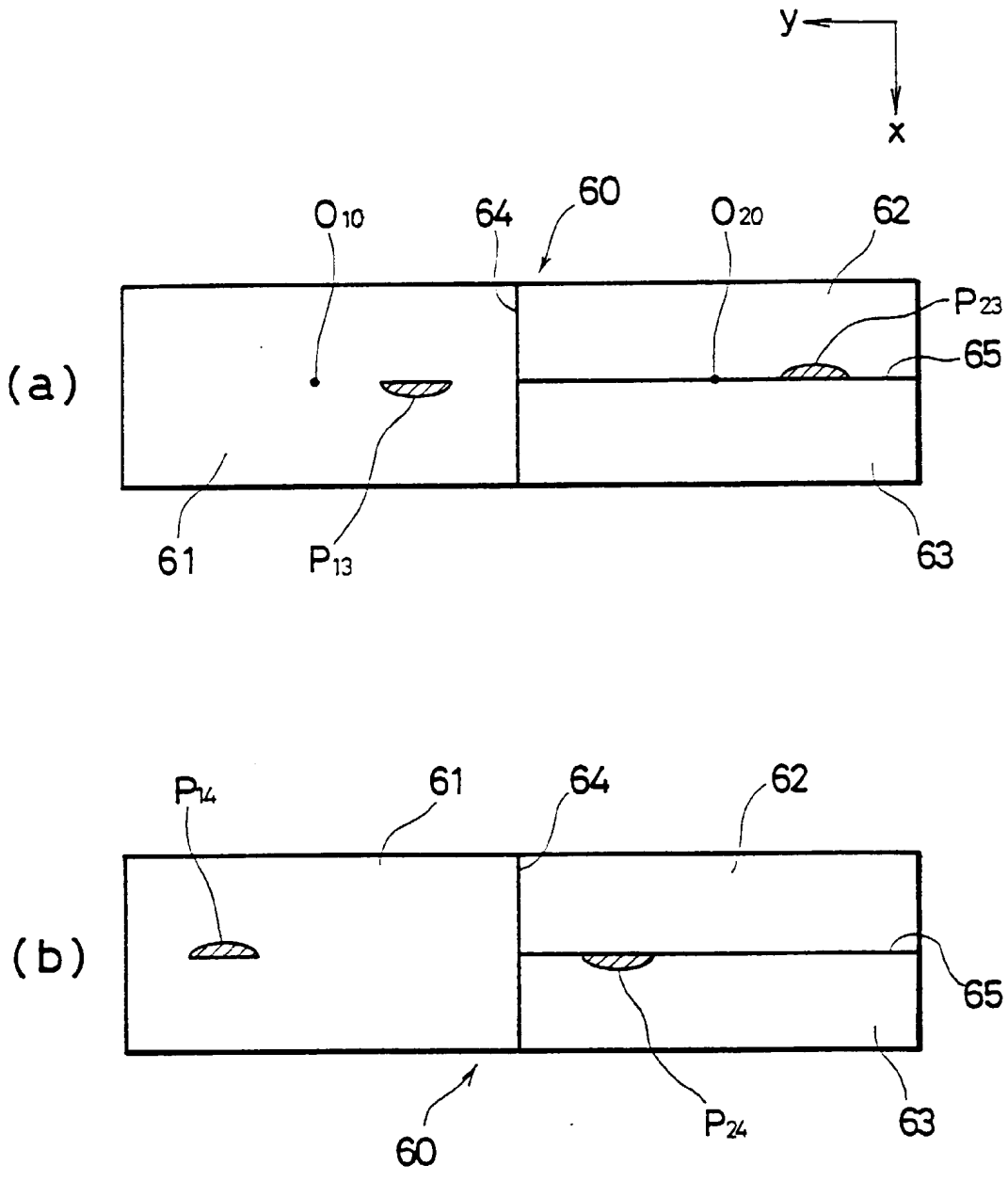


FIG. 7

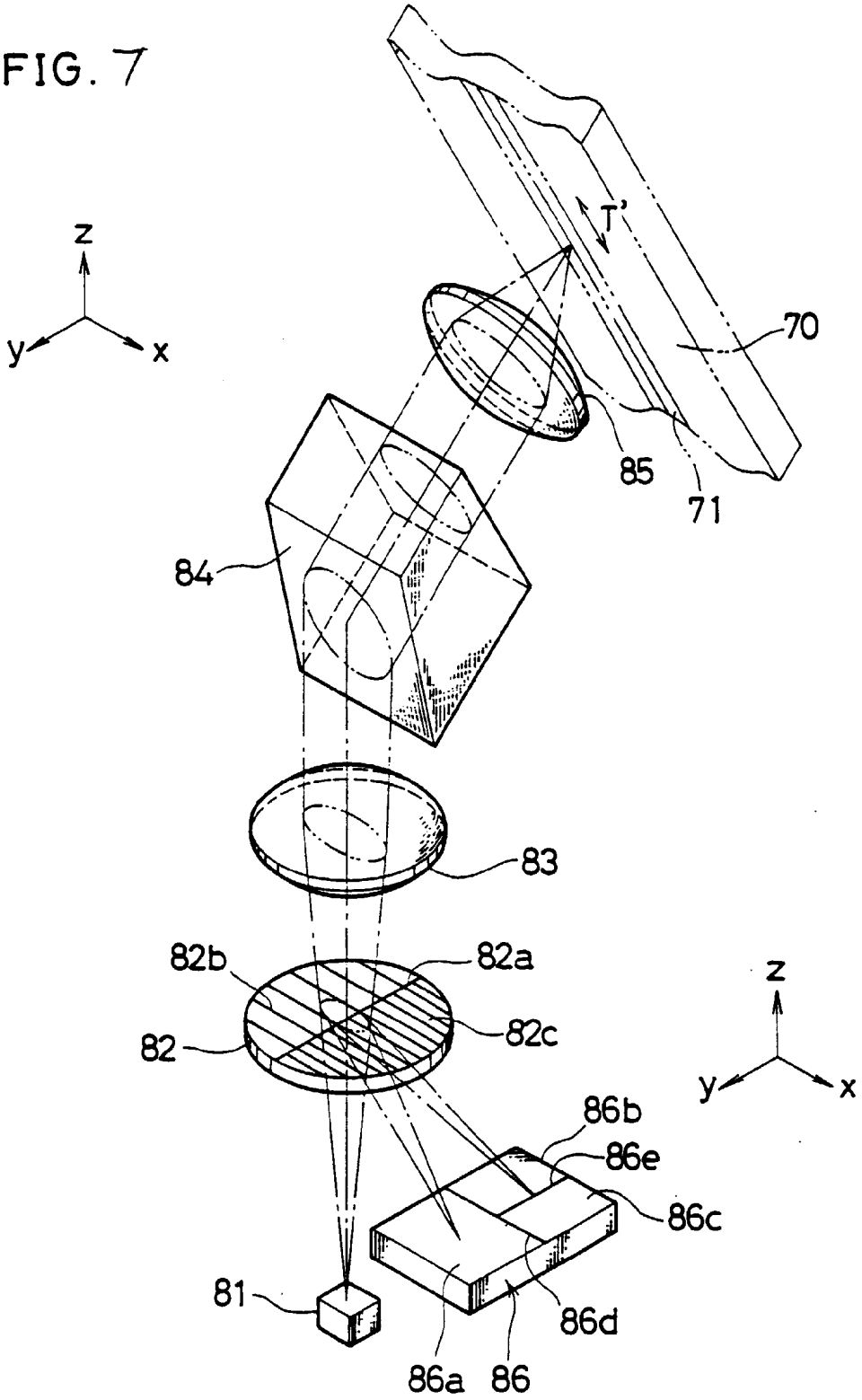


FIG. 8

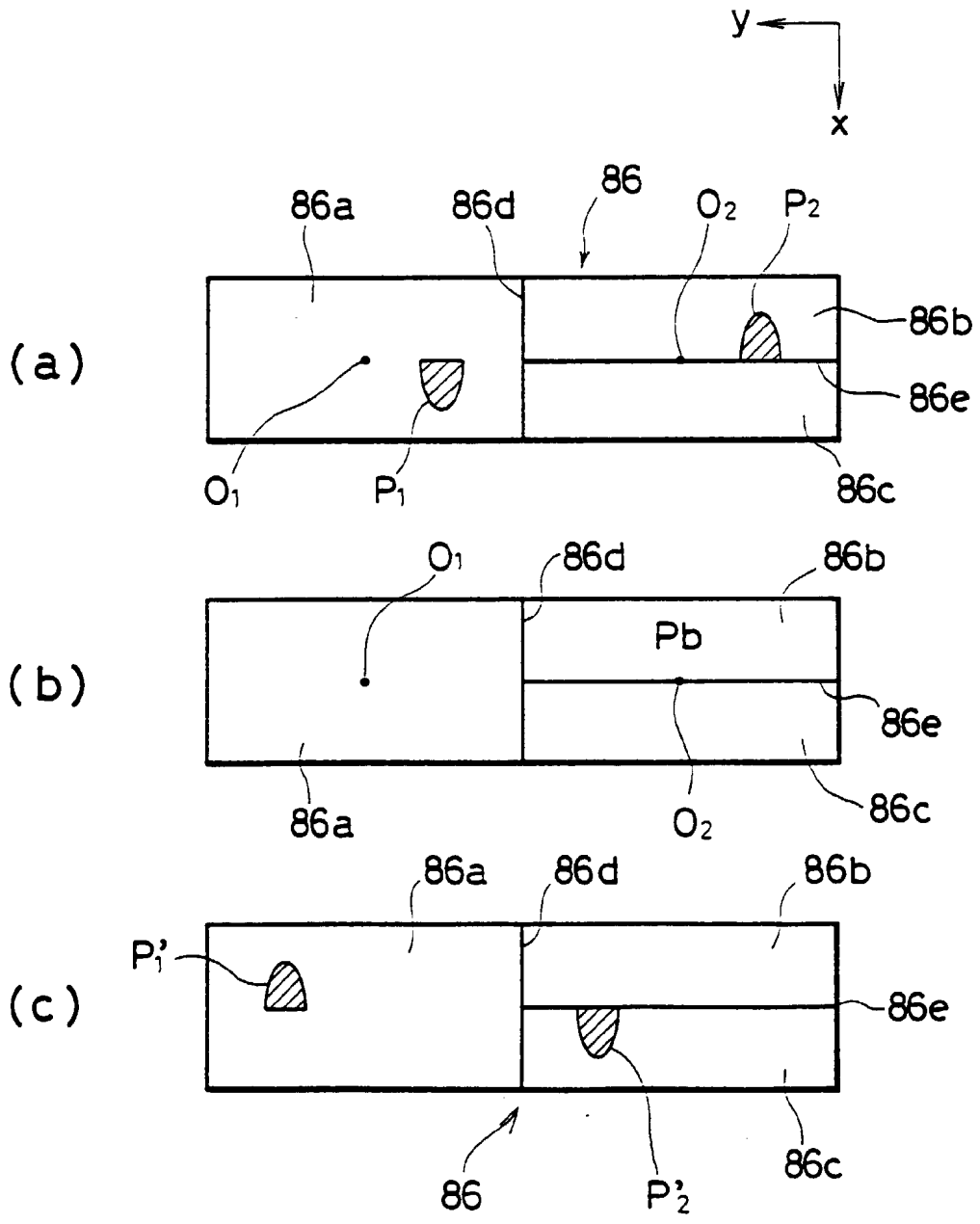

y
x
(a)
86a
86d
86
O2
P2
86b
86e
O1
P1
86c
(b)
O1
86d
86b
Pb
86e
86a
O2
86c
(c)
86a
86d
86b
P1'
86e
86
P'2
86c

FIG. 9

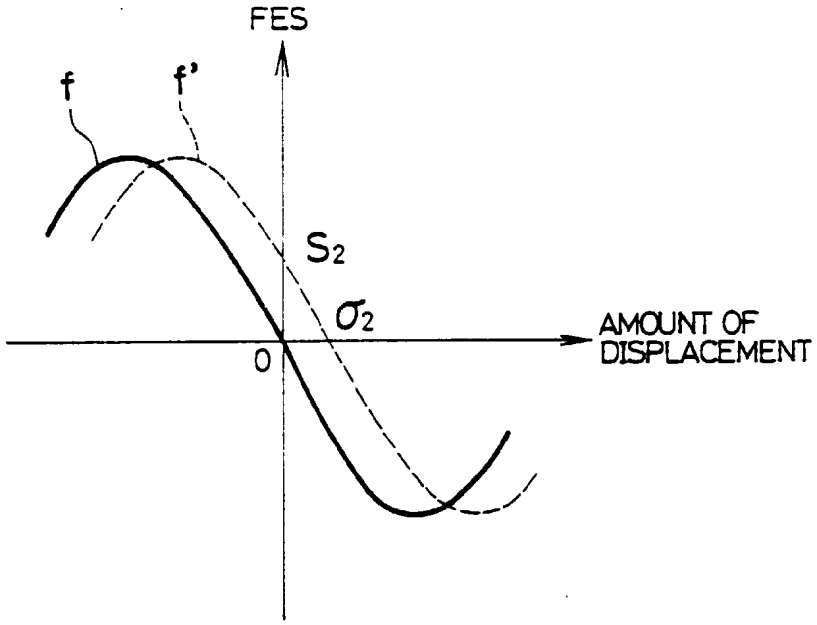


FIG. 10

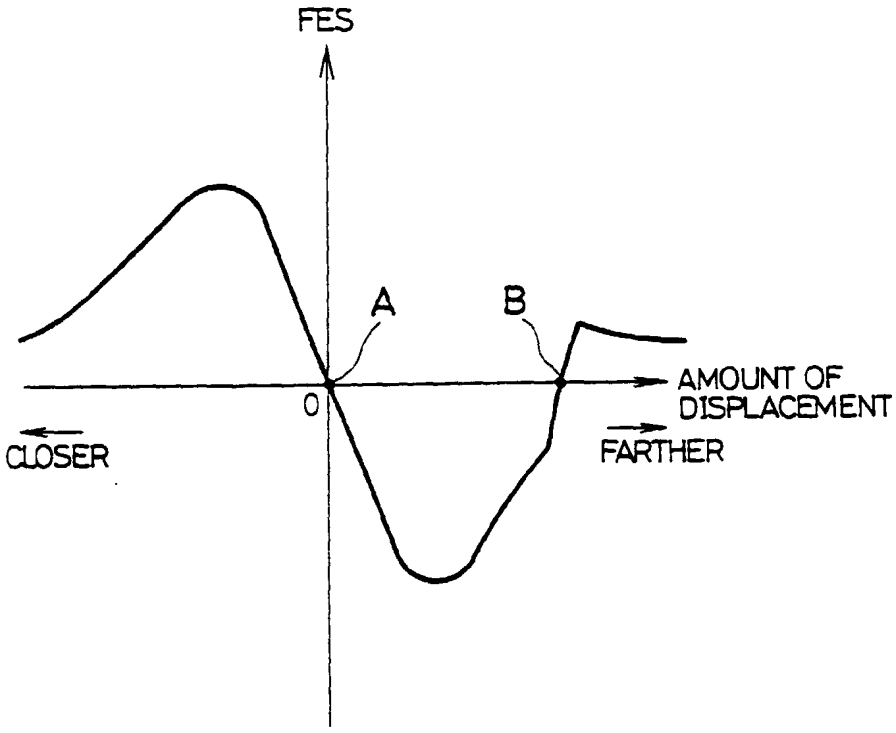

FES
A
B
0
AMOUNT OF DISPLACEMENT
CLOSER
FARTHER

FIG. 11

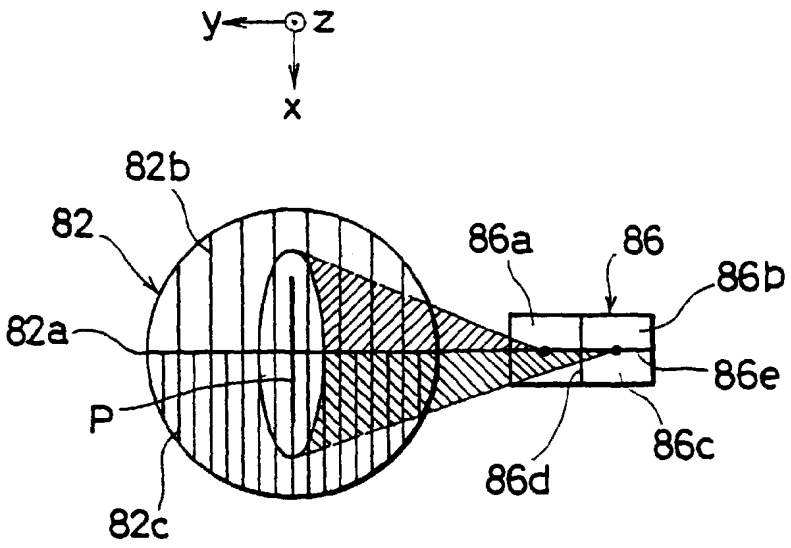

y
z
x
82b
82
82a
P
82c
86a
86
86b
86e
86c
86d

FIG. 12

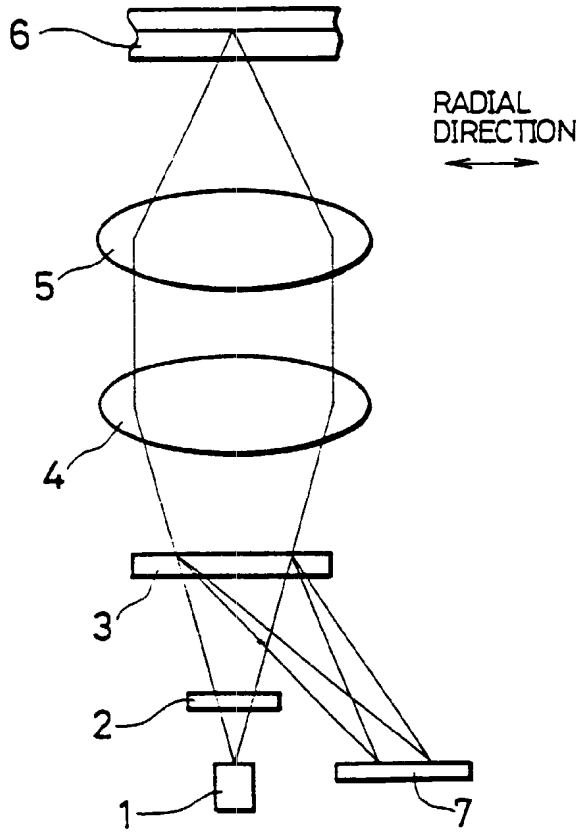


FIG.13

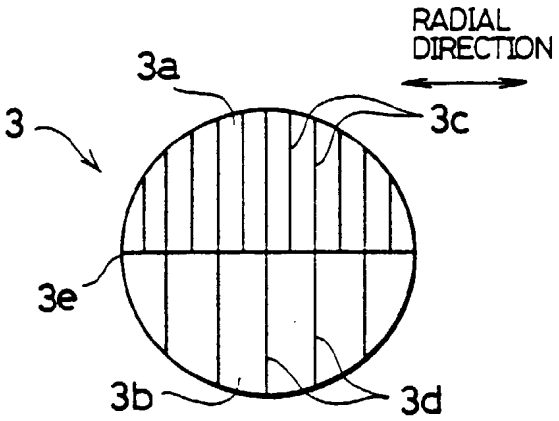
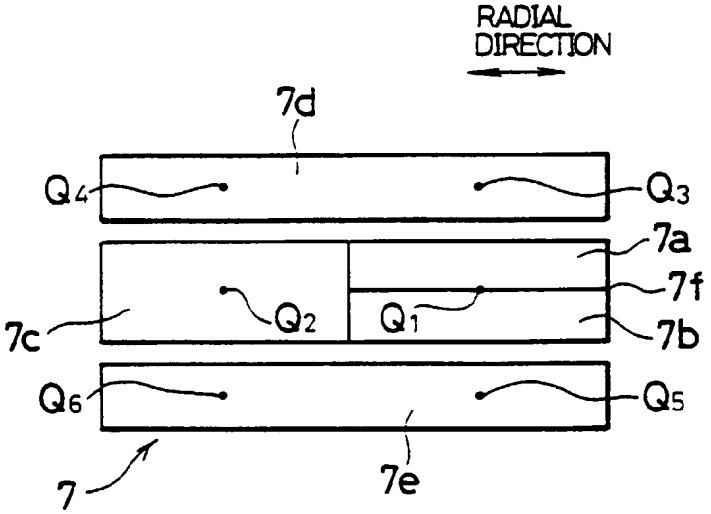

RADIAL
DIRECTION
3a
3
3c
3e
3b
3d

FIG.14


RADIAL
DIRECTION
7d
Q4
Q3
7a
7f
7c
Q2
Q1
7b
Q6
Q5
7
7e

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(54) Optical head apparatus

(57) An data-processing apparatus comprising an optical head (1) to oppose an optical disk (D). The optical head (1) has a laser-detector unit (10). The unit (10) comprises of a semiconductor laser (2), a first photodetector (4) and a second photodetector (6), which are located close to one another and in almost the same plane. The first photodetector (4) is designed to generate data signals from a light beam, and the second photodetector (6) is designed to generate monitor signals from a light beam. An optical diffraction element (12), a collimating lens (14), an aperture controller (16), a correction prism (18) and a focusing lens (20) are arranged in the optical path extending from the laser (2) to the optical disk (D). The prism (18) has a reflecting surface (18a) which is inclined at a prescribed angle to a plane perpendicular to the optical axis. The collimating lens (14) focuses a light beam reflected by the reflecting surface (18a), on the light-receiving surface of the second photodetector (6).

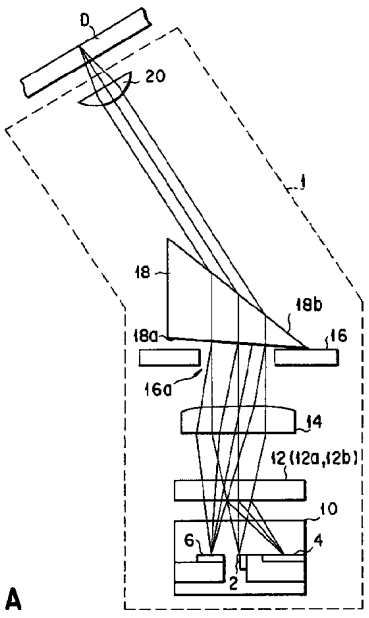


FIG. 1A

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Description

The present invention relates to a data-processing apparatus and, more particularly to an optical head apparatus which optically records data on a recording medium such as an optical disk and which optically reproduces the data from the recording medium.

Generally, a data-processing apparatus for optically record data on a recording medium such as an optical disk and for reproducing the data from the medium has an optical head. The optical head applies a light beam to the recording/reproducing surface of the medium to record data on the medium, and applies a light scan to the surface of the medium and receives the light reflected by the medium to reproduce the data from the recording medium.

The optical head comprises a semiconductor laser, a collimating lens, a focusing lens, a deflecting element, and a photodetector. The semiconductor laser is used as the light source. The collimating lens collimates the dispersing light emitted from the laser. The focusing lens receives the collimated light (i.e., a parallel beam) from the collimating lens and focuses it on an optical disk which is used as a recording medium. The deflecting element receives the light beam reflected by the disk and applied through the focusing lens and deflects the light beam in a predetermined direction. The photodetector receives the deflected light beam and converts it into electric signals. The electric signals represent the data reproduced from the optical disk.

The optical head further comprises another photodetector and a laser driver. This photodetector monitors the amount of light the semiconductor laser has emitted. The laser driver controls the laser on the basis of the amount of light the photodetector has monitored, so that the laser may emit light in a predetermined amount.

Tow methods of monitoring the amount of light emitted from a semiconductor laser are known. The first method is to monitor the light emitted from the back of the semiconductor laser and traveling away from a recording medium. The second method is to monitor the light emitted from the front of the laser and traveling toward the recording medium. Hereinafter, the light traveling away from the medium will be referred to as "backward light," and the light traveling toward the medium as "forward light."

The first method is disadvantageous, however. It cannot detect the amount of backward light accurately and cannot serve to control the semiconductor laser such that the laser emits light in the predetermined amount. This is because the backward light is influenced by the forward light reflected by the medium and reaching, though in a small amount, the semiconductor laser. Consequently, the amount of the backward light cannot be proportionate to the amount of the forward light, making it difficult to cause the laser to emit light in a desired amount.

The second method is disadvantageous, too. To perform the second method, an optical head needs to have two additional components. The first additional component is a beam splitter for splitting the forward light beam into two beams. The second additional component is a focusing lens for focusing one of the beams obtained by the beam splitter at the light-receiving surface of the photodetector for monitoring the light emitted from the laser. The use of these additional components inevitably increases the size of the optical head and the manufacturing cost thereof.

In view of the foregoing, the present invention has been made, and its object is to provide a data-processing apparatus which is simple in structure and can be manufactured at low cost and which can reliably record and reproduce data.

To achieve the object, an data-processing apparatus comprising: a light source for emitting a light beam; adjusting means for adjusting an amount in which the light beam is emitted from the light source; a collimating lens for collimating the light beam; a focusing lens for focusing the light beam collimated by the collimating lens, on a recording medium; deflecting means for deflecting the light beam reflected by the recording medium and applied through the focusing lens, to a first position near the light source; first light-receiving means provided at the first position, for receiving the light beam deflected by the deflecting means and converting the same into an electric data signal; a reflecting surface provided between the collimating lens and the focusing lens, having a reflectance of at most 50% and inclined to a plane perpendicular to an optical axis of the collimating lens, for reflecting a part of the light beam applied through the collimating lens and focusing the same at a second position near the light source; a second light-receiving means provided at the second position, for receiving the light beam focused at the second position and converting the same into an electric monitor signal; and control means for controlling the adjusting means in accordance with the electric monitor signal, thereby to cause the adjusting means to adjust the amount in which the light beam is emitted from the light source.

A data-processing apparatus according to another aspect of the invention comprises: an data-processing apparatus comprising: a light source for emitting a light beam; adjusting means for adjusting an amount in which the light beam is emitted from the light source; a collimating lens for collimating the light beam; a focusing lens for focusing the light beam collimated by the collimating lens, on a recording medium; polarization plane rotating means for allowing passage of the light beam applied toward the recording medium, allowing passage of the light beam reflected by the recording medium and rotating a polarization plane of the light beam reflected by the recording medium; deflecting means provided between the light source and the polarization plane rotating means, for allowing passage of the light beam emitted from the light source and deflecting the light beam which reflected by the recording medium and whose polarization plane is rotated by the

polarization plane rotating means, to a first position near the light source; first light-receiving means provided at the first position, for receiving the light beam deflected by the deflecting means and converting the same into an electric data signal; a reflecting surface provided between the collimating lens and the polarization plane rotating means, having a reflectance of at most 50% and inclined to a plane perpendicular to an optical axis of the collimating lens, for reflecting a part of the light beam applied through the collimating lens and focusing the same at a second position near the light source; a second light-receiving means provided at the second position, for receiving the light beam focused at the second position and converting the same into an electric monitor signal; and control means for controlling the adjusting means in accordance with the electric monitor signal, thereby to cause the adjusting means to adjust the amount in which the light beam is emitted from the light source.

This invention can be more fully understood from the following detailed description when taken in conjunction with the accompanying drawings, in which:

FIG. 1A is a schematic view showing the internal structure of the optical head incorporated in a data-processing apparatus according to a first embodiment of the invention;

FIG. 1B is a plan view of the laser-detector unit built in the optical head shown in FIG. 1A;

FIG. 2 is a block diagram illustrating the control system provided in the data-processing apparatus according to a first embodiment of the invention;

FIG. 3A is a schematic view showing the internal structure of the optical head incorporated in a data-processing apparatus according to a second embodiment of the invention; and

FIG. 3B is a plan view of the laser-detector unit built in the optical head shown in FIG. 3A.

Embodiments of the present invention will be described in detail, with reference to the accompanying drawings.

FIGS. 1A and 1B show the optical head 1 incorporated in a data-processing apparatus which is the first embodiment of the invention. In use, the optical head 1 is located to oppose the recording/reproducing surface of an optical disk D used as a recording medium. As shown in FIG. 1A, the optical head 1 has a laser-detector unit 10. The laser-detector unit 10 comprises a semiconductor laser 2, a first photo-detector 4 and a second photodetector 6 which are located in almost the same plane as is illustrated in FIG. 1B. The semiconductor laser 2, the first photodetector 4 and the second photodetector 6 are used as the light source, the first light-receiving means for generating data signals, and the second light-receiving means for generating monitor signals, respectively. The photodetectors 4 and 6 are arranged at first and second positions, near the semiconductor laser 2.

The optical head 1 further comprises an optical diffraction element 12, a collimating lens 14, an aperture controller 16, a correction prism 18 and a focusing lens 20. These components 12, 14, 16 and 18 are arranged, in the order mentioned, in the optical path extending from the semiconductor laser 2 to the optical disk D. The collimating lens 14 converts the dispersing light emitted from the laser 2 to a parallel light beam. The aperture controller 16 functions as diaphragm means. The correction prism 18 serves as correction means. The focusing lens 20 focuses the light output from the collimating lens 14 and applied through the controller 16 and the prism 18, forming a beam spot on the recording/reproducing surface of the optical disk D. The correction prism 18 has a reflecting surface 18a and an inclined surface 18b. The surface 18a has a reflectance of 50% or less. The surface 18b is inclined at a predetermined angle to the reflecting surface 18a.

The optical diffraction element 12 is shaped like a plate. It extends substantially parallel to a plane which is perpendicular to the axis of the light beam emitted from the semiconductor laser 2 toward the optical disk D. The element 12 has a light-diffracting section 12a and a light-passing section 12b. The light-diffracting section 12a performs two functions. The first function is to allow the passage of the light beam emitted from the semiconductor laser 2. The second function is to deflect the light beam reflected by the optical disk D and thus representing data, toward the first photodetector 4. The light-passing section 12b allows the passage of the monitor light beam reflected by the reflecting surface 18a of the correction prism 18, without reflecting this light beam.

The collimating lens 14 is placed in a horizontal plane which is substantially at right angles to the optical axis of the semiconductor laser 2. Not only does the lens 14 collimates the dispersed light beam which has been emitted from the semiconductor laser 2 and which has passed through the light-diffracting section 12a of the optical diffraction element 12. But also does the lens 14 focus the light beam reflected by the reflecting surface 18a of the correction prism 18, at the light-receiving surface of the second photodetector 6 which is provided for detecting monitor signals. In other words, the collimating lens 14 works as a focusing lens, as well.

The light beam reflected by the reflecting surface 18a of the correction prism 18 and focused by the collimating lens 14 passes through the light-passing section 12b of the optical diffraction element 12. This light beam is then focused at the light-receiving surface of the second photodetector 6.

The correction prism 18 has its reflecting surface 18a inclined at a prescribed angle to the plane which extends at right angles to the optical axis of the semiconductor laser 2. Since the reflecting surface 18a is inclined so, all light beam it reflects is applied to the effective region of the collimating lens 14 and is focused by the collimating lens 14. This beam focused is applied to only the light-passing section 12b of the optical dif-

fraction element 12, not to the light-diffracting section 12a thereof. The beam passes through the light-passing section 12b and reach the light-receiving surface of the second photodetector 6 provided for generating monitor signals.

The correction prism 18 is a triangular prism. As indicated above, it has the reflecting surface 18a and the inclined surface 18b. The inclined surface 18b refracts the input light beam toward the focusing lens 20, the input light beam having been emitted from the semiconductor laser 2, passed through the light-diffracting section 12a and the collimating lens 14 and applied to the reflecting surface 18a of the correction lens 18. The inclined surface 18b also reduces the ellipticity of the light beam. Namely, the ellipticity of the beam can be changed by inclining the axis of the beam to the major axis of an elliptical area in a plane perpendicular to the axis of the beam. This is because the intensity of a light beam emitted from a semiconductor laser 2 is distributed generally in that elliptical area. Hence, the intensity of the light beam in the cross section thereof can be distributed, desirably in a substantially circular area by setting the inclination angle of the surface 18b of the correction prism 18.

The aperture controller 16 provided between the collimating lens 14 and the correction prism 18 extend almost parallel to a plane which is at right angles to the optical axis of the semiconductor laser 2. The aperture controller 16 has a substantially circular aperture 16a and an adjusting mechanism (not shown) designed to adjust the opening of the aperture 16a. The opening of the aperture 16a is adjusted so that the beam reflected by the reflecting surface 18a of the correction lens 18 is applied in its entirely within the effective region of the collimating lens 14, that the beam focused by the collimating lens 14 may pass through the light-passing section 12b of the optical diffraction element 12, and that the beam may be applied to the second photodetector 6 provided for generating monitor signals.

The data-processing apparatus having the optical head described above has a control system, which will be described below.

As shown in FIG. 2, the control system has a CPU 30 used as control means. An input interface 31 is connected to the CPU 30. Connected to the input interface 31 are the first detector 4, the second detector 6, a data signal generating section 34, and a ROM 36. The section 34 is provided to store data to be recorded on the optical disk D. The ROM 36 stores various control data items, among which are a first reference value for the amount of a data-recording light beam and a second reference value for the amount of a data-reproducing light beam.

An output interface 32 is connected to the CPU 30. Connected to the output section 32 are a laser driver 38 and a signal reproducing section 40. The driver 38 is means for adjusting the amount of a light beam emitted from the laser 2. The section 40 processes the data signals generated by the first photodetector 4, thereby to reproduce the data signals.

The operation of the data-processing apparatus will be described.

At first, the semiconductor laser 2 emits a light beam, the amount of which is adjusted by the laser driver 38 adjusts. The light beam passes through the light-diffracting section 12a of the optical diffraction element 12, the collimating lens 14, and the reflecting surface 18a and the inclined surface 18b of the correction lens 18. The light beam is then focused the focusing lens 20 and thereby applied to the recording/reproducing surface of the optical disk D.

Part of the light beam emitted from the semiconductor laser 2 is reflected by the reflecting surface 18a of the correction lens 18 and applied back to the collimating lens 14. It then passes through the collimating lens 14 and through the light-passing section 12b of the optical diffraction element 12. Finally it is focused on the second photodetector 6 by the collimating lens 14.

To record data on the optical disk D, the signals representing the data are generated by the data signal generating section 34. The data signals are supplied to the laser driver 38 through the input interface 31 and CPU 30, output interface 32. The laser driver 38 emits light beam the amount of which is modulated in accordance with the data signals. Meanwhile, the second photodetector 6 monitors the light beam emitted from the semiconductor laser 2, detecting the amount of the light beam. The amount of light detected by the photodetector 6 is compared with the first reference value stored in the ROM 36. The CPU 30 controls the laser driver 38, whereby the amount of light beam emitted from the laser 2 becomes equal to the desired amount for the data-recording light beam. The light beam thus controlled in amount is focused on the recording/reproducing surface of the optical disk D. As a result, the data is recorded on the optical disk D.

To reproduce the data from the optical disk D, too, the second photodetector 6 monitors the light emitted from the semiconductor laser 2, detecting the amount of the beam. The amount of the light beam is compared with the second reference value stored in the ROM 36. The CPU 30 controls the laser driver 38, whereby the amount of light beam emitted from the laser 2 becomes equal to the desired amount for the data-reproducing light beam. The light beam thus controlled in amount is focused on the recording/reproducing surface of the optical disk D. As a result, the data is reproduced from the optical disk D.

The light beam reflected by the optical disk D is focused on the first photodetector 4 after passing through the focusing lens 20, correction prism 18, aperture controller 16, collimating lens 14 and the light-diffracting section 12a of the optical diffraction element 12. The first photodetector 4 generates data signals from the light beam. These data signals (i.e., the reproduced data signals) are processed by the signal reproducing section 40. As a result, the data recorded on the optical disk D is reproduced.

In the data-processing apparatus, the collimating lens 14 is used not only to collimate the light beam emitted from the semiconductor laser 2, but also to focus the light beam reflected by the disk D on the second photodetector 6 provided for generating monitor signals. Thus, the apparatus requires neither a beam splitter element for splitting the monitor light beam into two beams nor a focusing lens for focusing one of the beams obtained by the beam splitter to the second photodetector 6. The apparatus is therefore simple in structure and can be manufactured at low cost.

As described above, the semiconductor laser 2, first photodetector 4 and second detector 6 are provided together within the laser-detector unit 10, not spaced apart from one another. This helps to render the apparatus small.

An data-processing apparatus according to the second embodiment of the invention will be described. The components which are similar or identical to those of the first embodiment will be designated at the same reference numerals and will not be described in detail.

The apparatus according to the second embodiment has an optical head 101 shown in FIG. 3A. As shown in FIG. 3A. In use, the optical head 101 is located to oppose an optical disk D. The head 101 has a laser-detector unit 110. The laser-detector unit 110 comprises a semiconductor laser 2, a first photodetector 4 and a second photodetector 6. In the present embodiment, the photodetectors 4 and 6 are located close to each other, in almost the same plane and on the right side of the laser 2, as is illustrated in FIG. 3B.

The second photodetector 6 has a light-receiving region, extending in the direction in which the light beam reflected from the reflecting surface 118a of a correction prism 118 is deflected. Being elongate, the light-receiving region can compensate for an error, if any, of the inclination angle of the reflecting surface 118a. Therefore, no particular means is required to adjust the inclination angle of the reflecting surface 118a with high accuracy.

The optical head 101 further comprises a polarizing hologram 112, a collimating lens 14, an aperture controller 16, the correction prism 118, a quarter-wavelength plate 102, and a focusing lens 20 -- all arranged in the order mentioned in an optical path extending from the semiconductor laser 2 toward the optical disk D. Since both photodetectors 4 and 6 are located on the right side of the semiconductor laser 2, the surface 118a of the prism 118 is inclined in the direction opposite to the direction in which its counter part is inclined in the first embodiment.

The polarizing hologram 112 allows the passage of the entire light beam emitted from the laser 2 and also the light beam reflected by the reflecting surface 118a and deflects the light beam reflected by the optical disk D toward the first photodetector 4. In other words, the hologram 112 allows the passage of a component of the beam which is polarized in a certain direction and deflects in a prescribed direction another component of the beam which extends at right angles to that component. Thus, a light beam twice applied through the quarter-wave plate 102 and polarized is deflected by the hologram 112 toward the first photodetector 4.

The operation of the optical head 101 described above will be explained.

First, the semiconductor laser 2 emits a light beam in an mount adjusted by the laser driver 38. The light beam is applied to the focusing lens 20 through the polarizing hologram 112, collimating lens 14, aperture controller 16, correction prism 118 and quarter-wave plate 102. The focusing lens 20 focuses the light beam on the recording/reproducing surface of the optical disk D.

The recording/reproducing surface of the optical disk D reflects the light beam. The beam reflected is applied through the focusing lens 20, quarter-wave plate 102, correction prism 118, aperture controller 16, collimating lens 14 and polarizing hologram 112 and is focused on the first photodetector 4. As the light beam passes twice through the quarter-wave plate 102, it is polarized. The light beam is deflected by the polarizing hologram 112.

Part of the light beam emitted from the semiconductor laser 2 is reflected by the reflecting surface 118a of the correction prism 118, focused by the collimating lens 14, supplied through the polarizing hologram 112 and focused on the second photodetector 6. This part of the light beam, which does not pass through the quarter-wave plate 102, is not polarized and therefore passes through the polarizing hologram 112, not deflected at all.

The second embodiment (FIG. 3A) can attain the same advantages as the first embodiment (FIG. 1A). Further, the polarizing hologram 112 and the quarter-wave plate 102 render it easy to adjust the other components of the optical head 101. Moreover, the error of adjusting the inclination angle of the reflecting surface 118a of the correction prism 118 can be compensated for, since the light-receiving region of the second photodetector 6 is, extending in the direction of deflecting the light beam reflected from the reflecting surface 118a.

The present invention is not limited to the embodiments described above. Various changes and modifications can be made within the scope of the invention.

Claims

1. An data-processing apparatus characterized by comprising:

   a light source (2) for emitting a light beam;
   adjusting means (38) for adjusting an amount in which the light beam is emitted from said light source;
   a collimating lens (14) for collimating the light beam;
   a focusing lens (20) for focusing the light beam collimated by said collimating lens, on a record-

ing medium (D);

deflecting means (12a, 112) for deflecting the light beam reflected by the recording medium (D) and applied through said focusing lens (20), to a first position near said light source (2);

first light-receiving means (4) provided at the first position, for receiving the light beam deflected by said deflecting means and converting the same into an electric data signal;

a reflecting surface (18a) provided between said collimating lens (14) and said focusing lens (20), having a reflectance of at most 50% and inclined to a plane perpendicular to an optical axis of said collimating lens (14), for reflecting a part of the light beam applied through said collimating lens (14) and focusing the same at a second position near said light source (2);

a second light-receiving means (6) provided at the second position, for receiving the light beam focused at the second position and converting the same into an electric monitor signal; and

control means (30) for controlling said adjusting means (38) in accordance with the electric monitor signal, thereby to cause said adjusting means (30) to adjust the amount in which the light beam is emitted from said light source (2).

2. The apparatus according to claim 1, characterized by further comprising processing means (40) for processing the electric data signal.

3. The apparatus according to claim 1, characterized in that said deflecting means (12a) has an optical diffraction element provided between said light source (2) and said collimating lens (14), for deflecting to the first position the light beam reflected by the recording medium (D) and applied through said focusing lens (20).

4. The apparatus according to claim 2, characterized in that said deflecting means (12a) has an optical diffraction element provided between said light source (2) and said collimating lens (14), for deflecting to the first position the light beam reflected by the recording medium (D) and applied through said focusing lens (20).

5. The apparatus according to claim 1, which further comprises aperture controlling means (16) provided between said collimating lens (14) and said reflecting surface (18a) and having an aperture whose diameter is less than an effective diameter of said collimating lens (14) and equal to or greater than an effective diameter of said focusing lens (20), and in which said reflecting surface (18a) and said aperture controlling means (16) have such inclination angles and such aperture diameters that the entire light beam reflected by said reflecting surface (18a) passes through said collimating lens (14).

6. The apparatus according to claim 5, characterized in that said deflecting means (12a) has an optical diffraction element provided between said light source (2) and said collimating lens (14), for deflecting to the first position the light beam reflected by the recording medium (D) and applied through said focusing lens (20).

7. The apparatus according to claim 6, characterized by further comprising processing means (40) for processing the electric data signal.

8. The apparatus according to claim 1, characterized in that said reflecting surface (18a) is inclined to reflect and apply the light beam through said collimating lens (14) and to prevent the light beam from passing through said deflecting means (12a).

9. The apparatus according to claim 2, characterized in that said reflecting surface (18a) is inclined to reflect and apply the light beam through said collimating lens (14) and to prevent the light beam from passing through said deflecting means (12a).

10. The apparatus according to claim 1, characterized by further comprising correction means (18) having said reflecting surface (18a) and a inclined surface (18b) provided between said reflecting surface (18a) and said focusing lens (20), for correcting an ellipticity of the light beam applied through said reflecting surface (18a), said inclined surface (18b) inclined to said reflecting surface (18a) at such angle that the light beam emitted through said inclined surface (18b) has less ellipticity than the light beam applied through said reflecting surface (18a).

11. The apparatus according to claim 2, characterized by further comprising correction means (18) having said reflecting surface (18a) and a inclined surface (18b) provided between said reflecting surface (18a) and said focusing lens (20), for correcting an ellipticity of the light beam applied through said reflecting surface (18a), said inclined surface (18b) inclined to said reflecting surface (18a) at such angle that the light beam emitted through said inclined surface (18b) has less ellipticity than the light beam applied through said reflecting surface (18a).

12. An data-processing apparatus characterized by comprising:

a light source (2) for emitting a light beam;

adjusting means (38) for adjusting an amount in which the light beam is emitted from said light source;

a collimating lens (14) for collimating the light beam;

a focusing lens (20) for focusing the light beam collimated by said collimating lens, on a recording medium (D);

polarization plane rotating means (102) for allowing passage of the light beam applied toward the recording medium (D), allowing passage of the light beam reflected by the recording medium and rotating a polarization plane of the light beam reflected by the recording medium;

deflecting means (112) provided between said light source (2) and said polarization plane rotating means (102), for allowing passage of the light beam emitted from said light source (2) and deflecting the light beam which reflected by the recording medium (D) and whose polarization plane is rotated by said polarization plane rotating means (102), to a first position near said light source (2);

first light-receiving means (4) provided at the first position, for receiving the light beam deflected by said deflecting means and converting the same into an electric data signal;

a reflecting surface (18a) provided between said collimating lens (14) and said polarization plane rotating means (102), having a reflectance of at most 50% and inclined to a plane perpendicular to an optical axis of said collimating lens (14), for reflecting a part of the light beam applied through said collimating lens (14) and focusing the same at a second position near said light source (2);

a second light-receiving means (6) provided at the second position, for receiving the light beam focused at the second position and converting the same into an electric monitor signal; and

control means (30) for controlling said adjusting means (38) in accordance with the electric monitor signal, thereby to cause said adjusting means (38) to adjust the amount in which the light beam is emitted from said light source (2).

13. The apparatus according to claim 12, characterized by further comprising processing means (40) for processing the electric data signal.

14. The apparatus according to claim 12, characterized in that said second light-receiving means (6) has an elongated light-receiving region extending in a direction in which the light beam reflected by said reflecting surface (18a) is deflected.

15. The apparatus according to claim 13, characterized in that said second light-receiving means (6) has an elongated light-receiving region extending in a direction in which the light beam reflected by said reflecting surface (18a) is deflected.

16. The apparatus according to claim 12, characterized in that said deflecting means (112) provided between said light source (2) and said collimating lens (14), for allowing passage of the light beam emitted from said light source (2) and the light beam reflected by said reflecting surface (18a) and applied through said collimating lens (14) and for deflecting the light beam which reflected by the recording medium (D), whose polarization plane is rotated by said polarization plane rotating means (102) and applied through said collimating lens (14), to a first position near said light source (2).

17. The apparatus according to claim 16, characterized by further comprising processing means (40) for processing the electric data signal.

18. The apparatus according to claim 16, characterized in that said second light-receiving means (6) has an elongated light-receiving region extending in a direction in which the light beam reflected by said reflecting surface (18a) is deflected.

19. The apparatus according to claim 17, characterized in that said second light-receiving means (6) has an elongated light-receiving region extending in a direction in which the light beam reflected by said reflecting surface (18a) is deflected.

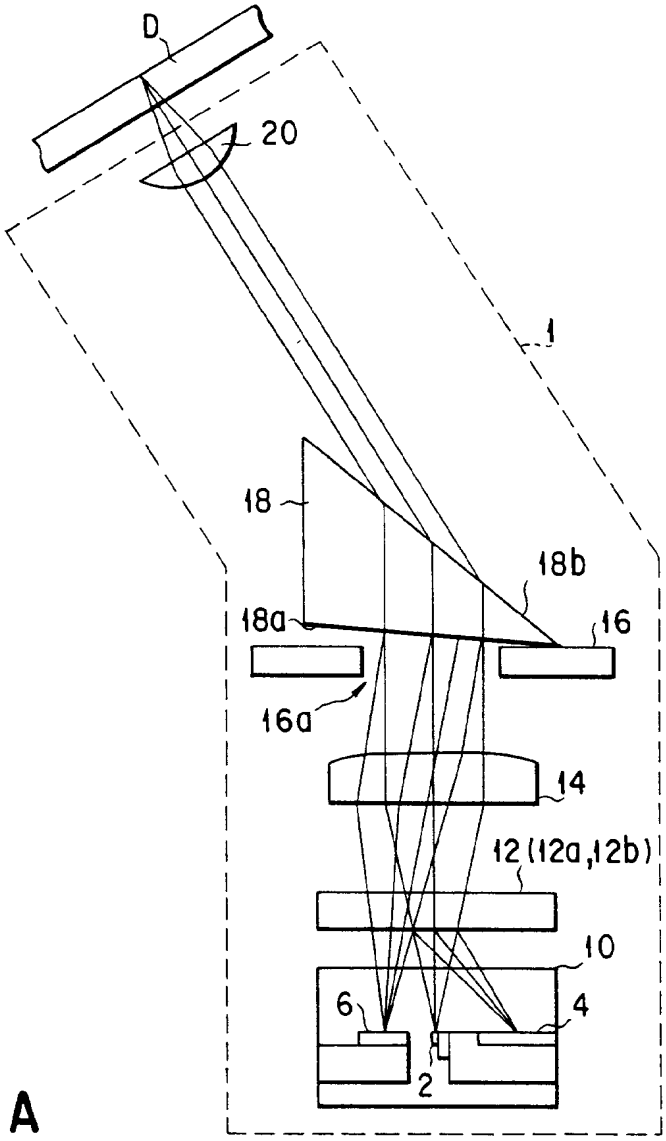


FIG. 1A

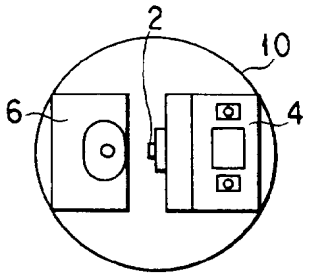


FIG. 1B

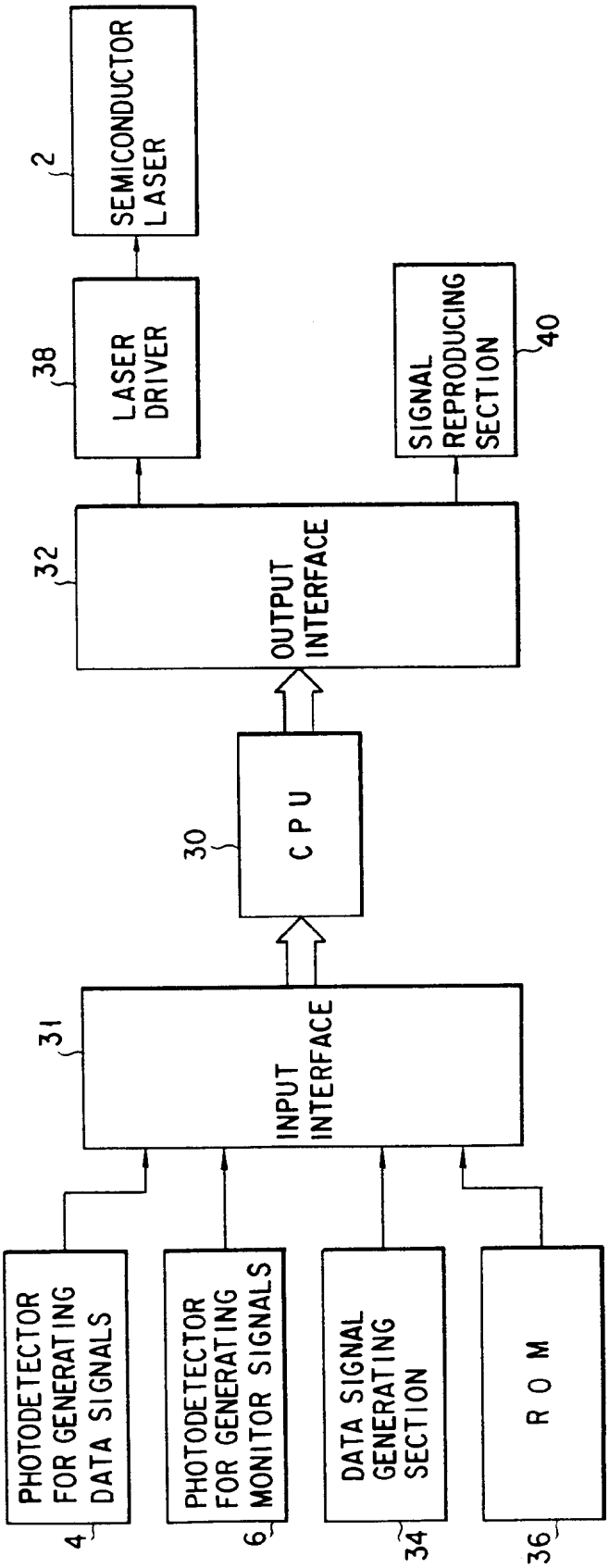

SEMICONDUCTOR LASER
2
LASER DRIVER
38
SIGNAL REPRODUCING SECTION
40
OUTPUT INTERFACE
32
CPU
30
INPUT INTERFACE
31
PHOTODETECTOR FOR GENERATING DATA SIGNALS
4
PHOTODETECTOR FOR GENERATING MONITOR SIGNALS
6
DATA SIGNAL GENERATING SECTION
34
ROM
36

FIG. 2

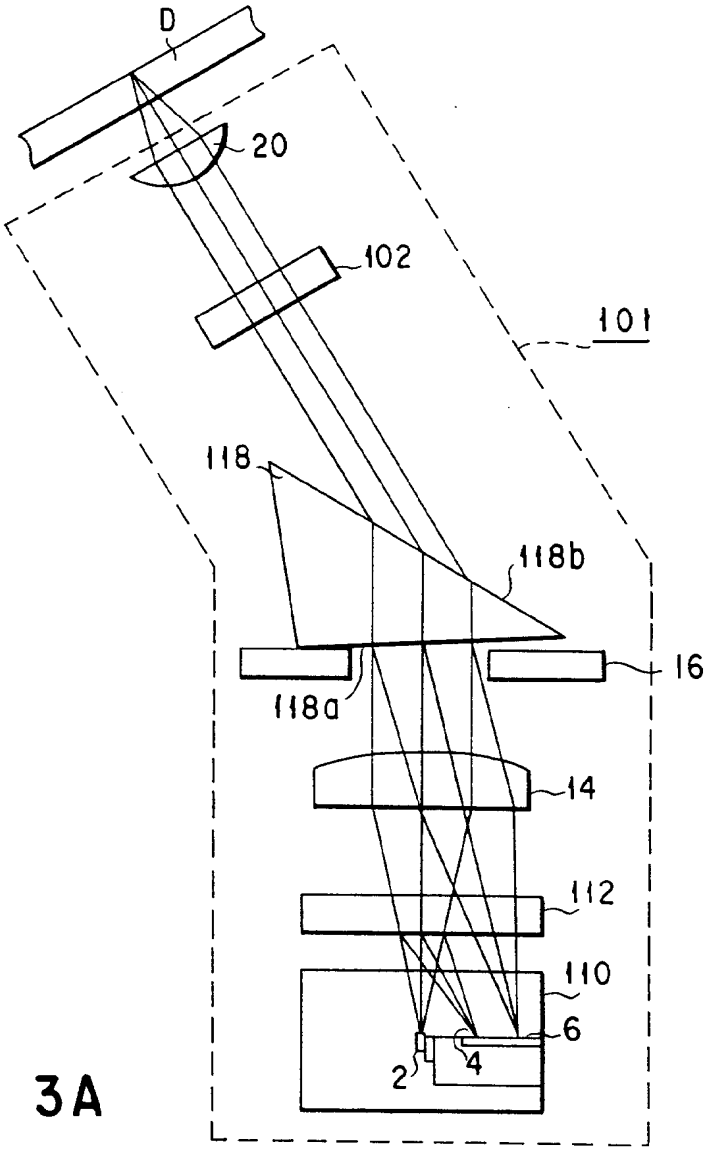


FIG. 3A

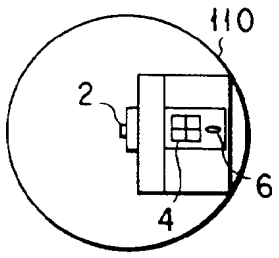


FIG. 3B

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 96 11 1949

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P,X	US-A-5 475 210 (TAGUCHI TOYOKI ET AL) 12 December 1995 * column 26, line 60 - column 27, line 53; figures 27,28 * & JP-A-06 267 100 (TOSHIBA) 22 September 1994	1,2,12, 13	G11B7/125 G11B7/135 G11B7/12
X	US-A-5 293 372 (HOSHINO ISAO ET AL) 8 March 1994 * figure 9 *	1,2,10, 11	
X	DE-A-43 36 546 (HYUNDAI ELECTRONICS AMERICA IN) 19 May 1994	1,10	
A	* the whole document *	11	
A	NL-A-8 501 473 (PHILIPS NV) 16 December 1986 * the whole document *	1,12	
A	US-A-5 136 152 (LEE WAI-HON) 4 August 1992 * column 8, line 30 - line 44; figure 11 * * column 5, line 28 - line 37; figure 6A *	1-4,6,7, 10-13,17	TECHNICAL FIELDS SEARCHED (Int.Cl.6) G11B
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15 November 1996	Holubov, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 757 346 B1

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(54) **Optical head apparatus**

Anordnung für optischen Kopf

Dispositif de tête optique

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05.02.1997 Bulletin 1997/06

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US-A- 5 136 152
US-A- 5 293 372
US-A- 5 475 210

- **PATENT ABSTRACTS OF JAPAN vol. 96, no. 8, 30 August 1996 & JP-A-08 087768 (TOSHIBA CORP), 2 April 1996,**

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 0 757 346 B1

Description

[0001] The present invention relates to a data-processing apparatus and, more particularly to an optical head apparatus which optically records data on a recording medium such as an optical disk and which optically reproduces the data from the recording medium.

[0002] Generally, a data-processing apparatus for optically record data on a recording medium such as an optical disk and for reproducing the data from the medium has an optical head. The optical head applies a light beam to the recording/reproducing surface of the medium to record data on the medium, and applies a light scan to the surface of the medium and receives the light reflected by the medium to reproduce the data from the recording medium.

[0003] The optical head comprises a semiconductor laser, a collimating lens, a focusing lens, a deflecting element, and a photodetector. The semiconductor laser is used as the light source. The collimating lens collimates the dispersing light emitted from the laser. The focusing lens receives the collimated light (i.e., a parallel beam) from the collimating lens and focuses it on an optical disk which is used as a recording medium. The deflecting element receives the light beam reflected by the disk and applied through the focusing lens and deflects the light beam in a predetermined direction. The photodetector receives the deflected light beam and converts it into electric signals. The electric signals represent the data reproduced from the optical disk.

[0004] The optical head further comprises another photodetector and a laser driver. This photodetector monitors the amount of light the semiconductor laser has emitted. The laser driver controls the laser on the basis of the amount of light the photodetector has monitored, so that the laser may emit light in a predetermined amount.

[0005] Two methods of monitoring the amount of light emitted from a semiconductor laser are known. The first method is to monitor the light emitted from the back of the semiconductor laser and traveling away from a recording medium. The second method is to monitor the light emitted from the front of the laser and traveling toward the recording medium. Hereinafter, the light traveling away from the medium will be referred to as "backward light," and the light traveling toward the medium as "forward light."

[0006] The first method is disadvantageous, however. It cannot detect the amount of backward light accurately and cannot serve to control the semiconductor laser such that the laser emits light in the predetermined amount. This is because the backward light is influenced by the forward light reflected by the medium and reaching, though in a small amount, the semiconductor laser. Consequently, the amount of the backward light cannot be proportionate to the amount of the forward light, making it difficult to cause the laser to emit light in a desired amount.

[0007] The second method is disadvantageous, too. To perform the second method, an optical head needs to have two additional components. The first additional component is a beam splitter for splitting the forward light beam into two beams. The second additional component is a focusing lens for focusing one of the beams obtained by the beam splitter at the light-receiving surface of the photodetector for monitoring the light emitted from the laser. The use of these additional components inevitably increases the size of the optical head and the manufacturing cost thereof.

[0008] JP-A-06.267.100 discloses an optical data processing apparatus which is reflected by the preamble of claim 1.

[0009] In view of the foregoing, the present invention has been made, and its object is to provide a data-processing apparatus which is simple in structure and can be manufactured at low cost and which can reliably record and reproduce data.

[0010] To achieve the object, an data-processing apparatus comprising: a light source for emitting a light beam; adjusting means for adjusting an amount in which the light beam is emitted from the light source; a collimating lens for collimating the light beam; a focusing lens for focusing the light beam collimated by the collimating lens, on a recording medium; deflecting means for deflecting the light beam reflected by the recording medium and applied through the focusing lens, to a first position; first light-receiving means for receiving the light beam deflected by the deflecting means, at the first position, and converting the same into an electric data signal; a reflecting surface provided between the collimating lens and the focusing lens, and inclined to a plane perpendicular to an optical axis of the collimating lens, for reflecting a part of the light beam emitted from the light source and applying the same to a second position; a second light-receiving means for receiving the light beam reflected by said reflecting surface at the second position and converting the same into an electric monitor signal; and control means for controlling the adjusting means in accordance with the electric monitor signal, thereby to adjust the amount in which the light beam is emitted from the light source, wherein said first and second light receiving means are adjacent to the light source, and there is further provided a correction means having said reflecting surface, for correcting an ellipticity of the light beam that has passed through said reflecting surface.

[0011] Further embodiments of the invention are defined in the appended dependent claims 2 to 11.

[0012] This invention can be more fully understood from the following detailed description when taken in conjunction with the accompanying drawings, in which:

FIG. 1A is a schematic view showing the internal structure of the optical head incorporated in a data-processing apparatus according to a first embodiment of the invention;

FIG. 1B is a plan view of the laser-detector unit built in the optical head shown in FIG. 1A;

FIG. 2 is a block diagram illustrating the control system provided in the data-processing apparatus according to a first embodiment of the invention;

FIG. 3A is a schematic view showing the internal structure of the optical head incorporated in a data-processing apparatus according to a second embodiment of the invention; and

FIG. 3B is a plan view of the laser-detector unit built in the optical head shown in FIG. 3A.

[0013] Embodiments of the present invention will be described in detail, with reference to the accompanying drawings.

[0014] FIGS. 1A and 1B show the optical head 1 incorporated in a data-processing apparatus which is the first embodiment of the invention. In use, the optical head 1 is located to oppose the recording/reproducing surface of an optical disk D used as a recording medium. As shown in FIG. 1A, the optical head 1 has a laser-detector unit 10. The laser-detector unit 10 comprises a semiconductor laser 2, a first photodetector 4 and a second photodetector 6 which are located in almost the same plane as is illustrated in FIG. 1B. The semiconductor laser 2, the first photodetector 4 and the second photodetector 6 are used as the light source, the first light-receiving means for generating data signals, and the second light-receiving means for generating monitor signals, respectively. The photodetectors 4 and 6 are arranged at first and second positions, near the semiconductor laser 2.

[0015] The optical head 1 further comprises an optical diffraction element 12, a collimating lens 14, an aperture controller 16, a correction prism 18 and a focusing lens 20. These components 12, 14, 16 and 18 are arranged, in the order mentioned, in the optical path extending from the semiconductor laser 2 to the optical disk D. The collimating lens 14 converts the dispersing light emitted from the laser 2 to a parallel light beam. The aperture controller 16 functions as diaphragm means. The correction prism 18 serves as correction means. The focusing lens 20 focuses the light output from the collimating lens 14 and applied through the controller 16 and the prism 18, forming a beam spot on the recording/reproducing surface of the optical disk D. The correction prism 18 has a reflecting surface 18a and an inclined surface 18b. The surface 18a has a reflectance of 50% or less. The surface 18b is inclined at a predetermined angle to the reflecting surface 18a.

[0016] The optical diffraction element 12 is shaped like a plate. It extends substantially parallel to a plane which is perpendicular to the axis of the light beam emitted from the semiconductor laser 2 toward the optical disk D. The element 12 has a light-diffracting section 12a and a light-passing section 12b. The light-diffracting section 12a performs two functions. The first function is to allow the passage of the light beam emitted from the semiconductor laser 2. The second function is to deflect the light beam reflected by the optical disk D and thus representing data, toward the first photodetector 4. The light-passing section 12b allows the passage of the monitor light beam reflected by the reflecting surface 18a of the correction prism 18, without reflecting this light beam.

[0017] The collimating lens 14 is placed in a horizontal plane which is substantially at right angles to the optical axis of the semiconductor laser 2. Not only does the lens 14 collimate the dispersed light beam which has been emitted from the semiconductor laser 2 and which has passed through the light-diffracting section 12a of the optical diffraction element 12. But also does the lens 14 focus the light beam reflected by the reflecting surface 18a of the correction prism 18, at the light-receiving surface of the second photodetector 6 which is provided for detecting monitor signals. In other words, the collimating lens 14 works as a focusing lens, as well.

[0018] The light beam reflected by the reflecting surface 18a of the correction prism 18 and focused by the collimating lens 14 passes through the light-passing section 12b of the optical diffraction element 12. This light beam is then focused at the light-receiving surface of the second photodetector 6.

[0019] The correction prism 18 has its reflecting surface 18a inclined at a prescribed angle to the plane which extends at right angles to the optical axis of the semiconductor laser 2. Since the reflecting surface 18a is inclined so, all light beam it reflects is applied to the effective region of the collimating lens 14 and is focused by the collimating lens 14. This beam focused is applied to only the light-passing section 12b of the optical diffraction element 12, not to the light-diffracting section 12a thereof. The beam passes through the light-passing section 12b and reaches the light-receiving surface of the second photodetector 6 provided for generating monitor signals.

[0020] The correction prism 18 is a triangular prism. As indicated above, it has the reflecting surface 18a and the inclined surface 18b. The inclined surface 18b refracts the input light beam toward the focusing lens 20, the input light beam having been emitted from the semiconductor laser 2, passed through the light-diffracting section 12a and the collimating lens 14 and applied to the reflecting surface 18a of the correction lens 18. The inclined surface 18b also reduces the ellipticity of the light beam. Namely, the ellipticity of the beam can be changed by inclining the axis of the beam to the major axis of an elliptical area in a plane perpendicular to the axis of the beam. This is because the intensity of a light beam emitted from a semiconductor laser 2 is distributed generally in that elliptical area. Hence, the intensity of the light beam in the cross section thereof can be distributed, desirably in a substantially circular area by setting the inclination angle of the surface 18b of the correction prism 18.

[0021] The aperture controller 16 provided between

the collimating lens 14 and the correction prism 18 extends almost parallel to a plane which is at right angles to the optical axis of the semiconductor laser 2. The aperture controller 16 has a substantially circular aperture 16a and an adjusting mechanism (not shown) designed to adjust the opening of the aperture 16a. The opening of the aperture 16a is adjusted so that the beam reflected by the reflecting surface 18a of the correction lens 18 is applied in its entirety within the effective region of the collimating lens 14, that the beam focused by the collimating lens 14 may pass through the light-passing section 12b of the optical diffraction element 12, and that the beam may be applied to the second photodetector 6 provided for generating monitor signals.

[0022] The data-processing apparatus having the optical head described above has a control system, which will be described below.

[0023] As shown in FIG. 2, the control system has a CPU 30 used as control means. An input interface 31 is connected to the CPU 30. Connected to the input interface 31 are the first detector 4, the second detector 6, a data signal generating section 34, and a ROM 36. The section 34 is provided to store data to be recorded on the optical disk D. The ROM 36 stores various control data items, among which are a first reference value for the amount of a data-recording light beam and a second reference value for the amount of a data-reproducing light beam.

[0024] An output interface 32 is connected to the CPU 30. Connected to the output section 32 are a laser driver 38 and a signal reproducing section 40. The driver 38 is means for adjusting the amount of a light beam emitted from the laser 2. The section 40 processes the data signals generated by the first photodetector 4, thereby to reproduce the data signals.

[0025] The operation of the data-processing apparatus will be described.

[0026] At first, the semiconductor laser 2 emits a light beam, the amount of which is adjusted by the laser driver 38. The light beam passes through the light-diffracting section 12a of the optical diffraction element 12, the collimating lens 14, and the reflecting surface 18a and the inclined surface 18b of the correction lens 18. The light beam is then focused by the focusing lens 20 and thereby applied to the recording/reproducing surface of the optical disk D.

[0027] Part of the light beam emitted from the semiconductor laser 2 is reflected by the reflecting surface 18a of the correction lens 18 and applied back to the collimating lens 14. It then passes through the collimating lens 14 and through the light-passing section 12b of the optical diffraction element 12. Finally it is focused on the second photodetector 6 by the collimating lens 14.

[0028] To record data on the optical disk D, the signals representing the data are generated by the data signal generating section 34. The data signals are supplied to the laser driver 38 through the input interface 31 and CPU 30, output interface 32. The laser driver 38 emits a light beam the amount of which is modulated in accordance with the data signals. Meanwhile, the second photodetector 6 monitors the light beam emitted from the semiconductor laser 2, detecting the amount of the light beam. The amount of light detected by the photodetector 6 is compared with the first reference value stored in the ROM 36. The CPU 30 controls the laser driver 38, whereby the amount of light beam emitted from the laser 2 becomes equal to the desired amount for the data-recording light beam. The light beam thus controlled in amount is focused on the recording/reproducing surface of the optical disk D. As a result, the data is recorded on the optical disk D.

[0029] To reproduce the data from the optical disk D, too, the second photodetector 6 monitors the light emitted from the semiconductor laser 2, detecting the amount of the beam. The amount of the light beam is compared with the second reference value stored in the ROM 36. The CPU 30 controls the laser driver 38, whereby the amount of light beam emitted from the laser 2 becomes equal to the desired amount for the data-reproducing light beam. The light beam thus controlled in amount is focused on the recording/reproducing surface of the optical disk D. As a result, the data is reproduced from the optical disk D.

[0030] The light beam reflected by the optical disk D is focused on the first photodetector 4 after passing through the focusing lens 20, correction prism 18, aperture controller 16, collimating lens 14 and the light-diffracting section 12a of the optical diffraction element 12. The first photodetector 4 generates data signals from the light beam. These data signals (i.e., the reproduced data signals) are processed by the signal reproducing section 40. As a result, the data recorded on the optical disk D is reproduced.

[0031] In the data-processing apparatus, the collimating lens 14 is used not only to collimate the light beam emitted from the semiconductor laser 2, but also to focus the light beam reflected by the disk D on the second photodetector 6 provided for generating monitor signals. Thus, the apparatus requires neither a beam splitter element for splitting the monitor light beam into two beams nor a focusing lens for focusing one of the beams obtained by the beam splitter to the second photodetector 6. The apparatus is therefore simple in structure and can be manufactured at low cost.

[0032] As described above, the semiconductor laser 2, first photodetector 4 and second detector 6 are provided together within the laser-detector unit 10, not spaced apart from one another. This helps to render the apparatus small.

[0033] An data-processing apparatus according to the second embodiment of the invention will be described. The components which are similar or identical to those of the first embodiment will be designated at the same reference numerals and will not be described in detail.

[0034] The apparatus according to the second em-

bodiment has an optical head 101 shown in FIG. 3A. As shown in FIG. 3A. In use, the optical head 101 is located to oppose an optical disk D. The head 101 has a laser-detector unit 110. The laser-detector unit 110 comprises a semiconductor laser 2, a first photodetector 4 and a second photodetector 6. In the present embodiment, the photodetectors 4 and 6 are located close to each other, in almost the same plane and on the right side of the laser 2, as is illustrated in FIG. 3B.

[0035] The second photodetector 6 has a light-receiving region, extending in the direction in which the light beam reflected from the reflecting surface 118a of a correction prism 118 is deflected. Being elongate, the light-receiving region can compensate for an error, if any, of the inclination angle of the reflecting surface 118a. Therefore, no particular means is required to adjust the inclination angle of the reflecting surface 118a with high accuracy.

[0036] The optical head 101 further comprises a polarizing hologram 112, a collimating lens 14, an aperture controller 16, the correction prism 118, a quarter-wavelength plate 102, and a focusing lens 20 -- all arranged in the order mentioned in an optical path extending from the semiconductor laser 2 toward the optical disk D. Since both photodetectors 4 and 6 are located on the right side of the semiconductor laser 2, the surface 118a of the prism 118 is inclined in the direction opposite to the direction in which its counter part is inclined in the first embodiment.

[0037] The polarizing hologram 112 allows the passage of the entire light beam emitted from the laser 2 and also the light beam reflected by the reflecting surface 118a and deflects the light beam reflected by the optical disk D toward the first photodetector 4. In other words, the hologram 112 allows the passage of a component of the beam which is polarized in a certain direction and deflects in a prescribed direction another component of the beam which extends at right angles to that component. Thus, a light beam twice applied through the quarter-wave plate 102 and polarized is deflected by the hologram 112 toward the first photodetector 4.

[0038] The operation of the optical head 101 described above will be explained.

[0039] First, the semiconductor laser 2 emits a light beam in an mount adjusted by the laser driver 38. The light beam is applied to the focusing lens 20 through the polarizing hologram 112, collimating lens 14, aperture controller 16, correction prism 118 and quarter-wave plate 102. The focusing lens 20 focuses the light beam on the recording/reproducing surface of the optical disk D.

[0040] The recording/reproducing surface of the optical disk D reflects the light beam. The beam reflected is applied through the focusing lens 20, quarter-wave plate 102, correction prism 118, aperture controller 16, collimating lens 14 and polarizing hologram 112 and is focused on the first photodetector 4. As the light beam passes twice through the quarter-wave plate 102, it is polarized. The light beam is deflected by the polarizing hologram 112.

[0041] Part of the light beam emitted from the semiconductor laser 2 is reflected by the reflecting surface 118a of the correction prism 118, focused by the collimating lens 14, supplied through the polarizing hologram 112 and focused on the second photodetector 6. This part of the light beam, which does not pass through the quarter-wave plate 102, is not polarized and therefore passes through the polarizing hologram 112, not deflected at all.

[0042] The second embodiment (FIG. 3A) can attain the same advantages as the first embodiment (FIG. 1A). Further, the polarizing hologram 112 and the quarter-wave plate 102 render it easy to adjust the other components of the optical head 101. Moreover, the error of adjusting the inclination angle of the reflecting surface 118a of the correction prism 118 can be compensated for, since the light-receiving region of the second photodetector 6 is, extending in the direction of deflecting the light beam reflected from the reflecting surface 118a.

Claims

1. A data-processing apparatus comprising:

a light source (2) for emitting a light beam;
adjusting means (38) for adjusting an amount in which the light beam is emitted from said light source (2);
a collimating lens (14) for collimating the light beam;
a focusing lens (20) for focusing the light beam collimated by said collimating lens (14), on a recording medium (D);
deflecting means (12a;112) for deflecting the light beam reflected by the recording medium (D) and applied through said focusing lens (20) to a first position;
first light-receiving means (4) for receiving the light beam deflected by said deflecting means (12a;112), at the first position, and converting the same into an electric data signal;
a reflecting surface (18a;118a) provided between said collimating lens (14) and said focusing lens (20) and inclined to a plane perpendicular to an optical axis of said collimating lens (14), for reflecting a part of the light beam emitted from said light source (2) and applying the same to a second position;
a second light-receiving means (6) for receiving the light beam reflected by said reflecting surface (18a;118a) at the second position and converting the same into an electric monitor signal; and
control means (30) for controlling said adjusting means (38) in accordance with the electric

monitor signal, thereby to adjust the amount in which the light beam is emitted from said light source (2)

characterized in that

said first and second light receiving means (4,6) are adjacent to the light source (2), and
there is further provided a correction means (18; 118) having said reflecting surface (18a; 118a), for correcting an ellipticity of the light beam that has passed through said reflecting surface (18a;118a).

2. The apparatus according to claim 1, **characterized in that** said deflecting means (12a) has an optical diffraction element provided between said light source (2) and said collimating lens (14), for deflecting to the first position the light beam reflected by the recording medium (D) and applied through said focusing lens (20).

3. The apparatus according to claim 1, **characterized by** comprising

polarization plane rotating means (102) for allowing passage of the light beam applied toward the recording medium (D), allowing passage of the light beam reflected by the recording medium (D) and rotating a polarization plane of the light beam reflected by the recording medium (D);
wherein said deflecting means (112) is provided between said light source (2) and said polarization plane rotating means (102), for allowing passage of the light beam emitted from said light source (2) and deflecting the light beam which is reflected by the recording medium (D) and whose polarization plane is rotated by said polarization plane rotating means (102), to said first position adjacent said light source (2); and said reflecting surface (118a) is provided between said collimating lens (14) and said polarization plane rotating means (102).

4. The apparatus according to claim 3, **characterized in that** said second light-receiving means (6) has an elongated light-receiving region extending in a direction in which the light beam reflected by said reflecting surface (118a) is deflected.

5. The apparatus according to claim 3 or 4, **characterized in that** said deflecting means (112) is provided between said light source (2) and said collimating lens (14), for allowing passage of the light beam emitted from said light source (2) and the light beam reflected by said reflecting surface (118a) and applied through said collimating lens (14) and for deflecting the light beam which reflected by the recording medium (D), whose polarization plane is rotated by said polarization plane rotating means (102) and applied through said collimating lens (14), to said first position adjacent said light source (2).

6. The apparatus according to any one of claims 1 to 5, which further comprises aperture controlling means (16) provided between said collimating lens (14) and said reflecting surface (18a;118a) and having an aperture whose diameter is less than an effective diameter of said collimating lens (14) and equal to or greater than an effective diameter of said focusing lens (20), and in which said reflecting surface (18a;118a) and said aperture controlling means (16) have such inclination angles and such aperture diameters that the entire light beam reflected by said reflecting surface (18a;118a) passes through said collimating lens (14).

7. The apparatus according to any one of claims 1 to 6, **characterized in that** said reflecting surface (18a;118a) is inclined to reflect and apply the light beam through said collimating lens (14) and to prevent the light beam from passing through said deflecting means (12a;112).

8. The apparatus according to any one of claims 1 to 7, **characterized in that** said correction means comprises a correction prism (18;118) which has said reflecting surface (18a;118a) and an inclined surface (18b;118b) provided between said reflecting surface (18a;118a) and said focusing lens (20), said inclined surface (18b;118b) being inclined to said reflecting surface (18a;118a) at such an angle that the light beam applied through said inclined surface (18b;118b) has less ellipticity than the light beam applied through said reflecting surface (18a; 118a).

9. The apparatus according to any one of claims 1 to 8, **characterized by** further comprising processing means (40) for processing the electric data signal.

10. The apparatus according to any one of claims 1 to 9, **characterized in that** said collimating lens (14) functions as a focusing lens that allows passage of the light beam reflected by the recording medium (D) and applied through said focusing lens (20), focuses the light beam at the first position, and focuses the part of the light beam reflected by said reflecting surface (18a;118a) at the second position.

11. The apparatus according to any one of claims 1 to 10, **characterized in that** said reflecting surface (18a;118a) has a reflectance of at most 50%.

Patentansprüche

1. Datenverarbeitungsgerät mit:

einer Lichtquelle (2) zum Emittieren eines Lichtstrahls,
einer Einstell- bzw. Anpassungseinrichtung (38) zum Einstellen bzw. Anpassen einer Stärke, mit der der Lichtstrahl von der Lichtquelle (2) emittiert wird,
einer Kollimatorlinse (14) zum Kollimieren des Lichtstrahls,
einer Fokussierlinse (20) zum Fokussieren des durch die Kollimatorlinse (14) kollimatierten Lichtstrahls auf ein Aufzeichnungsmedium (D),
einer Ablenkeinrichtung (12a; 112) zum Ablenken des vom Aufzeichnungsmedium (D) reflektierten und durch die Fokussierlinse (20) gerichteten Lichtstrahls auf eine erste Position,
einer ersten Lichtempfangseinrichtung (4) zum Empfangen des von der Ablenkeinrichtung (12a;112) abgelenkten Lichtstrahls an der ersten Position und zum Konvertieren desselben in ein elektrisches Datensignal,
einer Reflexionsfläche (18a;118a), die zwischen der Kollimatorlinse (14) und der Fokussierlinse (20) vorgesehen und zu einer Ebene hin geneigt ist, die senkrecht zu einer optischen Achse der Kollimatorlinse (14) ist, um einen Teil des von der Lichtquelle (2) emittierten Lichtstrahls zu reflektieren und denselben auf eine zweite Position zu richten,
einer zweiten Lichtempfangseinrichtung (6) zum Empfangen des von der Reflexionsfläche (18a;118a) reflektierten Lichtstrahls an der zweiten Position und zum Konvertieren desselben in ein elektrisches Überwachungssignal, und
einer Steuereinrichtung (30) zum Steuern der Einstelleinrichtung (38) gemäß dem elektrischen Überwachungssignal, wodurch die Stärke, mit der der Lichtstrahl von der Lichtquelle (2) emittiert wird, eingestellt bzw. angepasst wird,

dadurch gekennzeichnet, dass

sich die ersten und zweiten Lichtempfangseinrichtungen (4,6) neben der Lichtquelle (2) befinden, und
ferner eine Korrektureinrichtung (18:118) mit der Reflexionsfläche (18a;118a) vorgesehen ist, um eine Elliptizität des Lichtstrahls, der die Reflexionsfläche (18a;118a) passiert hat, zu korrigieren.

2. Gerät gemäß Anspruch 1, **dadurch gekennzeichnet, dass** die Ablenkeinrichtung (12a) ein optisches Beugungselement aufweist, das zwischen der Lichtquelle (2) und der Kollimatorlinse (14) vorgesehen ist, um den von dem Aufzeichnuhgsmedium (D) reflektierten und durch die Fokussierlinse (20) gerichteten Lichtstrahl zu der ersten Position abzulenken.

3. Gerät gemäß Anspruch 1, **dadurch gekennzeichnet, dass** sie umfasst:

eine Polarisierungsebenen-Dreheinrichtung (102), um ein Passieren des auf das Aufzeichnungsmedium (D) gerichteten Lichtstrahls sowie des durch das Aufzeichnungsmedium (D) reflektierten Lichtstrahls zu gestatten, und um eine Polarisierungsebene des von dem Aufzeichnungsmedium (D) reflektierten Lichtstrahls zu drehen,
wobei die Ablenkeinrichtung (112) zwischen der Lichtquelle (2) und der Polarisierungsebenen-Dreheinrichtung (102) vorgesehen ist, um ein Passieren des von der Lichtquelle (2) emittierten Lichtstrahls zu gestatten und den von dem Aufzeichnungsmedium (D) reflektierten Lichtstrahl, und dessen Polarisierungsebene durch die Polärisierungsebenen-Dreheinrichtung (102) gedreht wird, zu der ersten Position in Nähe der Lichtquelle (2) abzulenken und wobei
die Reflexionsfläche (118a) zwischen der Kollimatorlinse (14) und der Polarisierungsebenen-Dreheinrichtung (102) vorgesehen ist.

4. Gerät gemäß Anspruch 3, **dadurch gekennzeichnet, dass** die zweite Lichtempfangseinrichtung (6) einen länglichen Lichtempfangsbereich aufweist, der sich in einer Richtung erstreckt, in der der von der Reflexionsfläche (118a) reflektierte Lichtstrahl abgelenkt wird.

5. Gerät gemäß Anspruch 3 oder 4, **dadurch gekennzeichnet, dass** die Ablenkeinrichtung (112) zwischen der Lichtquelle (2) und der Kollimatorlinse (14) vorgesehen ist, um das Passieren des von der Lichtquelle (2) emittierten Lichtstrahls und des von der Reflexionsfläche (118a) reflektierten und durch die Kollimatorlinse (14) gerichteten Lichtstrahls zu gestatten, und um den durch das Aufzeichnungsmedium (D) reflektierten Lichtstrahl, dessen Polarisierungsebene durch die Polarisierungsebenen-Dreheinrichtung (102) gedreht und der durch die Kollimatorlinse (14) gerichtet wird, auf die erste Position neben der Lichtquelle (2) abzulenken.

6. Gerät gemäß einem der Ansprüche 1 bis 5, das ferner eine zwischen der Kollimatorlinse (14) und der Reflexionsfläche (18a;118a) vorgesehene Aperture-Steuereinrichtung (16) aufweist, die eine Blenden-

bzw. Aperturöffnung hat, deren Durchmesser kleiner als ein effektiver Durchmesser der Kollimatorlinse (14) und gleich oder größer als ein effektiver Durchmesser der Fokussierlinse (20) ist, und bei der die Reflexionsfläche (18a;118a) und die Apertur-Steuereinrichtung (16) solche Neigungswinkel und Aperturdurchmesser aufweisen, dass der gesamte von der Reflexionsfläche (18a,118a) reflektierte Lichtstrahl durch die Kollimatorlinse (14) hindurchgeht.

7. Gerät gemäß einem der Ansprüche 1 bis 6, **dadurch gekennzeichnet, dass** die Reflexionsfläche (18a;118a) so geneigt ist, dass sie den Lichtstrahl reflektiert und durch die Kollimatorlinse (14) richtet, und verhindert, dass der Lichtstrahl durch die Ablenkeinrichtung (12a;112) hindurchgeht.

8. Gerät gemäß einem der Ansprüche 1 bis 7, **dadurch gekennzeichnet, dass** die Korrektureinrichtung ein Korrekturprisma (18, 118) aufweist, welches die Reflexionsfläche (18a; 118a) und eine geneigte Fläche (18b; 118b) aufweist, die zwischen der Reflexionsfläche (18a;118a) und der Fokussierlinse (20) vorgesehen ist, wobei die geneigte Fläche (18b;118b) gegenüber der Reflexionsfläche (18a;118a) unter einem Winkel so geneigt ist, dass der durch die geneigte Fläche (18b;118b) gerichtete Lichtstrahl eine geringere Elliptizität als der durch die Reflexionsfläche (18a;118a) gerichtete Lichtstrahl aufweist.

9. Gerät gemäß einem der Ansprüche 1 bis 8, **dadurch gekennzeichnet, dass** es ferner eine Verarbeitungseinrichtung (40) zum Verarbeiten des elektrischen Datensignals aufweist.

10. Gerät gemäß einem der Ansprüche 1 bis 9, **dadurch gekennzeichnet, dass** die Kollimatorlinse (14) als Fokussierlinse fungiert, die ein Passieren des durch das Aufzeichnungsmedium (D) reflektierten und durch die Fokussierlinse (20) gerichteten Lichtstrahls gestattet, den Lichtstrahl an der ersten Position fokussiert und den Teil des von der Reflexionsfläche (18a;118a) reflektierten Lichtstrahls an der zweiten Position fokussiert.

11. Gerät gemäß einem der Ansprüche 1 bis 10, **dadurch gekennzeichnet, dass** die Reflexionsfläche (18a;118a) eine Reflektanz von höchstens 50% aufweist.

Revendications

1. Dispositif de traitement de données comprenant :

une source de-lumière (2) pour émettre un faisceau de lumière ;
des moyens de réglage (38) pour régler la quantité dont le faisceau de lumière est émis par ladite source de lumière (2) ;
une lentille collimatrice (14) pour collimater le faisceau de lumière ;
une lentille de focalisation (20) pour focaliser le faisceau de lumière collimaté par ladite lentille collimatrice (14), sur un support d'enregistrement (D) ;
des moyens déflecteurs (12a; 112) pour dévier le faisceau de lumière réfléchi par le support d'enregistrement (D) et appliqué à travers ladite lentille de focalisation (20), vers une première position ;
des premiers moyens récepteurs de lumière (4) destinés à recevoir le faisceau de lumière dévié par lesdits moyens déflecteurs (12a; 112) dans la première position et à les convertir en un signal de données électrique ;
une surface réfléchissante (18a; 118a) disposée entre ladite lentille collimatrice (14) et ladite lentille de focalisation (20), et inclinée par rapport à un plan perpendiculaire à l'axe optique de ladite lentille collimatrice (14), pour réfléchir une partie du faisceau de lumière émis par ladite source de lumière (2) et l'appliquer dans une seconde position ;
des seconds moyens récepteurs de lumière (6) destinés à recevoir le faisceau de lumière réfléchi par ladite surface réfléchissante (18a; 118a) dans la seconde position et à le convertir en un signal de surveillance électrique ; et
des moyens de commande (30) pour commander lesdits moyens de réglage (38) en fonction du signal de surveillance électrique, de manière à régler la quantité dont le faisceau de lumière est émis par ladite source de lumière (2)

caractérisé en ce que

lesdits premiers et seconds moyens récepteurs de lumière (4, 6) sont adjacents à la source de lumière (2), et
sont en outre fournis des moyens de correction (18; 118) comportant ladite surface réfléchissante (18a; 118a), pour corriger l'ellipticité du faisceau de lumière ayant traversé ladite surface réfléchissante (18a; 118a).

2. Dispositif selon la revendication 1, **caractérisé en ce que** lesdits moyens déflecteurs (12a) comportent un élément de diffraction optique disposé entre ladite source de lumière (2) et ladite lentille collimatrice (14), pour dévier vers la première position le faisceau de lumière réfléchi par le support d'enregistrement (D) et appliqué à travers ladite lentille de focalisation (20).

3. Dispositif selon la revendication 1, **caractérisé en ce qu'**il comprend

des moyens de rotation de plan de polarisation (102) pour permettre le passage du faisceau de lumière appliqué vers le support d'enregistrement (D), permettre le passage du faisceau de lumière réfléchi par le support d'enregistrement (D) et faire tourner le plan de polarisation du faisceau de lumière réfléchi par le support d'enregistrement (D) ;
dans lequel lesdits moyens déflecteurs (112) sont prévus entre ladite source de lumière (2) et lesdits moyens de rotation du plan de polarisation (102), pour permettre le passage du faisceau de lumière émis par ladite source de lumière (2) et dévier le faisceau de lumière réfléchi par le support d'enregistrement (D) et dont le plan de polarisation est tourné par lesdits moyens de rotation de plan de polarisation (102), vers ladite première position adjacente à ladite source de lumière (2) ; et
ladite surface réfléchissante (118a) est prévue entre ladite lentille collimatrice (14) et lesdits moyens de rotation de plan de polarisation (102).

4. Dispositif selon la revendication 3, **caractérisé en ce que** lesdits seconds moyens récepteurs de lumière (6) comportent une région allongée réceptrice de lumière s'étendant dans une direction dans laquelle le faisceau de lumière réfléchi par ladite surface réfléchissante (118a) est dévié.

5. Dispositif selon la revendication 3 ou 4, **caractérisé en ce que** lesdits moyens déflecteurs (112) sont disposés entre ladite source de lumière (2) et ladite lentille collimatrice (14), pour permettre le passage du faisceau de lumière émis par ladite source de lumière (2) et du faisceau de lumière réfléchi par ladite surface réfléchissante (118a) et appliqué à travers ladite lentille collimatrice (14) et pour dévier le faisceau de lumière réfléchi par le support d'enregistrement (D), dont le plan de polarisation est tourné par lesdits moyens de rotation de plan de polarisation (102) et appliqué à travers ladite lentille collimatrice (14), vers ladite première position adjacente à la dite source de lumière (2).

6. Dispositif selon l'une quelconque des revendications 1 à 5, comprenant en outre des moyens de commande d'ouverture (16) disposés entre ladite lentille collimatrice (14) et ladite surface réfléchissante (18a; 118a) et comportant une ouverture dont le diamètre est inférieur au diamètre effectif de ladite lentille collimatrice (14) et égal ou supérieur au diamètre effectif de ladite lentille de focalisation (20) et dans lequel ladite surface réfléchissante (18a; 118a) et lesdits moyens de commande d'ouverture (16) ont des angles d'inclinaison et des diamètres d'ouverture tels que le faisceau de lumière complet réfléchi par ladite surface réfléchissante (18a; 118a) traverse ladite lentille collimatrice (14).

7. Dispositif selon l'une quelconque des revendications 1 à 6, **caractérisé en ce que** ladite surface réfléchissante (18a; 118a) est inclinée pour réfléchir et appliquer le faisceau de lumière à travers ladite lentille collimatrice (14) et pour empêcher le faisceau de lumière de traverser lesdits moyens déflecteurs (12a; 112).

8. Dispositif selon l'une quelconque des revendications 1 à 7, **caractérisé en ce que** lesdits moyens de correction comprennent un prisme de correction (18; 118) comportant ladite surface réfléchissante (18a; 118a) et une surface inclinée (18b; 118b) disposée entre ladite surface réfléchissante (18a; 118a) et ladite lentille de focalisation (20), ladite surface inclinée (18b; 118b) étant inclinée par rapport à ladite surface réfléchissante (18a; 118a) selon un angle tel que le faisceau de lumière appliqué à travers ladite surface inclinée (18b; 118b) a une ellipticité moindre que le faisceau de lumière appliqué à travers ladite surface réfléchissante (18a; 118a).

9. Dispositif selon l'une quelconque des revendications 1 à 8, **caractérisé en ce qu'**il comprend en outre des moyens de traitement (40) pour traiter le signal de données électrique.

10. Dispositif selon l'une quelconque des revendications 1 à 9, **caractérisé en ce que** ladite lentille collimatrice (14) agit comme une lentille de focalisation permettant le passage du faisceau de lumière réfléchi par le support d'enregistrement (D) et appliqué à travers ladite lentille de focalisation (20), focalise le faisceau de lumière dans la première position et focalise la partie du faisceau de lumière réfléchie par ladite surface réfléchissante (18a; 118a) dans la seconde position.

11. Dispositif selon l'une quelconque des revendications 1 à 10, **caractérisé en ce que** ladite surface réfléchissante (18a; 118a) a une réflectance d'au plus 50%.

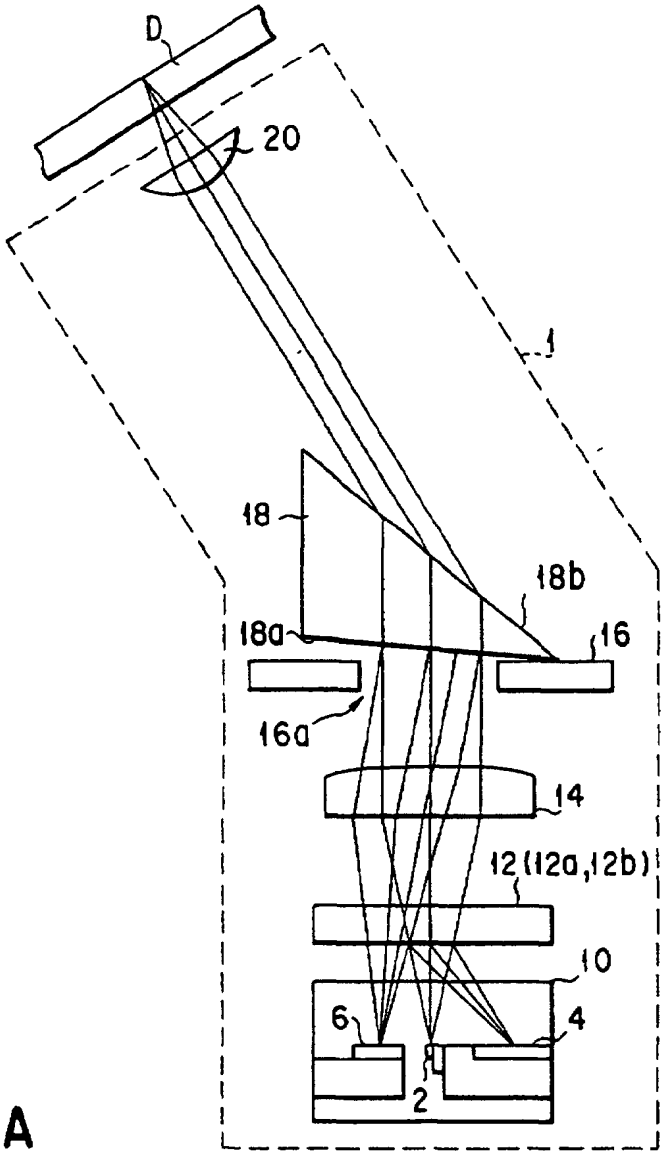


FIG. 1A

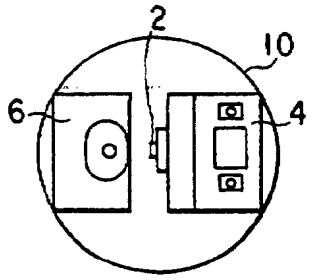


FIG. 1B

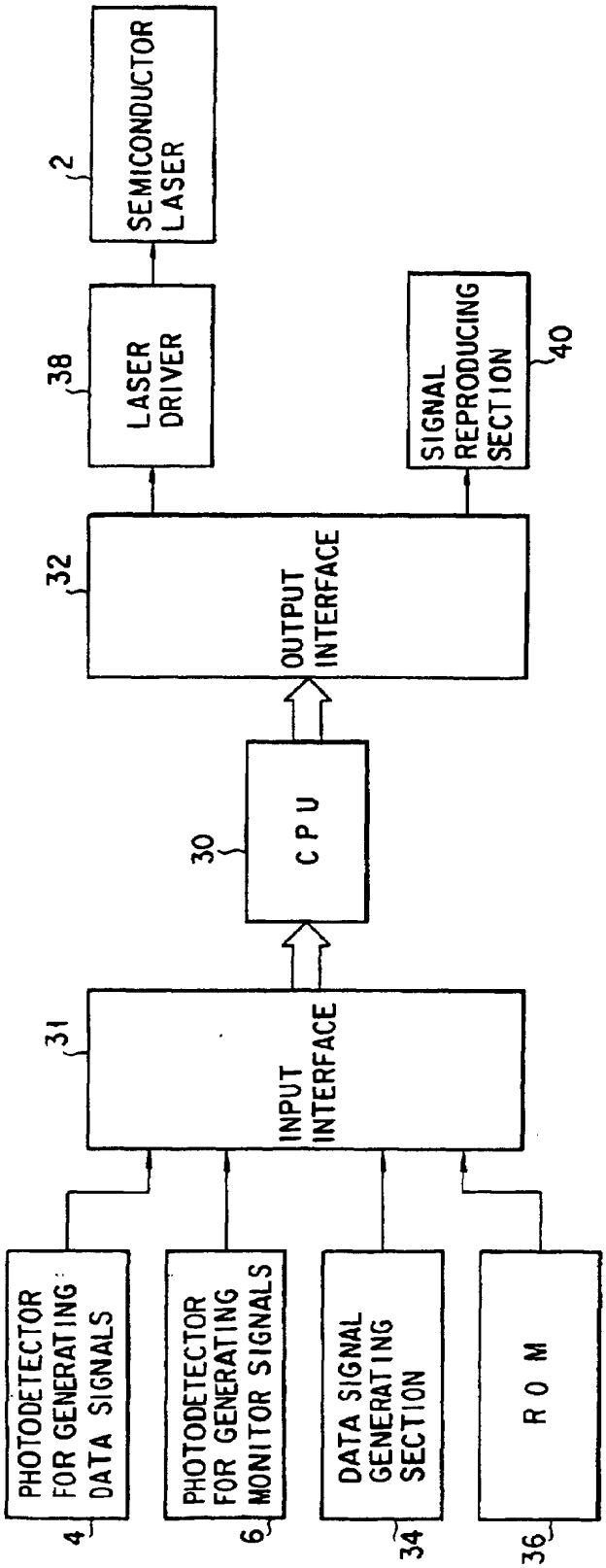

SEMICONDUCTOR LASER
2
LASER DRIVER
38
SIGNAL REPRODUCING SECTION
40
OUTPUT INTERFACE
32
CPU
30
INPUT INTERFACE
31
PHOTODETECTOR FOR GENERATING DATA SIGNALS
4
PHOTODETECTOR FOR GENERATING MONITOR SIGNALS
6
DATA SIGNAL GENERATING SECTION
34
ROM
36

FIG. 2

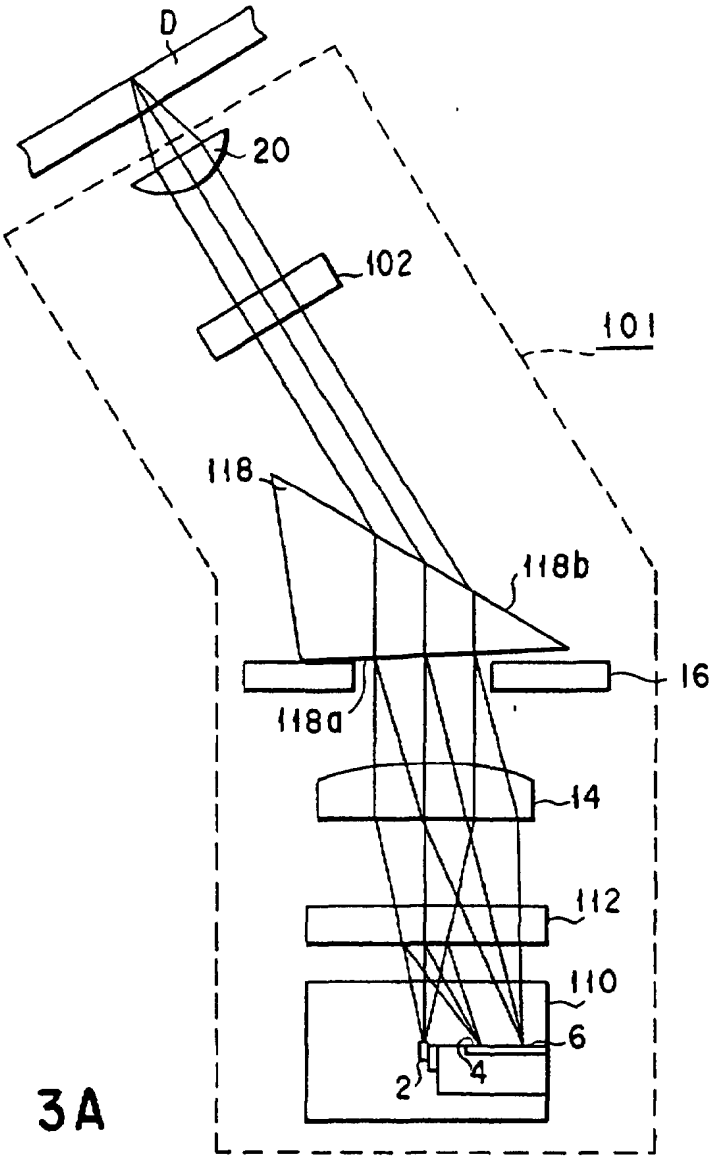


FIG. 3A

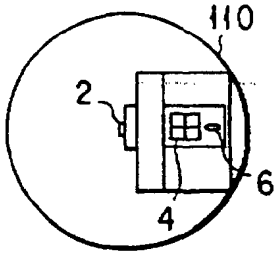


FIG. 3B

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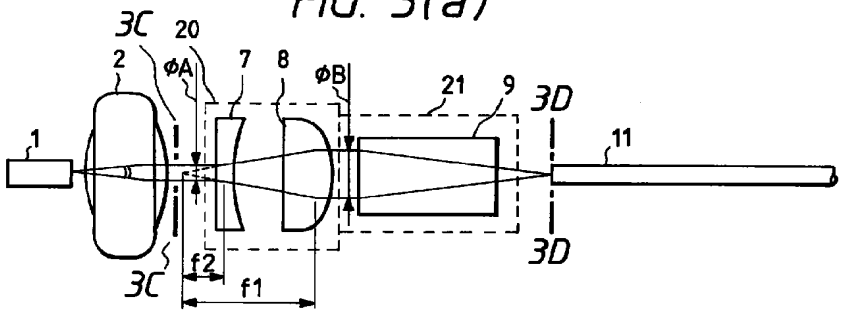
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(54) Optical coupling system and optical module

(57) An optical coupling system and optical module having a light emitting device for emitting light having different beam spread angles in vertical and horizontal directions. The optical coupling system and module include a first lens for changing the emitted light into parallel rays, a beam shaping and aberration correction device for expanding or contracting a beam cross section of the parallel rays only in a horizontal or vertical direction and for correcting for aberration. Further, a second lens is provided for converging the shaped aberration corrected beam onto an optical fiber for receiving the converged light with an improved coupling efficiency. The aberration correction corrects for astigmatism.

FIG. 3(a)



EP 0 758 755 A2

Description

The present invention relates to an optical coupling system and optical module and, more particularly, to an optical coupling system and optical module for guiding emitted light from a light emitting device which emits light having different beam spread angles in vertical and horizontal directions into an optical fiber or the like with high coupling efficiency, and for producing small variation in the optical output even if subjected to thermal stress.

BACKGROUND OF THE INVENTION

In a conventional coupling system for coupling a light beam from a light source, such as a laser diode emitting light with different beam spread angles in the vertical and horizontal directions to an optical fiber, generally a first and a second lens is utilized. As shown in Fig. 1(a), a light emitting device such as a laser diode emits a light beam to an optical fiber 11 via coupling lenses 2 and 9 where lens 2 is a collimating lens for forming generally parallel light rays such as an aspherical lens and providing an optical beam having a cross-sectional beam shape at line 1B-1B as shown in Fig. 1(b), where the dimensions in the vertical (h) and horizontal (w) directions are different so that the beam aspect ratio is about 4:1. A converging lens 9 such as a GRIN (gradient of refractive index) lens is utilized for converging the light from lens 2 onto the optical fiber 11 and this lens retains the previous cross-sectional shape beam aspect ratio as indicated at line 1C-1C, and as shown in Fig. 1(c). Due to the cross-sectional shape or large beam aspect ratio, the coupling efficiency is low.

In a prior arrangement of an optical coupling system, it has been proposed that a concave cylindrical lens 7 be inserted between the first lens 2 and the second lens 9 for expanding or contracting the cross section of the beam only in one of the vertical or horizontal directions, as shown in Figs. 2(a) and 2(b). In such a structure, light emitted from the light emitting device 1, which emits light having different beam spread angles in vertical and horizontal directions, is changed by the first lens 2 into parallel rays whose beam cross section takes on an elliptical or flattened circular form is shaped by the lens 7 to a beam having an improved beam aspect ratio in an attempt to achieve a circular form and, the converged light which is converged by the second lens 9 is introduced into the inlet end face of the optical fiber 11 to thereby improve the optical coupling efficiency. Such a structure is disclosed in Japanese Laid-open No. 5-333245 (1993), wherein the collimating lens and the cylindrical lens are held by the same holding member. Although the structure as shown in Fig. 2(a) and 2(b) wherein a cylindrical lens 7 is inserted between the first lens 2 and the second lens 9 improves the optical coupling efficiency, there is a disadvantage in that the focusing point of the converged light differs in the vertical and horizontal directions resulting in astigmatism. A loss of coupling efficiency is produced due to the astigmatism caused by the use of the cylindrical lens 7 for contracting or expanding the beam.

Japanese Laid-open No. 57-167014 (1982) discloses improvement of efficiency of coupling by arranging two semicylindrical lenses opposite to each other so that the axial cores thereof extend at right angles to one another for converting circular luminous flux from a light source to a vertical line or a horizontal line at different points so as to efficiently couple an optical fiber with a plane type waveguide, for example.

SUMMARY OF THE INVENTION

An object of the present invention is to provide an optical coupling system and optical module for guiding emitted light from a light emitting device which emits light having different beam spread angles in vertical and horizontal directions into an optical fiber or the like with high coupling efficiency, and for producing substantially no variation in the optical output even when subjected to thermal stress.

Another object of the present invention is to provide an optical coupling system and optical module for coupling light from a light emitting device such as a semiconductor laser to an optical fiber with high coupling efficiency utilizing a correction device for correcting for aberration, and in particular, correcting for astigmatism.

In accordance with the present invention, the optical coupling system and optical module comprise a light emitting device which emits light having different beam spread angles in vertical and horizontal directions, a first lens for changing the emitted light into parallel rays, a beam shaping and aberration correction device for expanding or contracting the cross section of the beam of the parallel rays in only one of the vertical and horizontal directions and for correcting aberration such as astigmatism, a second lens for converging the aberration corrected beam, and an optical fiber for receiving the converged beam.

According to another feature of the present invention, the optical module includes a fixing member for integrally fixing the beam shaping and aberration correcting device and the second lens thereon.

According to a further feature of the present invention, it is desirable to form the beam shaping and aberration correcting device of a cylindrical lens. More particularly, concave and convex cylindrical lenses serving respectively as the beam shaping device and the aberration correcting device are disposed between the first and second lenses.

In accordance with the present invention, the light from the light emitting device enters the first lens and the beam converged by the second lens enters the optical fiber. When the beam spread angles of the emitted light from the light emitting device are different in vertical and horizontal directions, the beam shaping device first performs beam shaping on the parallel rays produced by the first lens by, for example, expanding or contract-

ing the cross section of the beam only in its vertical or horizontal direction, thereby decreasing the difference between its height and width or improving its roundness or circularity. By performing such an optical process, the converged light provided by the second lens takes virtually the same form as that of the mode field of the optical fiber and the optical coupling efficiency is improved. Further, by providing that aberration such as astigmatism is corrected by the aberration correcting device, the loss due to the aberration can be reduced and providing improved coupling efficiency.

According to another feature of the present invention, by fixing the beam shaping device, the aberration correcting device, and the second lens on a common fixing member, variation in relative positions between the optical devices become smaller because they move integrally with each other even if thermal stress is applied to them, and therefore deviation from the optical axis, deviation from the focal point and the like can be reduced and variation in the output optical power can be reduced.

These and other objects, features and advantages of the present invention will become more apparent from the following description when taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWING

Figs. 1(a)-1(c) illustrate a top view of a conventional coupling system having a cross-sectional beam shape as shown in Fig. 1(b) and Fig. 1(c);

Figs. 2(a) and 2(b) illustrate in top view and side view of a prior art coupling system having astigmatism;

Figs. 3(a)-3(d) illustrate a top view of the coupling system of the present invention showing the cross-sectional beam shape in Figs. 3(c) and 3(d); and

Figs. 4(a) and 4(b) illustrate a top view and side view of the coupling system and optical module of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring now to the drawings wherein like reference numerals are utilized to designate like parts, the present invention is described below with reference to Figs. 3(a)-3(d) and Fig. 4.

Figs. 3(a) and 3(b) show a top view and side view of the coupling system of the present invention, wherein light emitted from a light emitting device such as a semiconductor laser 1 enters a first lens 2 which is an aspherical lens and providing a light beam shape which is elliptical having a beam aspect ratio of 4:1, wherein the height (h) of the beam in the vertical direction is 4 times as great the width (w) of the beam in the horizontal direction, as illustrated in Fig. 3(c). A beam shaping and aberration correcting device 20 including a concave cylindrical lens 7 having a focal length f2 and a convex cylindrical lens 8 having a focal length f1, as illustrated in Fig. 3(a), are arranged so as to reduce the beam aspect ratio and correct for astigmatism while providing an expansion of $\phi B/\phi A = f1/f2 = 3$. A converging device 21 such as second lens 9 in the form of a GRIN lens converges the light so as to enter the optical fiber 12 and to have a cross-sectional beam shape of substantial roundness or circularity, as illustrated in Fig. 3(d), where the astigmatic corrected beam has an aspect ratio of 4:3, thereby providing improved coupling efficiency.

Figs. 4(a) and 4(b) show a top view and side view of an optical module in accordance with the present invention, mounting thereon the semiconductor laser 1 of which the ratio between the height and width of the emitted beam spread angle is 4:1. The semiconductor laser 1, which is a pumping laser, is a light emitting device of which the ratio between the height and width of the emitted beam spread angle is 4:1. A laser drive circuit and necessary wirings are not shown in the drawing. A thermistor 3 serves for detecting temperature and is fixed in place on a stem 4. The stem 4 is fixed on a cooling surface of a thermoelectron cooling device 5, and the thermoelectron cooling device 5, integrally formed with the stem 4, is fixed on a bottom face of a case or housing 6. The thermoelectron cooling device 5 may be a Peltier device.

Although there is provided a control circuit for controlling the thermoelectron cooling device 5 in accordance with the temperature detected by the thermistor 3, such a control circuit is known and not related with the characteristic point of the present invention so that the control circuit and necessary wirings are not shown in the drawing.

As illustrated, the first lens 2, disposed on the stem 4, is a collimating device such as an aspherical lens for changing the emitted light from the semiconductor laser 1 into parallel rays. Although parallel rays are desired from an idealistic point of view, the rays may be pseudo parallel rays.

The concave cylindrical lens 7 is a device for expanding or contracting the beam of the parallel rays (or pseudo parallel rays) only in a width or height direction in the beam cross section. In the present embodiment, the lens 7 is set to have the function to expand the beam cross-section three times in its width direction. The convex cylindrical lens 8 is a device for correcting astigmatism and its actual form is determined in accordance with the required correction of the astigmatism. The second lens 9 is a device such as a GRIN lens for converging the light corrected for astigmatism and enabling the converged light to enter the end face of the optical fiber 12.

The concave cylindrical lens 7, the convex cylindrical lens 8, and the second lens 9 are integrally fixed in predetermined positions on a cylindrical supporting member 10 with a strong bonding agent such as glass with a low melting point and Au/Sn solder. Further, the cylindrical supporting member 10 is fixed on the case 6 by, for example, YAG laser welding. The optical fiber 12,

which is provided with a ferrule 11, is fixedly supported by a fiber holder 13. The fiber holder 13 is fixed to the end portion of the cylindrical supporting member 10 by Au/Sn soldering.

The operation of the optical module is such that, at first, a laser beam emitted from the semiconductor laser 1 of which the beam spread angle has a height-to-width ratio of 4:1 is changed by the first lens 2 into parallel rays with an elliptical beam cross section. Then, the parallel rays are expanded only in the width direction in the beam cross section by the concave cylindrical lens 7. Thereafter, astigmatism is corrected by the convex cylindrical lens 8. Then, the beam corrected for astigmatism is converged by the second lens 9 and enters the optical fiber 12 so that high efficiency optical coupling is achieved.

At this time, the beam converged into the inlet end face of the optical fiber 12 is shaped so that the ratio of the height to width of the cross section of the beam becomes 4:3. Namely, since the concave cylindrical lens 7 has the function to triple the cross section of the beam only in the width direction, the beam with the ratio of the height to width of 4:1 is shaped so as to have the height-to-width ratio being 4:1 x 3 = 4:3 with the astigmatism being corrected.

According to experiments conducted by the present inventors, an experimental value of the optical coupling efficiency of -1.2 dB was obtained and it was determined that the optical coupling efficiency was improved by 1.5 dB as compared with the case where the beam shaping is not effected.

Further, by integrally fixing the concave cylindrical lens 7, the convex cylindrical lens 8, and the second lens 9 on the supporting member 10, variation in the relative positions between the devices can be kept low even when subjected to thermal stress, and variation in the optical output can be greatly reduced. According to experiments conducted by the present inventors, it was determined that the variation in the optical output from the fiber could be kept 10% or below when the ambient temperature was changed from -20 degrees to +85 degrees.

According to the present invention, as described above, since there is provided a device for beam shape correction and astigmatism correction including at least one cylindrical lens inserted between the first lens and the second lens, the efficiency of the optical coupling with another optical wave guide device such as an optical fiber can be improved.

Further, by the provision of the fixing member for integrally fixing the beam shaping device, the aberration correcting device, and the second lens thereon, variation in relative positions between the devices can be kept down even when subjected to thermal stress and therefore variation in the optical output can be greatly reduced.

While we have shown and described several embodiments in accordance with the present invention, it is understood that the same is not limited thereto but is susceptible of numerous changes and modifications as known to those skilled in the art, and we therefore do not wish to be limited to the details shown and described herein but intend to cover all such changes and modifications as are encompassed by the scope of the appended claims.

Claims

1. An optical coupling system comprising:

   a light emitting device for emitting light having different beam spread angles in vertical and horizontal directions;
   a first lens for changing the emitted light into parallel rays;
   a beam shaping and aberration correction device for expanding or contracting a beam cross section of the parallel rays only in a horizontal or vertical direction and for correcting for aberration;
   a second lens for converging the shaped aberration corrected beam; and
   an optical fiber for receiving the converged light with an improved coupling efficiency.

2. An optical coupling system according to claim 1, wherein the beam shaping and aberration correction device expands or constructs the beam cross section in only the horizontal or vertical direction and corrects for astigmatism.

3. An optical coupling system according to claim 2, wherein the beam shaping and aberration correction device includes at least one cylindrical lens.

4. An optical coupling system according to claim 2, wherein the beam shaping and aberration correction device includes a first part for expanding or contracting the beam cross section of the parallel rays so as to provide diverging or converging rays and a second part for correcting astigmatism and for providing parallel rays, the second lens converging the shaped astigmatism corrected beam so as to have substantially the same focusing point of the parallel light rays in both the vertical and horizontal directions.

5. An optical coupling system according to claim 4, wherein the first part includes a concave cylindrical lens and the second part includes a convex cylindrical lens.

6. An optical module comprising the coupling system according to claim 1, further comprising a fixing member for integrally fixing the beam shaping and correcting device and the second lens thereon.

7. An optical module according to claim 6, wherein the

beam shaping and aberration correction device includes at least one cylindrical lens.

8. An optical module according to claim 6, wherein the beam shaping and aberration correction device includes a first part for expanding or contracting the beam cross section of the parallel rays so as to provide diverging or converging rays and a second part for correcting astigmatism and providing parallel rays, the second lens converging the shaped astigmatism corrected beam so as to have substantially the same focusing point of the parallel light rays in both the vertical and horizontal directions.

9. An optical module according to claim 6, wherein the first part includes a concave cylindrical lens and the second part includes a convex cylindrical lens.

10. An optical coupling system comprising:

a light emitting device for emitting light having different beam spread angles in vertical and horizontal directions;
a first lens for changing the emitted light into parallel rays;
a beam shaping device for expanding or contracting the beam of said parallel rays in only the vertical or horizontal direction so as to provide diverging or converging light rays; and
coupling efficiency improving means for coupling the light rays shaped by the beam shaping device to an optical fiber so that the light rays are converged onto an optical fiber at substantially the same focus point in both the vertical and horizontal directions.

11. An optical coupling system according to claim 10, wherein the coupling efficiency improving means includes at least a cylindrical lens.

12. An optical coupling system according to claim 10, wherein the beam shaping device includes a concave cylindrical lens, and the coupling efficiency improving means includes a convex cylindrical lens and a second lens.

13. An optical module comprising the coupling system according to claim 12, further comprising a fixing member for integrally fixing the concave cylindrical lens, the convex cylindrical lens and the second lens thereon.

14. An optical module comprising the coupling system according to claim 12, wherein the second lens is a converging lens.

FIG. 1(a)

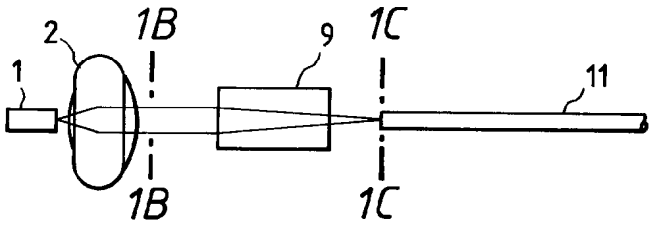


FIG. 1(b)

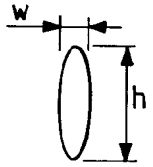


FIG. 1(c)

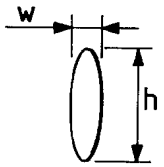
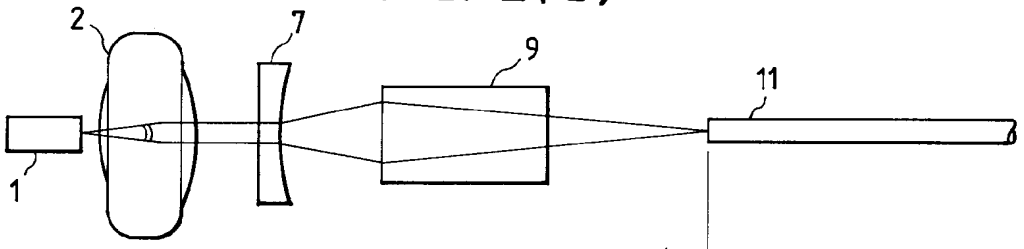


FIG. 2(a)



ΔZ = ASTIGMATISM

FIG. 2(b)

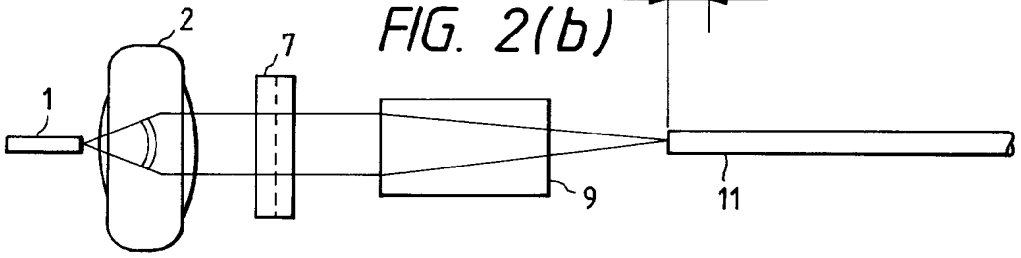


FIG. 3(a)

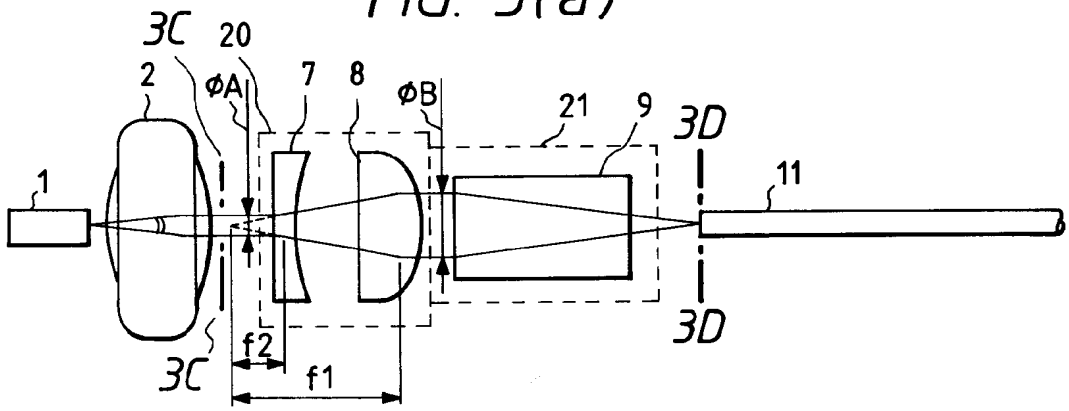


FIG. 3(b)

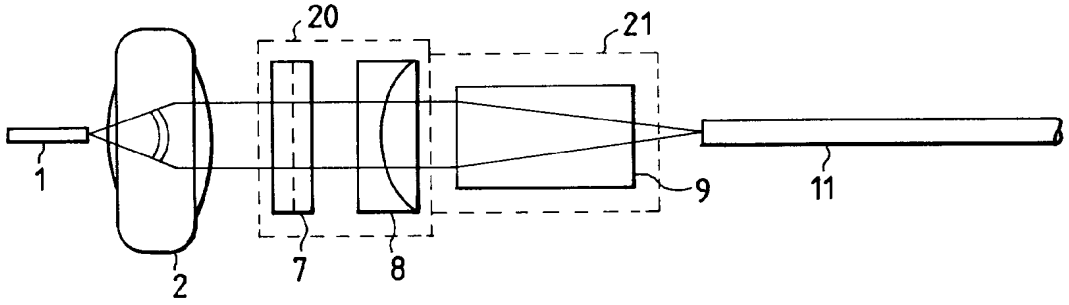


FIG. 3(c)

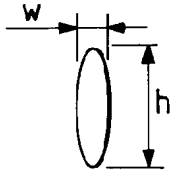


FIG. 3(d)

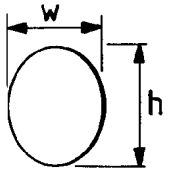


FIG. 4(a)

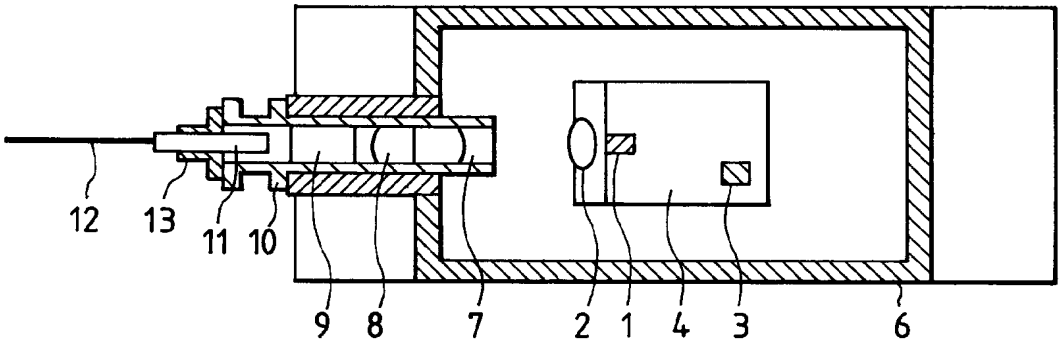
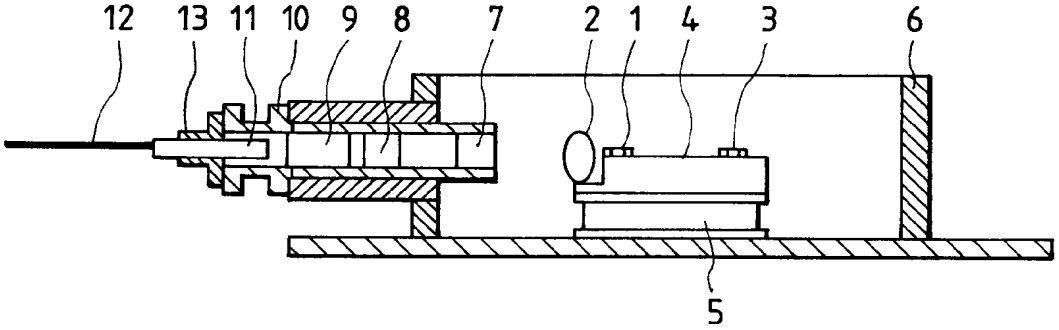


FIG. 4(b)



Espacenet

Bibliographic data: EP0889319 (A1) — 1999-01-07

Methods and divices for the determination of the concentration of a gas in a liquid drop containing gas mixture and their uses

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Applicant(s): ABB RESEARCH LTD [CH] ± (ABB RESEARCH LTD)

Classification: **- international:** ***G01N21/15; G01N21/31; G01N21/53;*** *G01N21/03; G01N21/33;* (IPC1-7): G01N21/15; G01N21/31

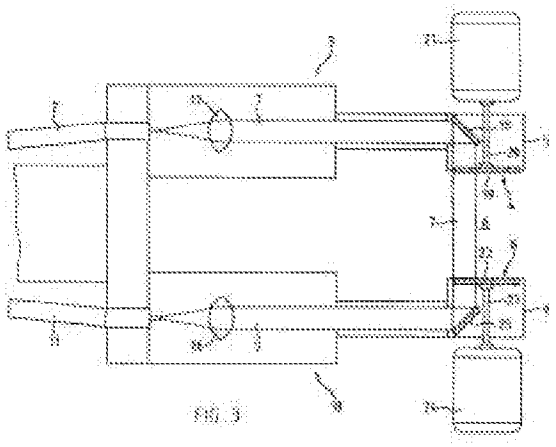
- cooperative: **G01N21/15**; **G01N21/314**; **G01N21/534**; G01N21/03; G01N21/33

Application number: EP19970810432 19970702

Priority number (s): EP19970810432 19970702

Abstract of EP0889319 (A1)

Process for measuring the concentration of a gas in a gas mixture containing liquid droplets comprises passing a light beam through the mixture, determining the intensities of its partial beams and calculating the concentration from these. Also claimed is an apparatus for carrying out the process comprising an emitter (3) for emitting a light beam made up of different wavelengths penetrating the gas mixture and a receiver (10) for receiving the beam and passing it to an evaluator (12) to determine the intensities of its partial beams and calculating the concentrations from these. Optical guide elements rotating at 3000 rpm are arranged between the emitter (3) and the receiver (10). Each element has a cleaning device having a nozzle to produce a liquid beam (16).



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(54) Verfahren und Vorrichtungen zur Ermittlung der Konzentration eines Gases in einer Flüssigkeitstropfen enthaltenden Gasmischung sowie Verwendung derselben

(57) Der SO_2-Gehalt einer Tropfenströmung (6) wird festgestellt, indem Intensitäten eines durch dieselbe gesandten Lichtstrahls (7) im Wellenlängenbereich von 280 - 300 nm, in dem für SO_2 spezifische Absorptionsbanden auftreten, gemessen und aus dem Vergleich der Schwächung der Intensitäten in verschieden stark absorbierten Teilbereichen die Konzentration ermittelt wird.

Zwischen einem Sender (3) und einem Empfänger (10) oder einem Spiegel (30) und der Tropfenströmung (6) sind jeweils Fenster (4; 8) vorgesehen, welche jeweils als kreisrunde Platten (19; 22) ausgebildet sind. Sie sind jeweils am Zentrum an einer Welle (20; 23) befestigt, die durch einen Elektromotor (21; 24) in eine Drehbewegung von zwischen 10'000 und 20'000 U/min versetzt wird. Durch die Drehbewegung wird ein Ansetzen von Tropfen aus der Tropfenströmung an den derselben zugewandten Grenzflächen der Fenster (4, 8) verhindert, welches sonst in kurzer Zeit ihre Transparenz so stark reduzieren würde, dass die Intensität des empfangenen Lichtes für eine zuverlässige Feststellung der SO_2-Konzentration nicht mehr genügen könnte. Es kann auch ein Spiegel (30) im Strahlengang liegen, welcher ebenfalls unmittelbar an die Tropfenströmung (16) grenzen kann. Er kann zur Bündelung des Lichtstrahls (7) als Hohlspiegel ausgebildet sein.

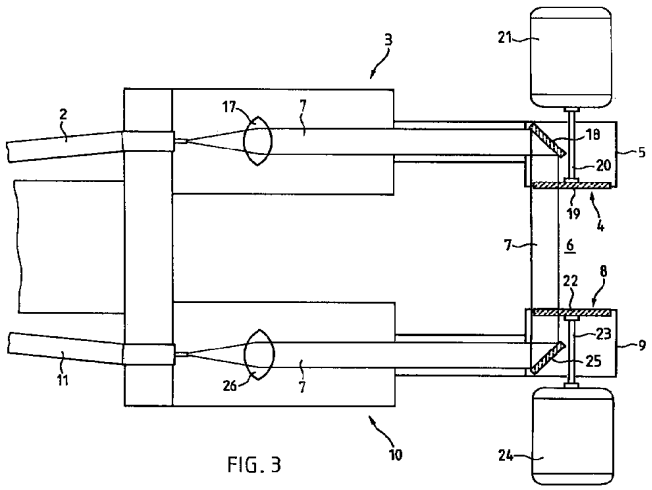


FIG. 3

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Beschreibung

Die Erfindung betrifft ein Verfahren zur Ermittlung der Konzentration eines Gases in einer Flüssigkeitstropfen enthaltenden Gasmischung gemäss dem Oberbegriff des Anspruchs 1 sowie Vorrichtungen zur Durchführung des Verfahrens nach dem Oberbegriff der Ansprüche 2 und 4 sowie Verwendungen derselben.

Es ist seit längerem bekannt, die Konzentration bestimmter Komponenten in einem Mischgas zu messen, indem durch dasselbe ein Lichtstrahl, z. B. von einer Xenonlampe erzeugtes Licht mit kontinuierlichem Spektrum, gesandt wird und die Intensitätsminderungen des Lichts in bestimmten schmalen Wellenlängenbereichen, das von besagten Komponenten dort unterschiedlich stark absorbiert wird, gemessen und aus den unterschiedlichen Werten die Konzentrationen der Komponenten berechnet werden.

Im einfachsten Fall, der im folgenden zur Illustration des Verfahrens genauer dargestellt wird, kann man sich mit zwei Wellenlängen begnügen, die von einer interessierenden Komponente des Mischgases unterschiedlich stark absorbiert werden. Wird z. B. vom fraglichen Gas Licht der Wellenlänge λ_1 mit einem ersten Absorptionskoeffizienten α_1 absorbiert und Licht der Wellenlänge λ_2 mit einem davon verschiedenen zweiten Absorptionskoeffizienten α_2, so ergibt sich für die Intensitäten der beiden durch das Mischgas geleiteten Teilstrahlen unter der Annahme, dass die übrigen Komponenten desselben die entsprechenden Wellenlängen nicht absorbieren und die Ausgangsintensitäten jeweils gleich I_0 sind:

$$I_1 = I_0 e^{-c\alpha_1 l} \quad (1)$$

und

$$I_2 = I_0 e^{-c\alpha_2 l} \quad (2)$$

wobei l die Absorptionslänge, d. h. die Länge des durch das Mischgas zurückgelegten Weges des Lichtstrahls ist und c die Konzentration des Gases.

Aus (1) und (2) ergibt sich

$$\log(I_1/I_2) = c(\alpha_2-\alpha_1)l \quad (3)$$

woraus ohne weiteres die Konzentration c bestimmt werden kann, ohne dass nicht wellenlängenspezifische Einflüsse wie Streuung das Resultat störend beeinflussen könnten.

In der Praxis wird der interessierende Teil des Spektrums der Lichtquelle in schmale Teilbereiche aufgeteilt und dann eine gerechnete Absorptionskurve an die gemessene Kurve angepasst, woraus sich dann die Konzentration der interessierenden Komponente ergibt. Auch die Konzentrationen mehrerer Komponenten können auf diese Weise gleichzeitig bestimmt werden.

Beim Versuch, die geschilderte Methode auf eine Flüssigkeitstropfen enthaltende Gasmischung anzuwenden, insbesondere auf eine Tropfenströmung, wie sie z. B. in einem Rauchgaswäscher auftritt, ergibt sich die Schwierigkeit, dass sich stets Tropfen mit suspendierten Feststoffteilchen an den Sender und den Empfänger oder an denselben im Strahlengang nach- bzw. vorgeordneten optischen Leitelementen ansetzen. Dies reduziert die Lichtintensität rasch so stark, dass sie für eine Messung nicht mehr ausreicht.

Aus der US-A-2 879 716 ist es bekannt, bei Messungen in Gasen, die Feststoffpartikel enthalten, zur Verhinderung einer Anlagerung derselben kreisrunde Glasfenster einzusetzen, welche an einer mit 1'420 U/min rotierenden Hohlwelle befestigt sind. Die Reinigung der gegen das Gas gerichteten Grenzfläche eines Fensters erfolgt jeweils durch eine Reinigungsflüssigkeit, die durch die Hohlwelle zugeführt wird und aus einem umlaufenden Spalt, der zwischen der Grenzfläche und einer an der Welle befestigten Kappe gebildet wird, austritt. Durch die Rotation wird die Flüssigkeit nach aussen getrieben und bildet einen Flüssigkeitsfilm.

Dieses Verfahren hat den Nachteil, dass die Flüssigkeit auf der gesamten Grenzfläche des Fensters eine ziemlich dicke, nicht unbedingt gleichmässige Schicht bildet und die Intensität des Lichtstrahls durch Absorption und eventuell Streuung schwächt. Ausserdem ist die Vorrichtung kompliziert im Aufbau, entsprechend teuer in der Herstellung und störungsanfällig.

Aus DE-A-29 31 266 ist es bekannt, in Vorrichtungen zur optischen Rauchdichtemessung langsam rotierende Scheiben einzusetzen, gegen welche zwei ebenfalls langsam rotierende kleinere Reinigungsscheiben gedrückt werden. Vor der ersten Reinigungsscheibe wird als Reinigungsflüssigkeit Spiritus aufgebracht, während die in Drehrichtung unmittelbar folgende zweite als Trockenreinigungsscheibe dient. Aus DE-B-15 72 845 ist eine ähnliche Einrichtung mit einer einzigen Reinigungsscheibe bekannt. Bei beiden Einrichtungen erfolgt die Reinigung im wesentlichen durch mechanische Mittel, so dass entsprechende Vorrichtungsteile im Rauch oder Gas selbst angeordnet werden müssen und sowohl dessen chemischer Einwirkung ausgesetzt sind als auch einer raschen Verschmutzung, die verhältnismässig häufige Wartung nötig macht, unterliegen. Ihr Aufbau ist verhältnismässig kompliziert.

Die beschriebenen vorbekannten Einrichtungen dienen der Entfernung von Feststoffpartikeln und sind für die Beseitigung von sich aus einer Gasmischung niederschlagenden Flüssigkeitstropfen sämtlich wenig geeignet.

Die Erfindung, wie sie in den Ansprüchen gekennzeichnet ist, schafft dagegen ein Verfahren, welches darauf gerichtet ist, die genaue Bestimmung der Konzentration eines Gases in einer Tropfen enthaltenden Gasmischung mit einfachen Mitteln zu ermöglichen und schafft auch entsprechende Vorrichtungen. Die Reini-

gung erfolgt so, dass mindestens Teile der Grenzflächen im wesentlichen von Flüssigkeit frei bleiben und die Lichtabsorption sehr gering gehalten wird.

Von besonderem Interesse ist die Verwendung des erfindungsgemässen Verfahrens sowie der erfindungsgemässen Vorrichtungen zur Bestimmung der Konzentration bestimmter Schadgase in Verbrennungsanlagen. So kann etwa die Konzentration von SO_2 in einem Rauchgaswäscher mit grosser Genauigkeit ermittelt und der festgestellte Wert für die Ueberwachung und Steuerung des Betriebs der Anlage herangezogen werden.

Im folgenden wird die Erfindung anhand von Zeichnungen, welche lediglich ein Ausführungsbeispiel darstellen, näher erläutert. Es zeigen

Fig. 1 schematisch den grundsätzlichen Aufbau einer erfindungsgemässen Vorrichtung nach einer ersten Ausführungsform,

Fig. 2a einen Längsschnitt durch ein Fenster mit Reinigungseinrichtung der erfindungsgemässen Vorrichtung nach Fig. 1,

Fig. 2b eine Vorderansicht des Fensters nach Fig. 2a,

Fig. 3 den Aufbau des Senders und des Empfängers gemäss einer zweiten Ausführungsform der erfindungsgemässen Vorrichtung,

Fig. 4 den Aufbau des Senders gemäss einer dritten Ausführungsform der erfindungsgemässen Vorrichtung,

Fig. 5 schematisch den Aufbau einer erfindungsgemässen Vorrichtung nach einer vierten Ausführungsform,

Fig. 6 schematisch den Aufbau einer erfindungsgemässen Vorrichtung nach einer fünften Ausführungsform, und

Fig. 7 schematisch den Aufbau einer erfindungsgemässen Vorrichtung nach einer sechsten Ausführungsform.

Die Erfindung wird am bereits oben angesprochenen Beispiel der Bestimmung der SO_2-Konzentration in einer Tropfenströmung, wie sie z. B. in einem Rauchgaswäscher auftritt, erläutert. In diesem Fall eignet sich (Fig. 1) als Lichtquelle 1 insbesondere eine Xenonlampe, deren Licht im UV-Bereich, insbesondere im Wellenlängenbereich von ca. 280 - 300 nm, in welchem SO_2 mehrere ausgeprägte Absorptionsbanden zeigt, ein kontinuierliches Spektrum aufweist. Das Licht derselben wird über einen Lichtleiter 2 einem Sender 3 zugeführt und gelangt durch ein erstes optisches Leitelement, nämlich ein Fenster 4 am Ende eines Rohrs 5 in die dort herrschende Tropfenströmung 6, die es als Lichtstrahl 7 durchdringt. Durch ein weiteres Leitelement, ein Fenster 8 in einem gegenüberliegenden Rohr 9 gelangt es weiter in einen Empfänger 10 und über einen weiteren Lichtleiter 11 in eine Auswerteeinheit 12.

In der Auswerteeinheit 12 werden die in schmalen Teilbereichen des oben angesprochenen Wellenlängenbereichs empfangenen Intensitäten gemessen, mit den bekannten Intensitäten des vom Sender im jeweils entsprechenden Teilbereich ausgesandten Lichts normalisiert, der auf unspezifische Streuung und Absorption zurückgehende Untergrund rechnerisch herausgefiltert und aus dem verbleibenden Signal, das die Absorptionsbanden des SO_2 deutlich wiederspiegelt, die Konzentration desselben berechnet.

Die Rohre 5 und 9 und mit ihnen die fest eingebauten Fenster 4, 8 werden dabei von einer geeigneten Antriebseinheit mit mindestens 3'000 U/min, vorzugsweise aber zwischen 10'000 U/min und 20'000 U/min gedreht, so dass sich an der der Tropfenströmung 6 zugewandten Grenzfläche derselben ansetzende Tropfen grösstenteils sofort weggeschleudert werden. Ein kleiner Teil bleibt zwar haften, bildet jedoch einen sehr dünnen Flüssigkeitsfilm gleichmässiger Dicke, der die optischen Eigenschaften des Fensters 4 kaum beeinflusst.

Zur Unterstützung der Reinigung ist (Fig. 2a,b) vor der der Tropfenströmung 6 zugewandten Grenzfläche des Fensters 4 am Ende des Rohres 5 eine Reinigungseinrichtung 13 fest angebracht mit einer Zuleitung 14, an welche eine Hohlnadel 15 anschliesst. Der Endbereich der Hohlnadel 15 bildet eine Düse von ca. 0,4 mm Innen- und ca. 0,6 mm Aussendurchmesser, die fast parallel zur Grenzfläche gerichtet ist; vorzugsweise ist sie mit 1°, höchstens ca. 3° gegen dieselbe geneigt. Die Zuleitung 14 ist mit einem Vorratsgefäss (nicht dargestellt) verbunden, in dem als Reinigungsflüssigkeit eventuell entionisiertes Wasser durch Pressluft unter einem Druck von mindestens 3, vorzugsweise 4-5 bar gehalten wird. Aus der Hohlnadel 15 tritt daher ein scharfer Flüssigkeitsstrahl 16 aus, welcher im wesentlichen gebündelt an der Grenzfläche des sich unter ihm durch drehenden Fensters 4 entlangläuft und ähnlich wie ein mechanischer Scheibenwischer Fremdkörper, vor allem Tropfen, die auch suspendierte Feststoffteilchen enthalten können, von demselben entfernt. Er wird unter dem Einfluss der Drehung geringfügig gespreizt, doch haftet er kaum an der Grenzfläche, so dass sich höchstens ein sehr geringer Anteil über die gesamte Grenzfläche verteilt und allenfalls einen äusserst dünnen, gleichmässigen Flüssigkeitsfilm bildet, der kaum Licht absorbiert oder streut. Der Schatten der dünnen Hohlnadel 15 und des im wesentlichen auf einen engen Sektor konzentrierten Flüssigkeitsstrahls 16 ist sehr schmal und bedeutet praktisch keine Schwächung der Gesamtintensität des Lichtstrahls 7. Am Fenster 8 ist eine genau gleich aufgebaute Reinigungseinrichtung

angebracht.

Im Zusammenhang mit einer zweiten Ausführungsform (Fig. 3) wird ein weiterer beispielsweiser Aufbau des Senders 3 und des Empfängers 10 genauer beschrieben. Der Sender 3 weist eine dem Ausgang des Lichtleiters 2 nachgeordnete Optik 17 auf, welche den Lichtstrahl 7 bündelt und auf einen Ablenkspiegel 18 wirft, von dem er durch das Fenster 4 in die Tropfenströmung 6 gelangt. Das Fenster 4 ist als kreisrunde Platte 19 aus Quarz von ca. 25 mm Durchmesser ausgebildet und ist somit für Ultraviolettlicht transparent.

Am Zentrum ist die Platte 19 an einer starren, geraden Welle 20 befestigt, welche von einem Motor, vorzugsweise einem Elektromotor 21 als Antriebseinheit angetrieben wird. Die Platte 19 ist hier in einer kreisrunden Oeffnung am Ende des Rohrs 5 angeordnet und zwar derart, dass zwischen ihrem Rand und dem Rand der Oeffnung ein Spalt von ca. 0,5 mm Breite freibleibt, so dass die Drehbewegung der Platte 19 nicht gehindert ist. Damit kein Gas aus dem Bereich der Tropfenströmung 6 durch den besagten Spalt dringen kann, ist der hinter dem Fenster 4 liegende Raum mit Stickstoff gefüllt und zwar steht derselbe unter einem Druck, der etwas über dem statischen Druck der Tropfenströmung 6 liegt. Die durch den Spalt in die Tropfenströmung 6 fliessende Stickstoffmenge ist dabei so gering, dass sie die Absorptionslänge und damit das Messergebnis nicht nennenswert beeinflusst.

Der Empfänger 10 ist hinter dem Fenster 8 - genau wie das Fenster 4 auf der Senderseite eine kreisrunde Platte 22 aus Quarz, welche über eine Welle 23 von einem Elektromotor 24 angetrieben wird - angeordnet und mit einem Ablenkspiegel 25 und einer Optik 26, welche den Lichtstrahl 7 auf das Ende des Lichtleiters 11 sammelt, genau gleich aufgebaut wie der Sender 3.

Die Elektromotoren 21, 24 versetzen die Platten 19, 22 ähnlich wie bereits im Zusammenhang mit der ersten Ausführungsform beschrieben in rasche Rotation. Vor den Fenstern 4, 8 sind den dort beschriebenen genau entsprechende Reinigungseinrichtungen angebracht, die der besseren Uebersichtlichkeit halber nicht dargestellt sind.

Gemäss einer dritten Ausführungsform (Fig. 4) ist im Sender 3 kein Ablenkspiegel vorgesehen, sondern die Optik 17 wirft den Lichtstrahl 7 direkt auf die Platte 19 des Fensters 4.

Die Welle 20 ist flexibel ausgebildet und wird aus der Rotationsachse heraus zum Elektromotor 21 geführt. Sie ist in mehreren Halterungen 27a, b, c drehbar gelagert. Zwischen der Optik 17 und dem Fenster 4 liegt ein Raum 28, der bis auf eine seitliche Durchführung für die Welle 20 und den die Platte 19 umgebenden Ringspalt am Fenster 4 geschlossen ist. Zur Aufrechterhaltung des erforderlichen Ueberdrucks, der wie oben erläutert ein Eindringen von Gas aus dem Bereich der Tropfenströmung 6 (Fig. 1) verhindert, ist eine Zuleitung 29 vorgesehen, über welche als Spülgas Stickstoff zugeführt wird. Der Empfänger kann gleich aufgebaut sein.

Bei der in Fig. 5 dargestellten vierten Ausführungsform dient der im Rohr 5 fest eingebaute Sender 3 gleichzeitig als Empfänger, während dem Fenster 4 als zweites optisches Leitelement ein Spiegel 30, vorzugsweise eine polierte Metallplatte, gegenüberliegt. Am hinteren Ende des Rohres 5 schliesst ein konischer Stutzen 31 mit einem axialen Lichtleiterabschnitt 32 an. An den von der Lichtquelle kommenden ersten Lichtleiter 2 ist über einen Strahlteiler 33 und eine Optik 34 ein zweiter Lichtleiter 35 gekoppelt, welcher bis an den Stutzen 31 führt. Der Lichtleiter 11 führt vom Strahlteiler 33 zur Auswerteeinheit.

Das Licht aus dem Lichtleiter 2 tritt zum Teil durch den Strahlteiler 33 durch und wird durch die Optik 34 in den Lichtleiter 35 eingekoppelt. An dessen Ende tritt es durch einen Luftspalt in den Lichtleiterabschnitt 32 des Senders 3 über. Anschliessend wird es als aus demselben austretender Lichtstrahl 7 durch die Optik 17 gebündelt und durch das Fenster 4 und die Tropfenströmung 6 zum Spiegel 30 geleitet. Dort reflektiert, läuft es den beschriebenen Weg bis zum Strahlteiler 33 zurück, der einen Teil des Lichtes dem Lichtleiter 11 zur Weiterleitung an die Auswerteeinheit zuführt. Der Spiegel 30 wie das Rohr 5 mit dem Sender 3 und dem Fenster 4 rotieren, von Elektromotoren angetrieben wie im Zusammenhang mit den vorgängig beschriebenen Ausführungsformen erläutert. Auch sind beide mit einer Reinigungseinrichtung der weiter oben beschriebenen Art ausgestattet.

Der Einsatz des Strahlteilers 33 verursacht zwar eine gewisse Einbusse an Lichtintensität, dafür ist die Vorrichtung äusserst einfach aufgebaut und entsprechend kostengünstig realisierbar.

Bei der fünften Ausführungsform (Fig. 6) ist der Einsatz eines Strahlteilers vermieden, indem die Lichtleiter 2 und, etwas versetzt, 11 durch die das Rohr 5 tragende Welle 20, welche zu diesem Zweck als Hohlwelle ausgebildet ist, durchgezogen sind Sie treten an der Rückseite des (nicht dargestellten) Elektromotors aus der Welle 20 aus und sind von dort zur Lichtquelle 1 bzw. der Auswerteeinheit 12 geführt. Der Lichtstrahl 7 tritt etwas schräg zur Achse aus dem Lichtleiter 2 aus, so dass er, von der Optik 17 gebündelt, nach dem Durchgang durch das Fenster 4 und die Tropfenströmung 6 auch schräg auf den Spiegel 30 trifft und nach Reflexion an demselben und neuerlichem Durchgang durch die Tropfenströmung 6 und das Fenster 4 von der Optik 17 auf das Ende des Lichtleiters 11 gesammelt wird.

Der Spiegel 30 ist genau gleich ausgebildet wie bei der vorstehenden Ausführungsform. Auch hier sind vor dem Fenster 4 und dem Spiegel 30 nicht dargestellte Reinigungseinrichtungen angeordnet.

Prinizipiell gleich aufgebaut wie gemäss der fünften Ausführungsform ist der auch hier mit dem Empfänger integrierte Sender 3 bei der sechsten (Fig. 7). Allerdings ist keine Optik vorgesehen und das Rohr 5 entsprechend kürzer.

Der Spiegel 30 dagegen ist als Hohlspiegel aus beschichtetem Glas ausgebildet. Er ist stationär hinter dem am Ende des Rohrs 9 angebrachten Fenster 8 angeordnet, welches das an die Tropfenströmung 6 grenzende zweite Leitelement bildet. Das Rohr 9 ist wiederum drehbar aufgehängt und von einem Elektromotor angetrieben.

Der auseinanderlaufende Lichtstrahl 7 tritt wieder etwas schräg aus dem Lichtleiter 2 und durch das Fenster 4, die Tropfenströmung 6 und das Fenster 8, so dass er vom Spiegel 30 auf das Ende des gegenüber dem Lichtleiter 2 versetzt angeordneten Lichtleiters 11 gesammelt wird. Da keine Linsenoptik erforderlich ist, wird bei dieser Ausführungsform nicht nur eine beträchtliche Einsparung erzielt, es wird auch die beim Einsatz von Linsensystemen unvermeidliche Lichtabschwächung vermieden.

Es gibt natürlich weitere Möglichkeiten, den Erfindungsgedanken ins Werk zu setzen. So können die beiden optischen Leitelemente über entsprechende Kraftübertragungssysteme von einem gemeinsamen Elektromotor angetrieben werden. Eventuell können auch beide an einer einzigen Welle befestigt sein. Die Reinigungseinrichtungen können u. U. auch entfallen, vor allem dann, wenn die Drehzahl der Leitelemente sehr hoch - in der Regel mindestens 3'000 U/min - ist. Dann kann die hohe Fliehkraft allein sicherstellen, dass sich höchstens ein sehr dünner, nicht mehr in störendem Ausmass Licht absorbierender Flüssigkeitsfilm bildet.

Patentansprüche

1. Verfahren zur Ermittlung der Konzentration eines Gases in einer Flüssigkeitstropfen enthaltenden Gasmischung, indem ein Lichtstrahl (7), welcher durch Ueberlagerung von mindestens zwei Anteilen unterschiedlicher Wellenlängen gebildet wird, durch die Gasmischung gesandt, die Intensitäten seiner Teilstrahlen bestimmt und die Konzentration aus denselben berechnet wird, wobei im Strahlengang unmittelbar angrenzend an die Gasmischung optische Leitelemente mit gegen dieselbe weisenden Grenzflächen angeordnet sind, **dadurch gekennzeichnet, dass** mindestens ein Teil jeder Grenzfläche von Flüssigkeit im wesentlichen frei gehalten wird, und zwar mindestens teilweise durch zyklische Bewegung der Leitelemente.

2. Vorrichtung zur Durchführung des Verfahrens nach Anspruch 1, mit einem Sender (3) zum Aussenden eines die Gasmischung durchdringenden Lichtstrahls (7), welcher durch Ueberlagerung von mindestens zwei Anteilen unterschiedlicher Wellenlängen gebildet wird und einem Empfänger (10) zum Empfangen und Weiterleiten desselben an eine Auswerteeinheit (12) zur Bestimmung der Intensitäten seiner Teilstrahlen und Berechnung der Konzentration aus denselben, wobei im Strahlengang zwischen dem Sender (3) und dem Empfänger (10) optische Leitelemente mit gegen die Gasmischung weisenden Grenzflächen, nämlich mindestens ein für den Lichtstrahl transparentes, zwischen dem Sender (3) und der Gasmischung angeordnetes erstes Fenster (4) sowie ein zweites Leitelement derart drehbar angeordnet sind, dass der Sender (3) und der Empfänger (10) von der Gasmischung getrennt sind, welchen Leitelementen mindestens eine Antriebseinrichtung zugeordnet ist, welche geeignet ist, jedes Leitelement einer Drehbewegung zu unterwerfen, **dadurch gekennzeichnet, dass** die Tourenzahl der Drehbewegung mindestens 3'000 U/min beträgt.

3. Vorrichtung nach Anspruch 2, **dadurch gekennzeichnet, dass** die Tourenzahl der Drehbewegung zwischen 10'000 und 20'000 U/min beträgt.

4. Vorrichtung zur Durchführung des Verfahrens nach Anspruch 1, mit einem Sender (3) zum Aussenden eines die Gasmischung durchdringenden Lichtstrahls (7), welcher durch Ueberlagerung von mindestens zwei Anteilen unterschiedlicher Wellenlängen gebildet wird und einem Empfänger (10) zum Empfangen und Weiterleiten desselben an eine Auswerteeinheit (12) zur Bestimmung der Intensitäten seiner Teilstrahlen und Berechnung der Konzentration aus denselben, wobei im Strahlengang zwischen dem Sender (3) und dem Empfänger (10) optische Leitelemente mit gegen die Gasmischung weisenden Grenzflächen, nämlich mindestens ein für den Lichtstrahl transparentes, zwischen dem Sender (3) und der Gasmischung angeordnetes erstes Fenster (4) sowie ein zweites Leitelement derart drehbar angeordnet sind, dass der Sender (3) und der Empfänger (10) von der Gasmischung getrennt sind, welchen Leitelementen mindestens eine Antriebseinrichtung zugeordnet ist, welche geeignet ist, jedes Leitelement einer Drehbewegung zu unterwerfen, **dadurch gekennzeichnet, dass** jedem Leitelement eine Reinigungseinrichtung (13) zugeordnet ist, die eine Düse zur Erzeugung eines Flüssigkeitsstrahls (16) umfasst, welcher gebündelt an der Grenzfläche entlangläuft.

5. Vorrichtung nach Anspruch 4, **dadurch gekennzeichnet, dass** die Düse jeweils unter einem Winkel von höchstens 3° schräg gegen die Grenzfläche gerichtet ist.

6. Vorrichtung nach Anspruch 4 oder 5, **dadurch gekennzeichnet, dass** der Innendurchmesser der Düse höchstens 0,5 mm beträgt.

7. Vorrichtung nach einem der Ansprüche 4 bis 6,

dadurch gekennzeichnet, dass die Düse durch eine Hohlnadel (15) gebildet wird.

8. Vorrichtung nach einem der Ansprüche 4 bis 7, **dadurch gekennzeichnet, dass** die den Flüssigkeitsstrahl (16) bildende Flüssigkeit unter einem Druck von mindestens 3 bar steht.

9. Vorrichtung nach einem der Ansprüche 2 bis 8, **dadurch gekennzeichnet, dass** das mindestens eine Fenster (4) am Ende eines drehbar aufgehängten, angetriebenen Rohres (5) fest eingebaut ist.

10. Vorrichtung nach einem der Ansprüche 2 bis 9, **dadurch gekennzeichnet, dass** die Grenzflächen mindestens eines der Leitelemente kreisrund und dasselbe jeweils mit einer von der mindestens einen Antriebseinrichtung angetriebenen, die Grenzfläche im Zentrum schneidenden Welle (20; 23) verbunden ist.

11. Vorrichtung nach einem der Ansprüche 2 bis 10, **dadurch gekennzeichnet, dass** das zweite Leitelement als dem ersten Fenster (4) gegenüberliegendes zweites Fenster (8) ausgebildet ist.

12. Vorrichtung nach Anspruch 11, **dadurch gekennzeichnet, dass** auf der der Gasmischung abgewandten Seite des zweiten Fensters (8) der Empfänger (10) angeordnet ist.

13. Vorrichung nach Anspruch 11, **dadurch gekennzeichnet, dass** auf der der Gasmischung abgewandten Seite des zweiten Fensters (8) ein Spiegel (30) angeordnet ist.

14. Vorrichtung nach einem der Ansprüche 2 bis 10, **dadurch gekennzeichnet, dass** das weitere optische Leitelement als Spiegel (30) ausgebildet ist.

15. Vorrichtung nach Anspruch 13 oder 14, **dadurch gekennzeichnet, dass** der Spiegel (30) als Hohlspiegel ausgebildet ist.

16. Vorrichtung nach einem der Ansprüche 2 bis 15, **dadurch gekennzeichnet, dass** das mindestens eine Fenster (4; 8) jeweils als zur Achse der Drehbewegung senkrechte ebene Platte (19; 22) ausgebildet ist.

17. Vorrichtung nach einem der Ansprüche 10 bis 16, **dadurch gekennzeichnet, dass** das mindestens eine Fenster (4; 8) jeweils in einer kreisrunden Oeffnung derart angeordnet ist, dass zwischen seinem Rand und dem Rand der Oeffnung ein Spalt freibleibt und dass an die von der Gasmischung abgewandte Seite des Fensters (4; 8) ein mit einem Gas gefüllter Raum (28) anschliesst, in welchem ein über dem statischen Druck in der Gasmischung liegender Druck aufrechterhalten wird.

18. Vorrichtung nach einem der Ansprüche 1 bis 17, **dadurch gekennzeichnet, dass** der Lichtstrahl (7) Ultraviolettlicht enthält und das Fenster (4; 8) jeweils im wesentlichen aus Quarz besteht.

19. Verwendung der Vorrichtung nach einem der Ansprüche 2 bis 18 zur Ermittlung der Konzentration von Schadgasen in einer Verbrennungsanlage.

20. Verwendung der Vorrichtung nach Anspruch 18 zur Ermittlung der Konzentration von SO_2 in einem Rauchgaswäscher.

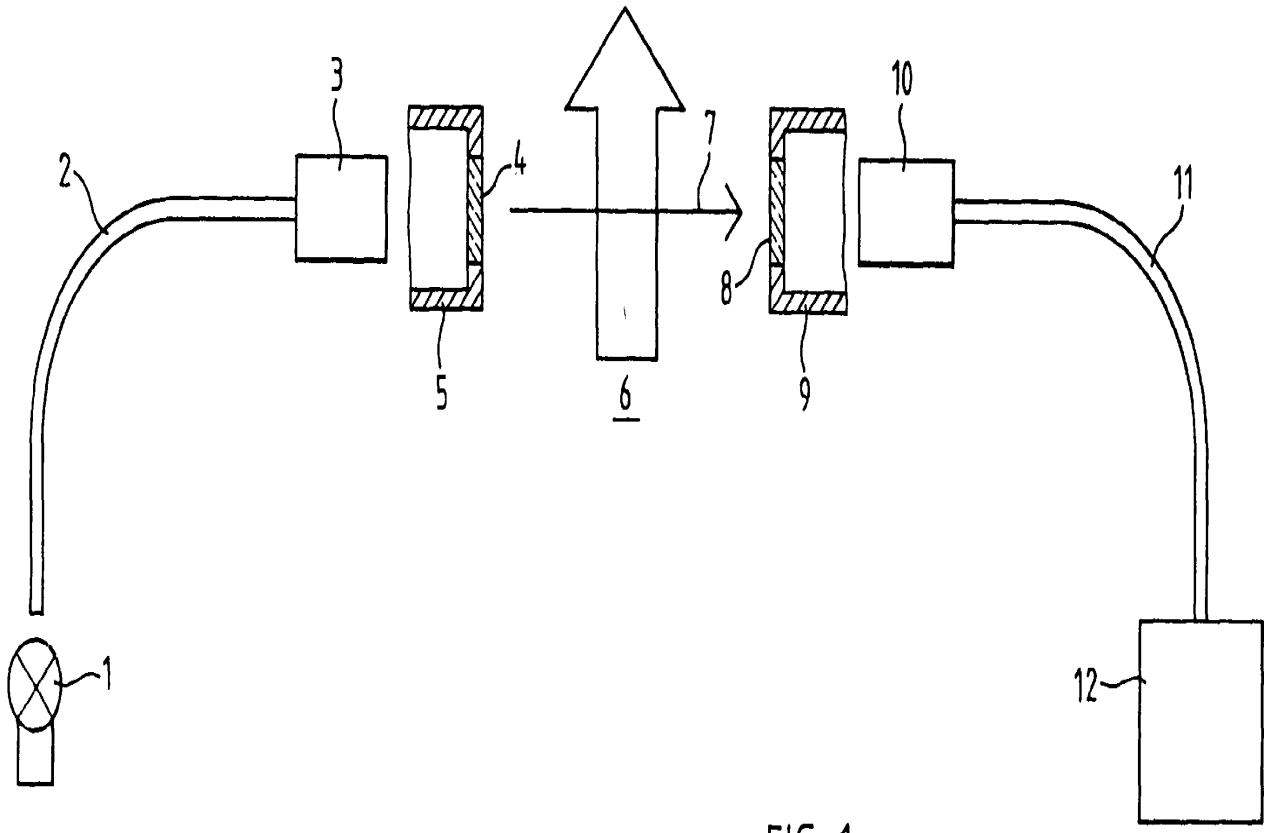


FIG. 1

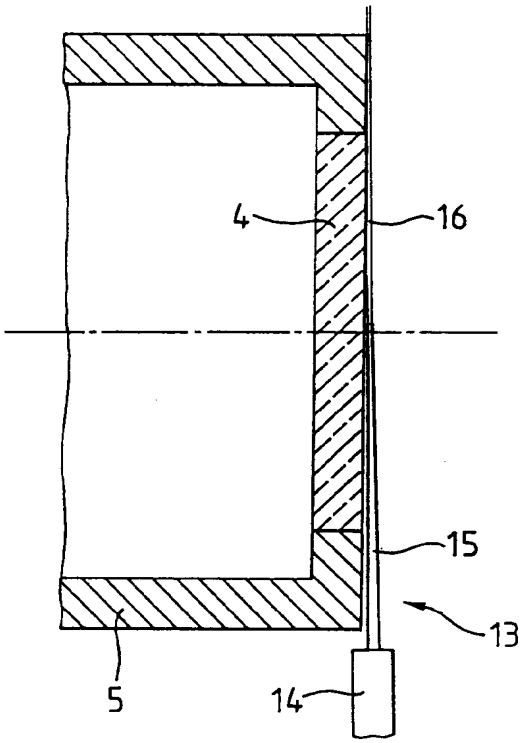


FIG. 2a

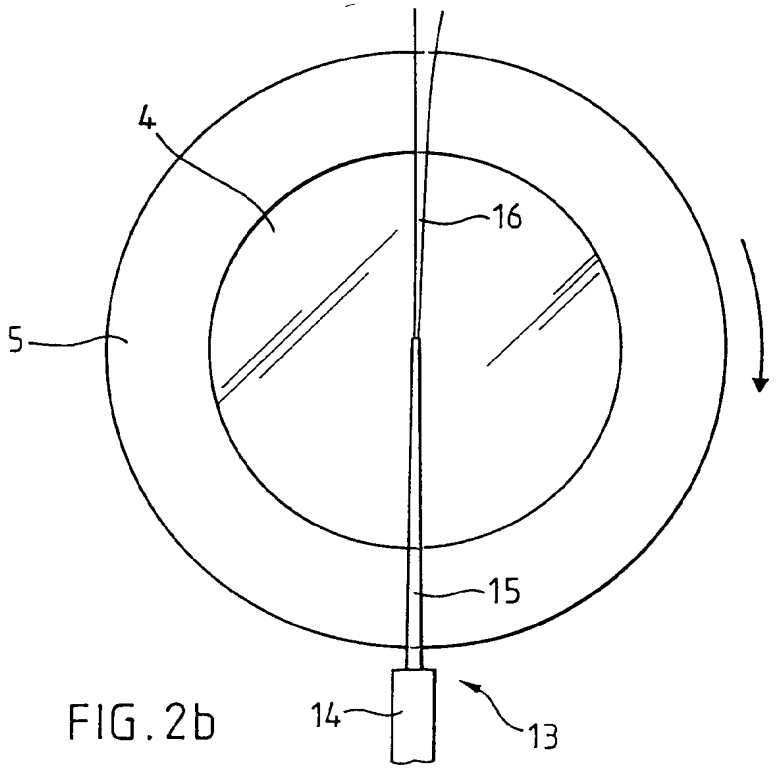


FIG. 2b

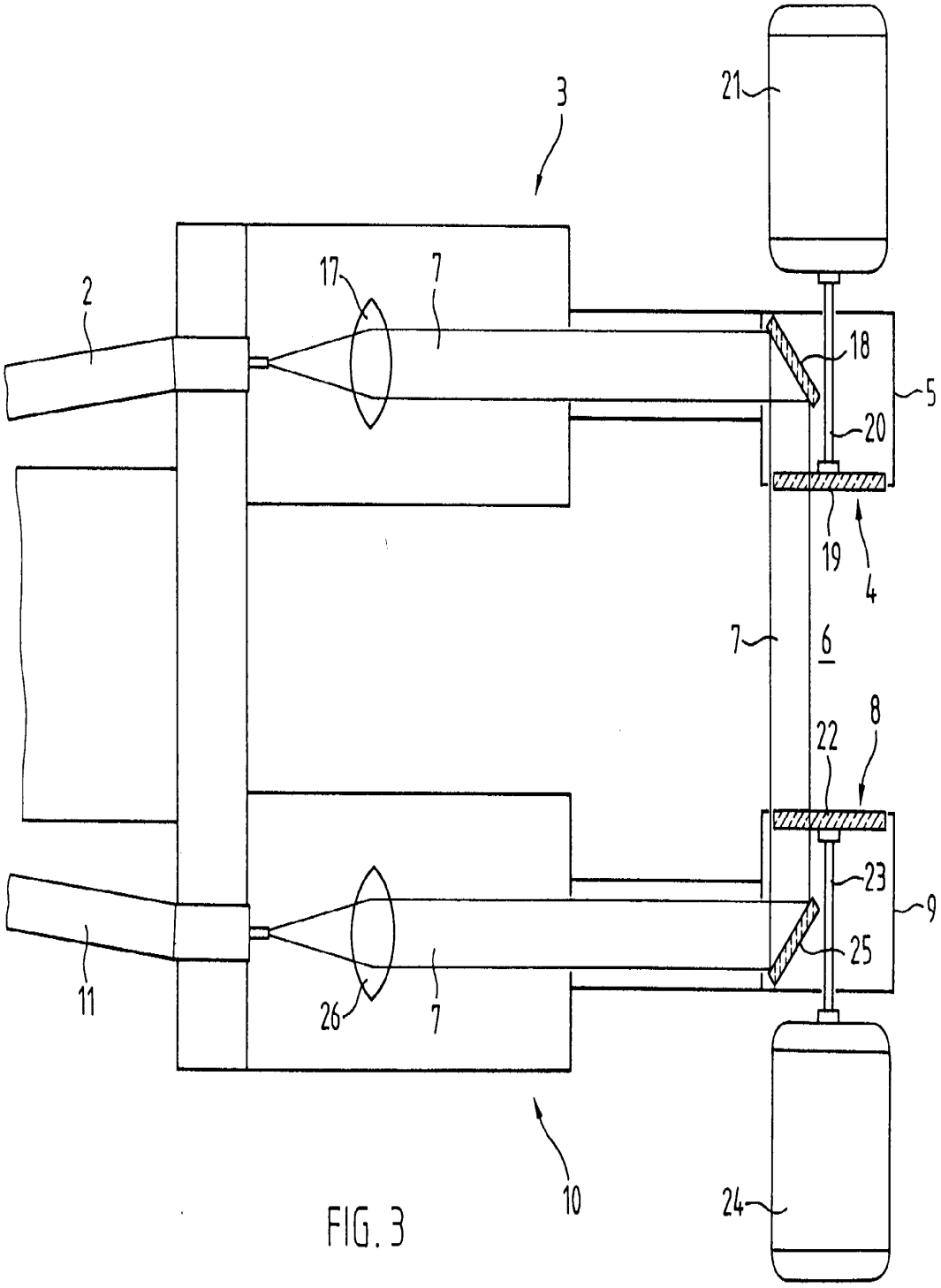


FIG. 3

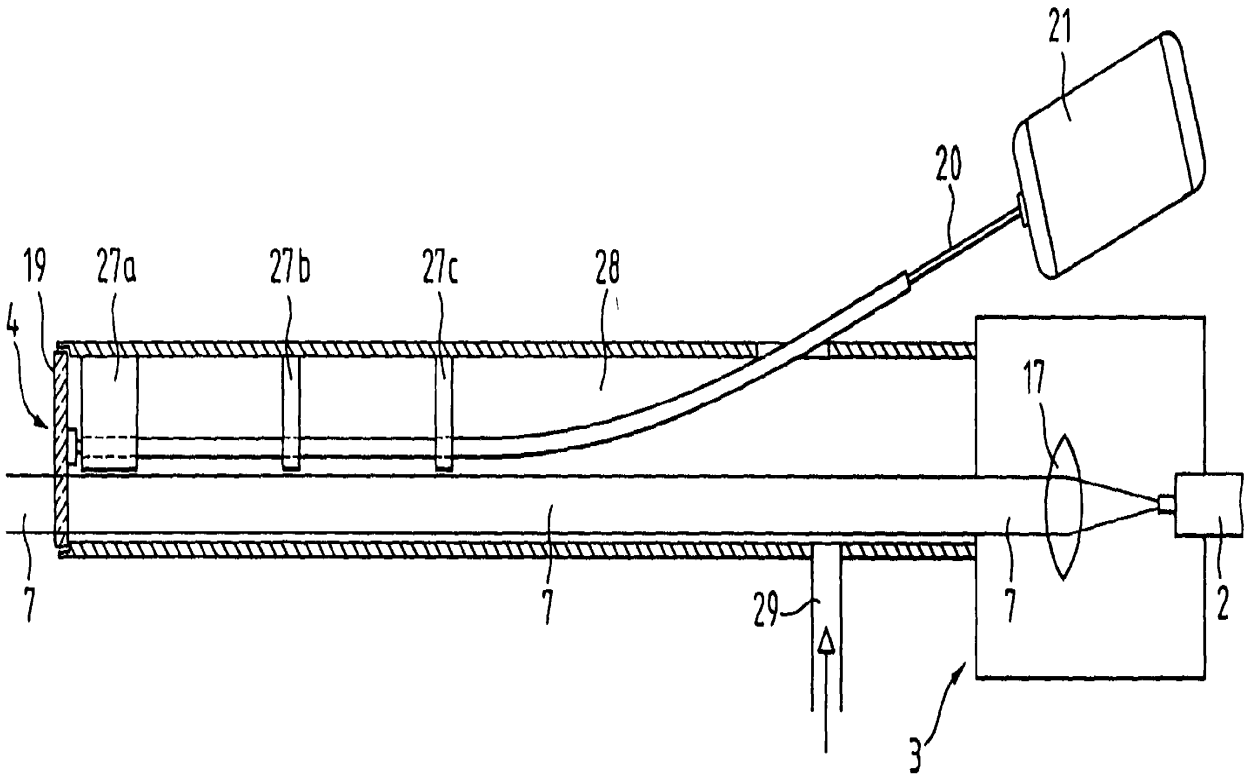


FIG. 4

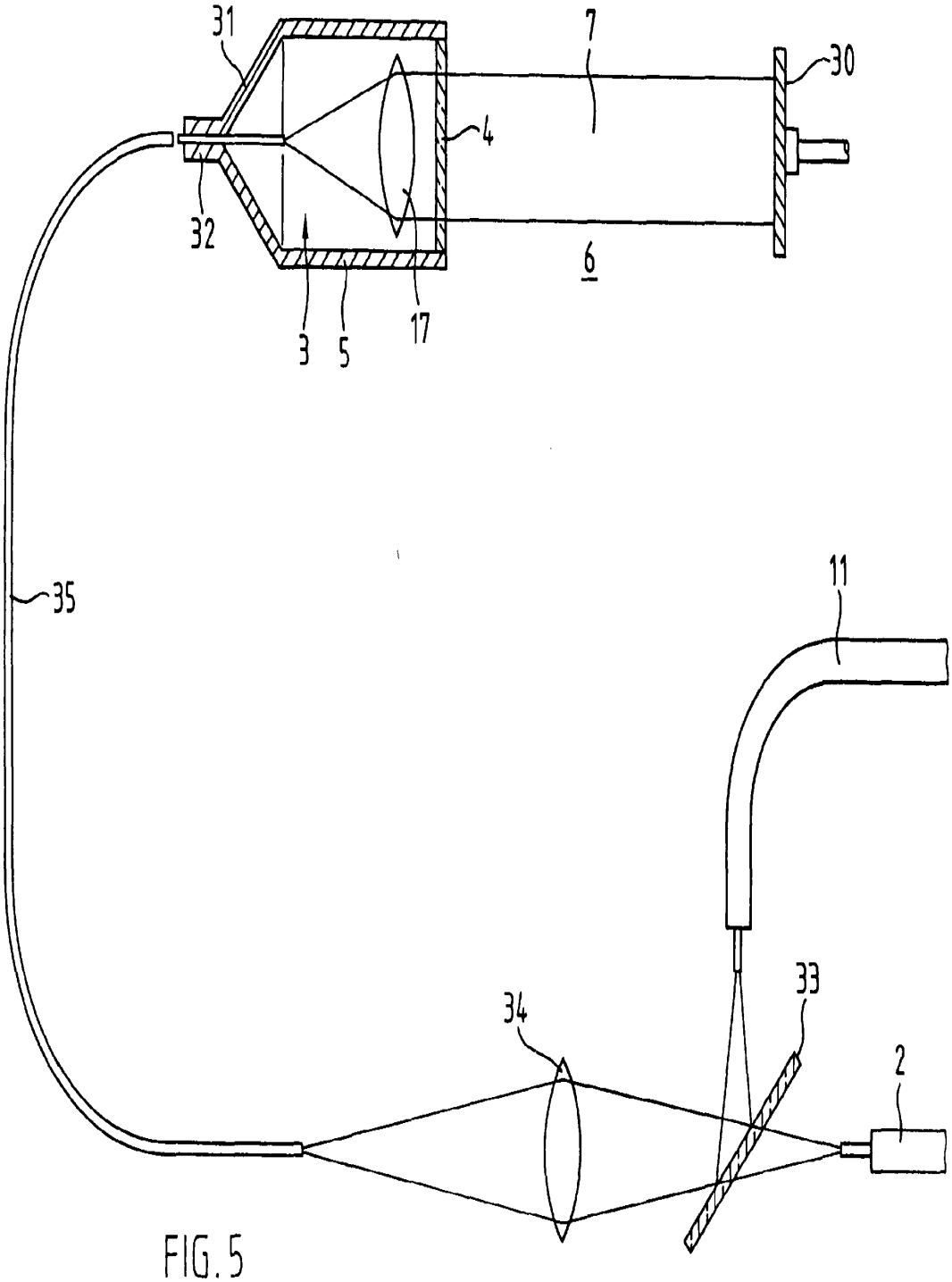


FIG. 5

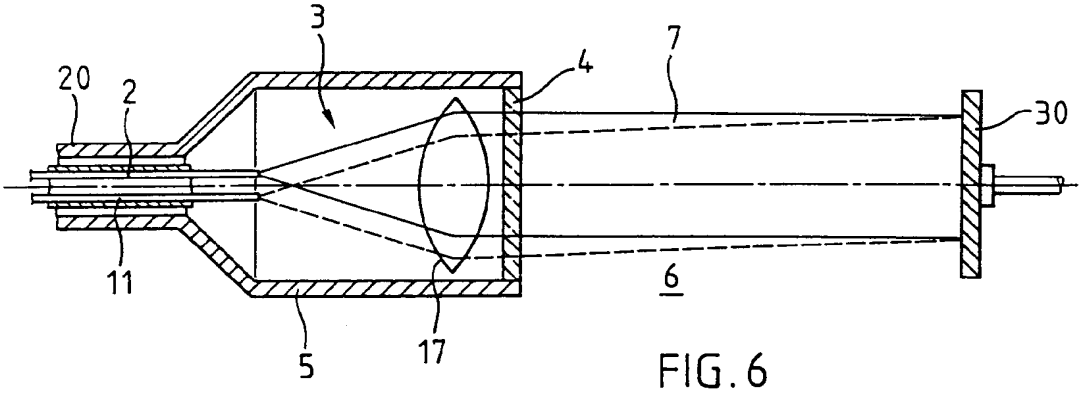


FIG. 6

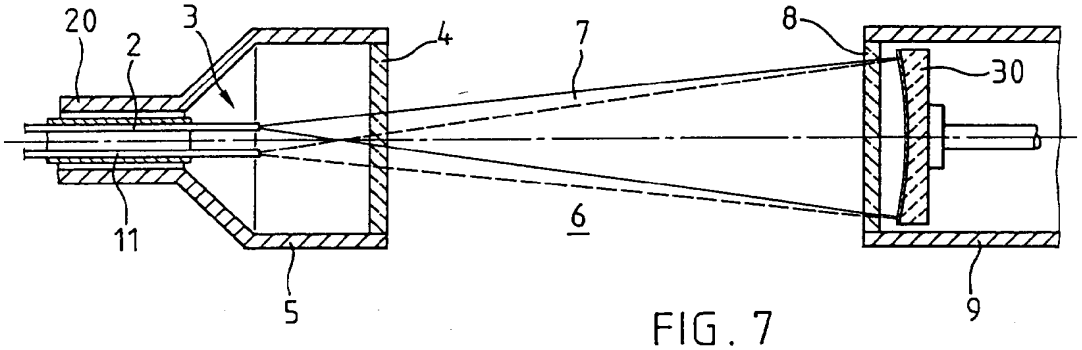


FIG. 7

Europäisches Patentamt

EUROPÄISCHER RECHERCHENBERICHT

Nummer der Anmeldung
EP 97 81 0432

EINSCHLÄGIGE DOKUMENTE

Kategorie	Kennzeichnung des Dokuments mit Angabe, soweit erforderlich, der maßgeblichen Teile	Betrifft Anspruch	KLASSIFIKATION DER ANMELDUNG (Int.Cl.6)
D,Y	US 2 878 716 A (F.C. LEONARD) 24.März 1959 * Spalte 2, Zeile 13 - Spalte 4, Zeile 59 * ---	1-20	G01N21/31 G01N21/15
Y	EP 0 337 108 A (WESTINGHOUSE ELECTRIC CORP) * Spalte 2, Zeile 13 - Spalte 4, Zeile 44; Abbildungen 3,4 * ---	1-20	
A	US 3 935 463 A (JACOBSEN JOHN KENNETH) * Spalte 1, Zeile 3 - Spalte 3, Zeile 24; Abbildung 1 * -----	4-8	

RECHERCHIERTE SACHGEBIETE (Int.Cl.6)

G01N

Der vorliegende Recherchenbericht wurde für alle Patentansprüche erstellt

Recherchenort	Abschlußdatum der Recherche	Prüfer
MÜNCHEN	1.Dezember 1997	Müller, T

KATEGORIE DER GENANNTEN DOKUMENTE

X : von besonderer Bedeutung allein betrachtet
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& : Mitglied der gleichen Patentfamilie, übereinstimmendes Dokument

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(54) Optical wavelength demultiplexer

Optischer Wellenlängen-Demultiplexer

Démultiplexeur de longueur d'ondes optiques

(84) Designated Contracting States:
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(30) Priority: **24.11.1998 US 199988**

(43) Date of publication of application:
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(56) References cited:
EP-A- 0 196 948
FR-A- 2 539 518
US-A- 4 786 133
US-A- 4 994 664
US-A- 5 835 517

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EP 1 004 907 B1

Description

TECHNICAL FIELD

[0001] The invention relates generally to wavelength division multiplexed optical communication systems and more particularly to an optical wavelength division demultiplexer.

DESCRIPTION OF THE RELATED ART

[0002] In a wavelength division multiplexed (WDM) optical system, light from several lasers, each having a different central wavelength, is combined into a single beam that is introduced into an optical fiber. Each wavelength is associated with an independent data signal through the fiber. At the exit end of the fiber, a demultiplexer is used to separate the beam by wavelength into the independent signals. In this way, the data transmission capacity of a fiber is increased by a factor equal to the number of single-wavelength signals combined into a single fiber.

[0003] Many examples of prior art optical demultiplexers exist. One example of a bulk optical filter-based demultiplexer is disclosed in U.S. Pat. No. 5,808,763, entitled "Optical Demultiplexer," issued to Duck et al. (hereinafter Duck). The optical demultiplexer of Duck receives collimated light into a glass block and directs the collimated light onto a single interference filter. The single interference filter has a wavelength filtering characteristic that is dependent upon the angle of incidence with which the collimated light impacts the filter. To manipulate the angle of incidence of the collimated light impacting the filter, a series of reflective surfaces are located opposite the filter and are angled such that the collimated light zigzags between the filter and the reflective surfaces within the glass block, reaching the filter each time at a different angle of incidence. The different angles of incidence are predetermined to enable separation of a multi-wavelength beam of light into its wavelength components. Although the demultiplexer of Duck works well for its intended purpose, the beam diameters involved with collimated light place physical constraints on the degree of miniaturization that can be achieved in a demultiplexer of this type. In addition, the reflective surfaces must be precisely angled to achieve light filtration at the desired wavelength.

[0004] In another known bulk optical filter-based demultiplexer, a compound objective lens collimates light from an optical fiber and then directs the light onto a succession of wavelength-specific optical filters at a particular angle. At each optical filter, light of one wavelength, or a group of wavelengths, is transmitted while light of the remaining wavelengths is reflected. The transmitted light from each optical filter is refocused by filter-specific compound objective lenses and coupled into outgoing fibers for subsequent use. The light reflected from each optical filter propagates back and forth between successive wavelength-specific optical filters in a zigzag fashion within the body of the demultiplexer. Although the demultiplexer works well for its intended purpose, the demultiplexer requires several discrete objective lenses which must be assembled and precisely aligned with respect to one another. In addition, as with the Duck demultiplexer, the use of collimated light limits the degree of miniaturization that can be achieved in a demultiplexer of this type.

[0005] Another example of a prior art demultiplexer is disclosed in U.S. Pat. No. 4,675,860, entitled "Compact Wavelength Multiplexer-Demultiplexer with Variable Filtration," issued to Laude et al. (hereinafter Laude). Laude discloses a demultiplexer that utilizes a number of spherical interference filters that are arranged in series along an optical path of a beam of light that is emitted from an optical fiber. Each filter is selective to a particular wavelength and reflects light of the particular wavelength back to a wavelength-specific output fiber, while the light of other wavelengths is passed onto the next filter in the series. While the demultiplexer works well for its intended purpose, since the filters are located in series along the direction of light propagation, light of a wavelength that is not initially reflected by a first filter will pass through each filter twice. For example, in a three-channel demultiplexer, portions of the original light beam must pass forwardly and rearwardly through two filters. In addition, since the filters refocus the diverging light upon reflection, the curvature of the filters must be precisely formed. Further, because the filters are arranged in a series along the optical path, the filters must be bonded to a device that is formed by combining multiple separately fabricated parts.

[0006] Document D1 (US 5,835,517) describes a compact optical device for multiplexing and demultiplexing an optical signal. On opposite sides of an optically active substrate an array of reflecting lenses is disposed in opposition to an array of optical filters, each of the filters having a different characteristic transmission/refraction wavelength. In the demultiplexer mode, an incoming light beam is reflected from the first filter and directed to a first reflecting, focussing and collimating lens. In turn, this lens directs the collimated light beam to a second filter which reflects the light to a second focussing reflecting and collimating lens and so on. Each filter transmits specific wavelengths and reflects others.

[0007] Document D2 (EP 0 196 948) describes a reversible demuliplexer for separating light with different wavelengths with one input and several outputs. Incoming light is reflected by a convex mirror to a set of stacked filters. The light beam is transmitted through the filters until a certain filter with a certain wavelength is met and the light with the corresponding wavelength is reflected to a next converging device, which outputs the light beams to the output ports.

[0008] Document D3 (US 4,786,133) describes a multiplexer-demultiplexer having one input and several outputs which separates the different wavelengths by a de-

fraction grating that redirects different wavelengths in different directions corresponding to the different outputs.

[0009] Document D4 (US 4,994,664) describes a detector array in which the light is transmitted through an array of refractive lenses that focus the light on light detectors, the lenses being located on one face of the substrate and the sensor elements are located on the opposite face of the substrate.

[0010] In view of the size constraints involved with bulk optical multiplexers and the drawbacks involved with utilizing filters arranged in series along an optical path, what is needed is an optical demultiplexer that can be easily produced with greater miniaturization.

SUMMARY OF THE INVENTION

[0011] A demultiplexer in accordance with the invention includes an optically transparent structure that utilizes focusing relay mirrors to relay a multi-wavelength light beam among laterally arranged wavelength-specific interference filters, with each filter separating out a specific wavelength component from the multi-wavelength beam. The relay mirrors are focusing mirrors, so that the demultiplexer can be operated with a non-collimated light beam in a manner that controls the tendency of such a beam to have a large angle of divergence, while taking advantage of the small beam diameter in order to create a demultiplexer with greater miniaturization.

[0012] A preferred demultiplexer includes a main optical block, wavelength-specific interference filters coupled to the main optical block, and a series of relay mirrors formed within the main optical block to direct and focus light onto the interference filters. The preferred main optical block of the demultiplexer is composed of a monolithic optically transparent material, such as plastic or glass. Mechanical features at an input end of the main optical block align and register an optical fiber, so that a beam of light from the fiber enters the block through a flat input surface of the block. An objective mirror is integrated into another surface of the main optical block to receive the beam of multi-wavelength optical energy from the input fiber and to direct the beam to the first one of the interference filters. The objective mirror is a convex surface relative to the exterior of the block and is shaped such that light that is incident on the first interference filter has the desired spatial and angular characteristics. The surface segment that forms the objective mirror is preferably coated so as to be internally reflective, however at sufficient angles of incidence, an uncoated mirror with total internal reflection can be used. In either embodiment, additional internally reflecting surfaces can be implemented to fold the incoming beam from the optical fiber, so that the necessary optical distance between the input fiber and the first interference filter is obtained in a relatively small space.

[0013] The main optical block also includes an output end. The output end of the main optical block preferably includes a flat output surface to which the interference filters are attached. In some cases, mechanical features can be integrated into the output end of the main optical block to aid in the alignment and registration of the interference filters.

[0014] The interference filters are wavelength-selective filters that are preferably connected to the output end of the MOB in a linear array with fixed center-to-center spacing. Each filter has high transmission and low reflection over a particular range of wavelengths and low transmission and high reflection over another range of wavelengths. The preferable transmission spectrum for WDM demultiplexer applications is a "flat top" shape in which very high and uniform transmission is achieved over one wavelength range, while immediately outside the range a very low transmission and high reflection is achieved. Although the interference filters are preferably separated into discrete pieces, the interference filters can also be integrated onto a single substrate that is attached to the output end of the main optical block. Moreover, the interference filters can be deposited directly onto the output end of the main optical block.

[0015] The relay mirrors are focusing mirrors that are preferably integrated into a surface of the main optical block that is parallel to the surface which is in contact with the interference filters. The relay mirrors are located and shaped to produce the desired spatial and angular characteristics of a beam that propagates in a zigzag fashion between the interference filters and the relay mirrors. The relay mirrors are preferably convex aspheric surfaces relative to the exterior of the block and are coated so that they are internally reflective. In situations in which the fiber core and the beam diameter at the filters can be treated as "points," because they are significantly smaller than any other dimension in the optical system, an ellipsoidal objective mirror and ellipsoidal relay mirrors with foci at the input fiber and the filter centers can be utilized to provide more precise imaging.

[0016] In an additional aspect of the invention, a lens array is coupled to the output end of the main optical block to direct and focus the filtered light that is output from the main optical block to an adjacent array of detectors. Preferably, the lens array is integrated into a lens array block that is a structure of plastic or glass similar to the main optical block. The lens array block and the main optical block can be made to have complementary mechanical features that enable the two parts to fit together with precise alignment. Additional mechanical features on the lens array block and/or the main optical block can be formed to act as spacers that fix the distance between the lens array and an optical detector array or an array of output optical fibers.

[0017] When implemented in a preferred four-channel WDM communications system, light from an optical fiber is coupled directly into the main optical block through the input surface without the light being collimated. The objective mirror integrated into the main optical block focuses and directs the light from the optical fiber to a first of four wavelength-specific interference filters that are

formed on the output end of the main optical block. The light propagates from the objective mirror to the first interference filter at a pre-established angle of incidence. The light that is transmitted through the first filter is focused by a lens of the attached lens array onto a detector for signal detection. The light that is not transmitted through the first filter is reflected from the first filter and re-reflected to a second filter by the first relay mirror. The second filter further separates the beam, and the same process is repeated between the second and third, and between the third and fourth wavelength-specific interference filters, using the second and third relay mirrors, respectively. As the light beam is relayed among the four wavelength-specific filters, the components of the light beam have been separated by wavelength and transmitted through the attached lens array to appropriate detectors of the detector array, thereby demultiplexing the WDM signal.

[0018] Advantages of the invention include the ability to fabricate the demultiplexer using high volume, low cost techniques, such as injection molding, without the need to rely on specialized materials. In addition, because dielectric interference filters have reflectivities higher than 99% outside of their passband, essentially all of the light of a particular wavelength reaches the appropriate filter, thereby limiting signal loss to the transmission loss of the filter. Because the demultiplexer uses integrated focusing reflectors in a folded geometry, the demultiplexer occupies less space than prior designs while providing the same performance. Further, assembly of the demultiplexer involves attaching the interference filters to the main optical block with only a very coarse alignment.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019]

Fig. 1 is a depiction of a four-channel demultiplexer with an objective mirror and separate MOB and lens array in accordance with the invention.

Fig. 2 is a depiction of a four-channel demultiplexer with an objective mirror and direct coupling to detectors in accordance with the invention.

DETAILED DESCRIPTION

[0020] Fig. 1 is a depiction of a demultiplexer 10 in accordance with a preferred embodiment of the invention. The demultiplexer includes a main optical block 14 (MOB), an input surface 38, an objective mirror 40, wavelength-specific dielectric interference filters 20, 22, 24 and 26 coupled to the MOB, and a series of relay mirrors 30, 32, and 36 integrated into the MOB to direct and focus light between the interference filters. The term "wavelength" is used herein to designate the approximate center wavelength of a wavelength distribution that is typical of an optical signal.

[0021] In brief, when implemented in a four-channel WDM communications system, light is coupled from an optical fiber 42 into the MOB through the input surface 38 without the light being collimated. The objective mirror 40 integrated into the MOB receives the input light 44 that is diverging from the input surface and focuses and directs the light 70 to a first filter 20 of the four wavelength-specific interference filters that are attached to an output end 46 of the MOB.

[0022] The light beam 58 that is transmitted through the first filter is focused by an adjacent lens 50 onto a detector 60 for signal detection. The light beam portion 68 that is not transmitted through the first filter is reflected by the first filter for impingement upon a second filter 22 via the first internally reflective concave relay mirror 30. The second filter further separates the beam based upon wavelength. The same process is repeated between the second and third 24 and between the third and fourth 26 wavelength-specific filters, using the second 32 and third 36 relay mirrors respectively. By directing the WDM signal to each of the wavelength-specific filters, the WDM signal is separated into its four channels. It is important to note that the relay mirrors are concave mirrors, so that the non-collimated and diverging light beam is continuously refocused while propagating between adjacent filters, allowing the demultiplexer to be extremely small as compared to prior art bulk optical demultiplexers for non-collimated beams. With the basic structure and function of a preferred demultiplexer briefly described, more details of the preferred embodiment and alternative embodiments are described below.

[0023] The MOB 14 of Fig. 1 utilizes a flat input surface 38 at the input end 39 of the MOB and an objective mirror 40 at another surface of the MOB to direct the incoming light to the first filter 20. The objective mirror 40 is a convex mirror (relative to the exterior of the MOB) integrated into the surface of the MOB in alignment to receive the multi-wavelength light 44 that is introduced at the input surface. The objective mirror can be coated so as to be internally reflective, or at sufficiently high incident angles an uncoated mirror with totally internal reflection can be used. As can be seen, the multi-wavelength light 44 is diverging as it travels from the input surface 38 to the objective mirror. Ideally, the mirror is configured such that the multi-wavelength light 70 is converging as the light propagates from the objective mirror 40 to the first filter. The input structure of the MOB is designed so that a converging light beam is incident on the first filter 20 at a non-perpendicular angle with respect to the filters 20-26. For each filter, the preferred angle of incidence relative to the output end 46 is approximately 78 degrees.

[0024] Mechanical features 45 can be formed in the input end 39 of the MOB 14 for aligning and registering the optical fiber 42. The preferred MOB is composed of a monolithic, homogeneous, and optically transparent material such as plastic or glass. Injection molding is preferably used to fabricate the MOB, but other molding techniques or precision machining techniques can also be used. The dimensions of the preferred MOB as shown

in Fig. 1 may be approximately 7 mm high, 5 mm long, and 1.75 mm wide, although the exact dimensions are not critical.

[0025] Fig. 2 is an alternative input arrangement in which like elements are numbered as in Fig. 1. In the arrangement of Fig. 2, the input fiber 42 is perpendicular to the output end 46 of the MOB. A flat objective mirror 88 and a convex integrated mirror 41 (concave relative to incident light) are utilized to direct and focus the light from the input surface 38 to the first filter 20. Fig. 2 is one example of a folded geometry that can be implemented, but various other arrangements are possible. For example, additional flat reflective surfaces can be used following the input surface to fold the beam, so that the required optical distance between the objective and the first filter can be obtained in a relatively small space.

[0026] Referring back to Fig. 1, the output end 46 of the MOB 14 preferably includes a flat output surface. The interference filters 20-26 are preferably attached to or deposited on the flat output surface. As an alternative, mechanical features can be integrated into the output end of the MOB to aid in the alignment and registration of the interference filters. For example, Fig. 2 depicts a design in which depressions are formed in the output end 46 of the MOB to precisely position the four distinct interference filters 20-26. Fig. 3 depicts another alternative design in which a single depression is formed in the output end 46 to receive an integrated filter array 84.

[0027] In some embodiments, the interference filters can be deposited directly onto the output end 46 of the MOB 14. When the filters are deposited directly onto the output end of the MOB, the output surfaces do not have to be flat. For example, curved output surfaces formed at the output end of the MOB may be used to maintain the desired beam characteristics within the MOB and to focus the beams of light that are output from the MOB. Although some examples of the output end arrangement are shown and described, the exact arrangement is not critical to the invention and may be modified as would be apparent to one of ordinary skill in the art.

[0028] Referring back to Fig. 1, the interference filters 20-26 are preferably dielectric filters that are used to separate one optical wavelength from another. The wavelength-selective filters are discrete pieces that are preferably arranged in a linear array with fixed center-to-center spacing. The interface of the interference filters and the output end 46 of the MOB 14 defines a mirror plane. Each filter has high transmission and low reflection over a selected set or sets of wavelengths, called the passband, and low transmission and high reflection over another set or sets of wavelengths, called the stopband. The preferable transmission spectrum for WDM demultiplexer applications is a "flat-top" shape in which very high and uniform transmission is achieved over the passband wavelength interval, while immediately outside the passband very low transmission and high reflection is achieved. The wavelength interval over which the transmission varies from high to low should be as small as possible to accommodate narrow channel spacing. In a preferred four-channel WDM system, the four channels are centered at approximately 1280, 1300, 1320, and 1340 nanometers (nm). Another preferred four-channel spacing distribution includes wavelengths of 820, 835, 850, and 865 nm. Although the demultiplexer is described with reference to a four-channel demultiplexer, more or fewer channels can be demultiplexed by adding or subtracting filters and relay mirrors.

[0029] Because the transmission spectrum of an interference filter changes as a function of the angle of incidence, the best performance is achieved when the angular extent of the light passing through a filter is minimized. In addition, the use of materials with high refractive indices will reduce the dependence of the transmission spectrum on the incident angle. Although the interference filters 20-26 are separated into discrete pieces in Figs. 1 and 2, in the demultiplexer of Fig. 3, the four interference filters 20-26 are monolithically integrated onto a single substrate to form a filter array 84 that is attached to the output surface of the MOB. Either arrangement can be implemented in accordance with the invention. In another alternative embodiment, the interference filters can be deposited directly onto the output end of the MOB.

[0030] The relay mirrors 30-36 in Figs. 1, 2, and 3 are converging or focusing mirrors that are preferably integrated into a surface of the MOB 14 that is parallel to the faces of the interference filters 20-26. The relay mirrors are located and shaped to produce the desired spatial and angular characteristics of a beam that propagates in a zigzag fashion between the interference filters and the relay mirrors. The relay mirrors are preferably convex aspheric surfaces that are coated so that they are internally reflective. The relay mirrors may also be diffractive mirrors or any other mirror that causes an increase in the angle of convergence or a decrease in the angle of divergence of a beam of light.

[0031] In the ideal limit, where the fiber core and the beam diameter at the filters can be treated as points, much smaller than any other dimension in an optical system, an ellipsoidal objective mirror and ellipsoidal relay mirrors with foci at the fiber and the filter centers can be utilized to provide more precise imaging. The objectives should be chosen such that the ratio of image-to-object distances gives the angular reduction factor necessary to achieve satisfactory filter transmission spectra. However, when space limitations dictate small dimensions for the MOB, this ideal limit is a poor approximation to the system.

[0032] Referring to Fig. 1, in an additional aspect of the invention, an array of lenses 50, 52, 54, and 56 is coupled to the output end 46 of the MOB 14 to direct and focus the filtered light to the adjacent array of detectors 60, 62, 64, and 66, or alternatively to other output devices, such as optical fibers. In the arrangement of Fig. 1, the lenses 50-56 are integrated into a lens array block 76. The lens array block is preferably a structure of plastic

or glass similar to the MOB. The lens array block and the MOB can be made to have complementary mechanical features 72, 74 and 92, 94 that enable the two parts to fit together with precise alignment. In the design of Fig. 1, the lens array block and the MOB are fit together and secured with adhesive. In an alternative to the two-part design of Fig. 1, a lens array can be integrated into the MOB, with the MOB having a slot where the interference filters are inserted between the output end of the MOB and the lens array. In either case, mechanical features 96 and 98 of the lens array block and/or the MOB can be formed to act as spacers that fix the distance between the lenses and the detectors.

[0033] The lenses 50-56 integrated into the lens array block 76 are preferably either plano-convex or bi-convex aspheres. The plano-convex aspheres have the advantage that the planar sides can face the interference filters 20-26 with index-matching adhesive filling in the intervening space to reduce loss due to Fresnel reflection. The convex side of the lenses can face the detectors 60-66, with the mechanical features 96 and 98 fixing the distance between the lenses and the detectors.

[0034] Referring to Fig. 2, in another alternative embodiment, light 110, 112, 114, and 116 output from the interference filters 20-26 propagates directly to the detectors 60-66. In order to implement direct coupling, the detector array 100 is preferably closely aligned with the filters and the spatial extent of the beam incident on the filters is sufficiently smaller than the detector diameter to allow for alignment tolerances and divergence between the filters and the detectors. Direct coupling sets a lower bound on the angular divergence of the beam that is incident on the interference filter.

[0035] As stated above, the light output from the interference filters 20-26 can be directed into optical fibers, instead of detectors. When outputting into optical fibers, the fiber core diameter and numerical aperture define limitations regarding the spatial and angular extent of the output beam. Mechanical features on the MOB and/or the lens array block can be formed to align and register the output fibers.

[0036] Although the MOB 14 is described with reference to a WDM demultiplexer, the MOB can be slightly modified and operated in reverse as a WDM multiplexer. When used as a WDM multiplexer, single-wavelength beams of light are input to the MOB behind the interference filters 20-26. The interference filters pass the input wavelengths and reflect other wavelengths within the MOB. The combined light is then reflected by the objective mirror 40 into the optical fiber 42, which acts as an output fiber.

Claims

1. An optical demultiplexer comprising:

   a main optical block (14) having an input for receiving a beam of multi-wavelength optical energy and a plurality of outputs for outputting a plurality of wavelength-specific optical energy beams;

   a plurality of wavelength-specific filters (20, 22, 24, and 26) aligned with said plurality of outputs and connected to said main optical block such that a first of said filters is impinged by said received beam of multi-wavelength optical energy, each of said wavelength-specific filters having optical characteristics that cause transmission of optical energy at a first set of wavelengths and reflection of optical energy at a second set of wavelengths outside of said first set;

   a plurality of converging reflectors (30, 32, and 36) formed at a surface of said main optical block wherein said converging reflectors are located relative to said wavelength-specific filters such that each converging reflector receives at least a portion of said beam of optical energy from one of said wavelength-specific filters and refocuses said received optical energy toward a different one of said wavelength-specific filters; and

   an input reflector (40) located relative to said input such that said beam of multi-wavelength optical energy is reflected from said input reflector to said first of said wavelength-specfic filters, wherein said input reflector (40) is an ellipsoidal reflector with a first focus point at said input and a second focus point at said first of said plurality of wavelength-specific filters.

2. The optical demultiplexer of claim 1 wherein said plurality of converging reflectors (30, 32, and 36) are formed in a linear array on a surface of said main optical block (14).

3. The optical demultiplexer of claim 1 wherein said input reflector (40) is formed at said surface of said main optical block (14), said main optical block being a unitary body that includes said input reflector and said converging reflectors (30, 32, and 36).

4. The optical demultiplexer of one of the preceding claims wherein said main optical block (14) is coupled to a lens array (50, 52, 54, and 56) that receives said wavelength-specific optical energy beams from said plurality of outputs and that directs and focuses said wavelength-specific optical energy beams to collectors (60, 62, 64, and 66).

5. The optical demultiplexer of claim 4 wherein said collectors (60, 62, 64, and 66) are optical fibers.

6. The optical demultiplexer of claim 4 wherein said collectors (60, 62, 64, and 66) are optical detectors.

7. The optical demultiplexer one of the preceding claims wherein said converging reflectors (30, 32, and 36) are concave mirrors.

8. The optical demultiplexer of one of claims 1 to 7 wherein said converging reflectors (30, 32, and 36) are diffractive mirrors.

Patentansprüche

1. Ein optischer Demultiplexer aufweisend:

einen Hauptoptikblock (14), der einen Eingang zum Empfangen eines Strahls einer optischen Mehrfachwellenlängen-Energie und einer Mehrzahl von Ausgängen zum Ausgeben einer Mehrzahl von wellenlängenspezifischen optischen Energiestrahlen;
eine Mehrzahl von wellenlängenspezifischen Filtern (20, 22, 24 und 26), die mit der Mehrzahl von Ausgängen ausgerichtet sind und mit dem Hauptoptikblock verbunden sind, so dass ein erster der Filter durch den empfangenen Strahl der optischen Mehrfachwellenlängen-Energie beeinflusst wird, wobei jeder der wellenlängenspezifischen Filter optische Charakteristika aufweist, die eine Übertragung von optischer Energie bei einem ersten Satz von Wellenlängen und eine Reflektion von optischer Energie bei einem zweiten Satz von Wellenlängen außerhalb des ersten Satzes bewirken;
eine Mehrzahl von konvergierenden Reflektoren (30, 32 und 36), die an einer Fläche des Hauptoptikblock s gebildet sind, wobei die konvergierenden Reflektoren relativ zu den wellenlängenspezifischen Filtern angeordnet sind, so dass jeder konvergierende Reflektor zumindest einen Teil des Strahls von optischer Energie von einem der wellenlängenspezifischen Filter empfängt und die empfangene optische Energie neu gegen einen anderen der wellenlängenspezifischen Filter fokussiert; und
einen Eingangsreflektor (40), der relativ zu dem Eingang angeordnet ist, so dass der Strahl der optischen Mehrfachwellenlängen-Energie von dem Eingangsreflektor zu dem ersten der wellenlängenspezifischen Filter reflektiert wird;
wobei der Eingangsreflektor (40) ein ellipsenförmiger Reflektor mit einem ersten Brennpunkt bei dem Eingang und einem zweiten Brennpunkt bei dem ersten der Mehrzahl von wellenlängenspezifischen Filtern ist.

2. Der optische Demultiplexer nach Anspruch 1, wobei die Mehrzahl von konvergierenden Reflektoren (30, 32 und 36) in einer linearen Anordnung auf einer Fläche des Hauptoptikblock (14) gebildet ist.

3. Der optische Demultiplexer nach Anspruch 1, wobei der Eingangsreflektor (40) an der Fläche des Hauptoptikblock s (14) gebildet ist, wobei der Hauptoptikblock ein einheitlicher Körper ist, der den Eingangsreflektor und die konvergierenden Reflektoren (30, 32 und 36) aufweist.

4. Der optische Demultiplexer nach einem der vorhergehenden Ansprüche, wobei der Hauptoptikblock (14) zu einer Linsenanordnung (50, 52, 53 und 56) gekoppelt ist, die die wellenlängenspezifischen optischen Energiestrahlen von der Mehrzahl der Ausgänge empfängt und die wellenlängenspezifischen optischen Energiestrahlen auf Kollektoren (60, 62, 64 und 66) richtet und fokussiert.

5. Der optische Demultiplexer nach Anspruch 4, wobei die Kollektoren (60, 62, 64 und 66) Lichtwellenleiter sind.

6. Der optische Demultuplexer nach Anspruch 4, wobei die Kollektoren (60, 62, 64 und 66) optische Detektoren sind.

7. Der optische Demultiplexer nach einem der vorhergehenden Ansprüche, wobei die konvergierenden Reflektoren (30, 32 und 36) konkave Spiegel sind.

8. Der optische Demultiplexer nach einem der Ansprüche 1 bis 7, wobei die konvergierenden Reflektoren (30, 32 und 36) beugende Spiegel sind.

Revendications

1. Démultiplexeur optique comprenant :

un bloc optique principal (14) muni d'une entrée pour recevoir un faisceau d'énergie optique à longueurs d'onde multiples et d'une pluralité de sorties pour produire une pluralité de faisceaux d'énergie optique spécifiques à la longueur d'onde ;
une pluralité de filtres (20, 22, 24 et 26) spécifiques à la longueur d'onde alignés avec ladite pluralité de sorties et connectés audit bloc optique principal de telle sorte qu'un premier desdits filtres est frappé par ledit faisceau d'énergie optique à longueurs d'onde multiples reçu, chacun desdits filtres spécifiques à la longueur d'onde ayant des caractéristiques optiques qui entraînent la transmission d'énergie optique à un premier ensemble de longueurs d'onde et la réflexion de l'énergie optique sur un deuxième ensemble de longueurs d'onde en dehors dudit premier ensemble ;
une pluralité de réflecteurs convergents (30, 32 et 36) formée à une surface dudit bloc optique

principal dans laquelle lesdits réflecteurs convergents sont situés par rapport auxdits filtres spécifiques à la longueur d'onde de telle sorte que chaque réflecteur convergent reçoit au moins une partie dudit faisceau d'énergie optique de l'un desdits filtres spécifiques à la longueur d'onde et refocalise ladite énergie optique reçue vers un filtre différent parmi lesdits filtres spécifiques à la longueur d'onde ; et

un réflecteur (40) d'entrée situé par rapport à ladite entrée de telle sorte que ledit faisceau d'énergie optique à longueurs d'onde multiples est réfléchi dudit réflecteur d'entrée audit premier desdits filtres spécifiques à la longueur d'onde,

dans lequel ledit réflecteur (40) d'entrée est un réflecteur ellipsoïdal avec un premier point de focalisation au niveau de ladite entrée et un deuxième point de focalisation au niveau dudit premier de ladite pluralité de filtres spécifiques à la longueur d'onde.

2. Démultiplexeur optique selon la revendication 1, dans lequel ladite pluralité de réflecteurs convergents (30, 32 et 36) est formée dans un réseau linéaire sur une surface dudit bloc optique principal (14).

3. Démultiplexeur optique selon la revendication 1, dans lequel ledit réflecteur (40) d'entrée est formé au niveau de ladite surface dudit bloc optique principal (14), ledit bloc optique principal étant un corps unitaire qui comprend ledit réflecteur d'entrée et lesdits réflecteurs convergents (30, 32 et 36).

4. Démultiplexeur optique selon l'une des revendications précédentes, dans lequel ledit bloc optique principal (14) est couplé à un réseau de lentilles (50, 52, 54 et 56) qui reçoit lesdits faisceaux d'énergie optique spécifiques à la longueur d'onde de ladite pluralité de sorties et qui dirige et focalise lesdits faisceaux d'énergie optique spécifiques à la longueur d'onde sur les collecteurs (60, 62, 64 et 66).

5. Démultiplexeur optique selon la revendication 4, dans lequel lesdits collecteurs (60, 62, 64 et 66) sont des fibres optiques.

6. Démultiplexeur optique selon la revendication 4, dans lequel lesdits collecteurs (60, 62, 64 et 66) sont des détecteurs optiques.

7. Démultiplexeur optique selon l'une des revendications précédentes, dans lequel lesdits réflecteurs convergents (30, 32 et 36) sont des miroirs concaves.

8. Démultiplexeur optique selon l'une des revendications 1 à 7, dans lequel lesdits réflecteurs convergents (30, 32 et 36) sont des miroirs diffractifs.

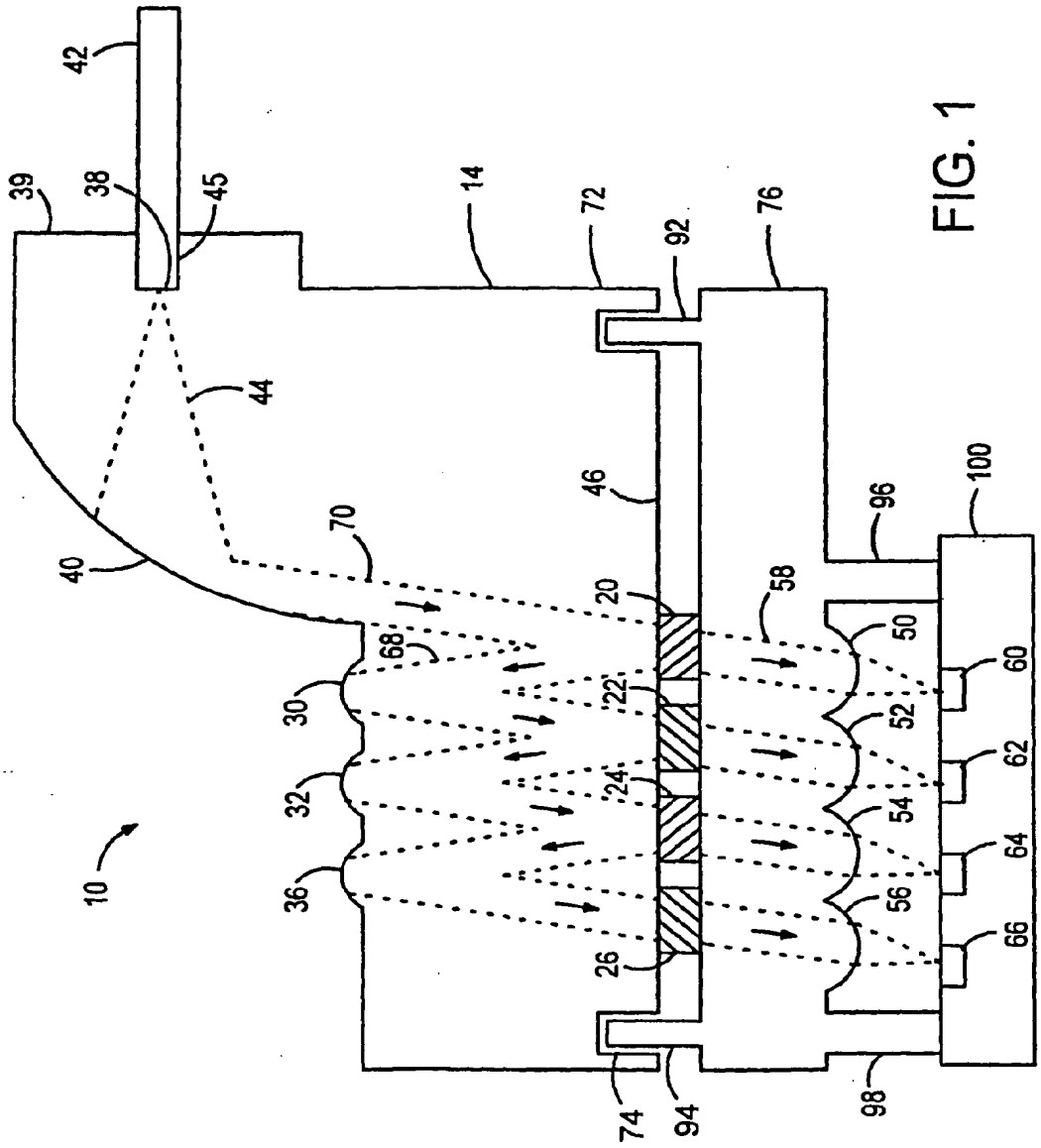

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FIG. 1

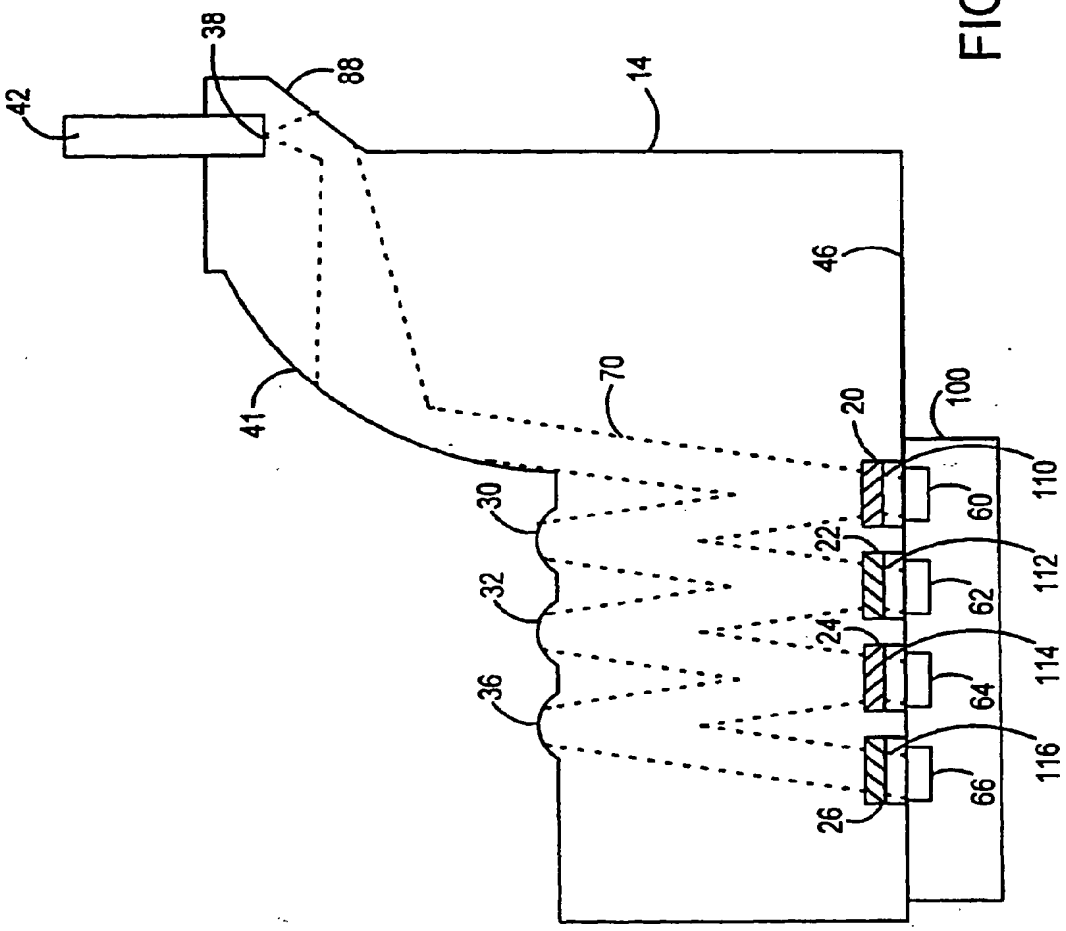

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FIG. 2

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

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(54) Hematological analyzer, method for analyzing body fluid and computer program product

(57) A hematological analyzer for measuring blood, sets a body fluid measurement mode; receives a measurement start instruction; irradiates a measurement sample with light and obtains optical information from cells contained in the measurement sample; and classifies at least white blood cells and nucleated cells other than white blood cells contained in the measurement sample, and counts the white blood cells and nucleated cells other than white blood cells based on the optical information obtained from the cells in the measurement sample prepared from a body fluid sample and white blood cell measuring reagent when the body fluid measurement mode has been set and the measurement start instruction has been selected, is disclosed. A method for analyzing body fluid and a computer program product are also disclosed.

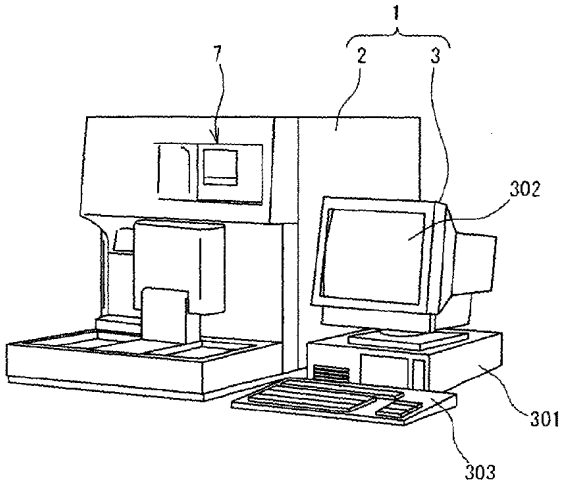


FIG.1

EP 1 953 526 A2

Description

FIELD OF THE INVENTION

[0001] The present invention relates to a hematological analyzer, a method for analyzing body fluid and a computer program product capable of measuring not only blood, but also body fluids other than blood such as cerebrospinal fluid (spinal fluid), fluid of the thoracic cavity (pleural fluid), abdominal fluid and the like.

BACKGROUND

[0002] In the field of clinical examinations, blood is collected from a body and used as a sample which is measured by a hematological analyzer, and the analysis result is used to aid diagnosis and monitor treatment.

[0003] In the area of clinical examinations there is a demand for a convenient way to measure body fluids other than blood, such as cerebrospinal fluid. Although cells are not normally found in body fluids, cells such as blood cells (hemorrhage), anomalous cells, and bacteria and the like appear in cases of disease, organ-related tumors, injury and the like.

[0004] US Patent Publication No. 2003-0215890 discloses art for measuring cells in body fluids using a blood cell analyzer. In US Patent Publication No. 2003-0215890, a measurement sample is prepared by mixing cerebrospinal fluid (CSF) with a reagent composition which contains an aldehyde, surface active agent, and cyclodextrin, analyzing the prepared measurement sample using a model ADVIA 120 cytometer analyzer, and classifying and counting the cells in the cerebrospinal fluid using the cytograms shown in FIGS. 11A through 11G.

[0005] Cerebrospinal fluid (CSF) is the only body fluid actually analyzed in the art disclosed in US Patent Publication No. 2003-0215890, inasmuch as, for example, abdominal fluid and thoracic fluid and the like are not analyzed. Although cerebrospinal fluid seldom contains particles other than blood cells, mesothelial cells, macrophages, tumor cells and the like may be present in body fluids other than cerebrospinal fluid, for example, abdominal fluid and thoracic fluid, depending on the disease of the patient. When a body fluid which has a particle composition other than blood cells is analyzed by the art disclosed in US Patent Publication No. 2003-0215890, there is a possibility, for example, that particles other than blood cells may appear in cell fraction regions of any cytogram, in which case an accurate analysis result can not be obtained.

SUMMARY OF THE INVENTION

[0006] The scope of the present invention is defined solely by the appended claims, and is not affected to any degree by the statements within this summary.

[0007] A first aspect of the present invention is a hematological analyzer for measuring blood, comprising: a measurement mode setting means for setting a body fluid measurement mode; a measurement starting means for receiving a measurement start instruction; an optical information obtaining means for irradiating a measurement sample with light and obtaining optical information from cells contained in the measurement sample; and an analyzing means for classifying at least white blood cells and nucleated cells other than white blood cells contained in the measurement sample, and counting the white blood cells and nucleated cells other than white blood cells based on the optical information obtained from the cells in the measurement sample prepared from a body fluid sample and white blood cell measuring reagent when the body fluid measurement mode has been set and the measurement starting means has received the measurement start instruction.

[0008] A second aspect of the present invention is a hematological analyzer comprising: a measurement mode setting means for setting a body fluid measurement mode; a measurement starting means for receiving a measurement start instruction; a sample aspirating means for aspirating a body fluid sample; a measurement sample preparing means for preparing a measurement sample using a white blood cell measuring reagent and the body fluid sample aspirated by the sample aspirating means; an optical information obtaining means for irradiating the measurement sample with light and obtaining optical information from cells contained in the measurement sample; and analyzing means for classifying the cells contained in the measurement sample based on the obtained optical information, and counting the classified cells; wherein the measurement sample preparing means prepares the measurement sample from the white blood cell measuring reagent and the body fluid sample aspirated by the sample aspirating means when the body fluid measurement mode has been set by the measurement mode setting means and the measurement start instruction has been received by the measurement starting means, and the analyzing means classifies the cells contained in the measurement sample as white blood cells and nucleated cells other than the white blood cells based on the optical information, and counts the white blood cells and the nucleated cells other than white blood cells.

[0009] A third aspect of the present invention is a method for analyzing body fluid comprising: (a) a step of setting a measurement mode to a body fluid measurement mode; (b) a step of receiving a measurement start instruction after the body fluid measurement mode has been set; (c) a step of irradiating a measurement sample prepared from a white blood cell measuring reagent and a body fluid sample and obtaining optical information from cells contained in the measurement sample after receiving the measurement start instruction; and (d) a step of classifying the cells contained in the measurement sample at least as white blood cells and nucleated cells other than the white blood cells based on the ob-

tained optical information, and counting the number of the white blood cells and the number of the nucleated cells other than white blood cells.

[0010] A fourth aspect of the present invention is a computer program product, comprising: a computer readable medium; and instructions, on the computer readable medium, adapted to enable a general purpose computer to perform operations, comprising (a) a step of setting a measurement mode to a body fluid measurement mode; (b) a step of receiving a measurement start instruction after the body fluid measurement mode has been set; (c) a step of irradiating a measurement sample prepared from a white blood cell measuring reagent and a body fluid sample and obtaining optical information from the cell contained in the measurement sample after the measurement start instruction has been received; and (d) a step of classifying the cells contained in the measurement sample at least as white blood cells and nucleated cells other than white blood cells based on the obtained optical information, and counting the number of the white blood cells and the number of the nucleated cells other than white blood cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

FIG. 1 is an exterior view of a blood cell analyzer of a first embodiment of the present invention;
FIG. 2 is a block diagram of the measuring unit of the analyzer;
FIG. 3 is a block diagram of the fluid supplying unit;
FIG. 4 shows the optical system of the white blood cell detection unit;
FIG. 5 shows the RBC/PLT detection unit;
FIG. 6 shows the HGB detection unit;
FIG. 7 is a flow chart of the sample measuring process;
FIG. 8 shows the display screen for setting the measurement mode;
FIG. 9 is a flow chart showing the pre sequence process;
FIG. 10 is a schematic view of a scattergram derived from measurements of a DIFF measurement sample prepared from body fluid;
FIG. 11 compares measurement results by the blood cell analyzer of the embodiment and measurement results by a reference method;
FIG. 12 is a schematic view of a scattergram derived from measurements of a DIFF measurement sample prepared from blood;
FIG. 13 is a display screen showing the measurement results in the blood measurement mode;
FIG. 14 is a display screen showing the measurement results in the body fluid measurement mode;
FIG. 15 is a display screen showing the measurement results in the body fluid measurement mode;
FIG. 16 is a display screen showing the measurement results in the body fluid measurement mode; and
FIG. 17 is a confirmation screen at the start of the blank check which is displayed in the body fluid measurement mode.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0012] The preferred embodiments of the present invention will be described hereinafter with reference to the drawings.

[0013] FIG. 1 shows a hematological analyzer 1. The hematological analyzer 1 is configured as an automatic multi-item blood cell analyzer which performs blood analysis by measuring blood samples held in sample containers (blood collection tubes), obtaining characteristics information representing the characteristics of the blood cells contained in the sample, and analyzing the characteristic information. The hematological analyzer 1 is also capable of analyzing body fluids. In the blood cell analyzer of the present embodiment, the body fluids used as analysis objects include, fluid within the body cavity other than blood. Specifically, cerebrospinal fluid (spinal fluid, CSF: fluid filling the ventricle or sublemmal cavity), fluid of the thoracic cavity (pleural fluid, PE: fluid collected in pleural cavity), abdominal fluid (fluid collected in the abdominal cavity), fluid of the cardiac sac (fluid collected in the cardiac sac), synovial fluid (fluid present in joints, synovial sac, peritenon) and the like. Among types of body fluid which can be analyzed are dialysate of peritoneal dialysis (CAPD), intraperitoneal rinse and the like. Cells are usually not observed in these body fluids, however, the fluids may contain blood cells, abnormal cells, and cells such as bacteria in the case of disease, tumor of related organs, or injury. For example, it is possible to clinically estimate the following from measurement results in the case of cerebrospinal fluid.

For example, sub-arachnoidal hemorrhage is indicted when there is an increase of red blood cells, meningitis is indicated when there is an increase of neutrophils, infectious disease (parasitic and fungal) is indicated when there is an increase of eosinophils, tuberculous meningitis and viral meningitis are indicated when there is an increase of monocytes, and advanced meningeal tumor is indicated when there is an increase of other cells. ed In the case of abdominal and thoracic fluids, cancers may be indicated when analysis of finds nucleated cells other than blood cells, that is, the fluid contains nucleated cells of mesothelial cells, macrophages, tumor cells and the like.

[0014] The hematological analyzer 1 is provided with a measuring unit 2 which has the function of measuring blood and body fluid samples, and a data processing unit 3 which obtains analysis results by processing the measurement results output from the measurement unit 2. The data processing unit 3 is provided with a control unit 301, a display unit 302, and an input unit 303. Although the

measuring unit 2 and data processing unit 3 are separate devices in FIG. 1, the both may also be integrated in a single apparatus.

[0015] FIG. 2 is a block diagram of the measuring unit 2 of the analyzer 1. As shown in FIG. 2, the measuring unit 2 is provided with a blood cell detecting unit 4, an analog processing unit 5 which processes the output (analog signals) of the detecting unit 4, microcomputer unit 6, display and operating unit 7, and a device 8 for measuring blood and body fluids. The device 8 includes a fluid supplying unit 81 which is described below.

[0016] FIG. 3 is a block diagram showing the structure of the fluid supplying unit 81. As shown in FIG. 3, the fluid supplying unit 81 is provided with a sample aspiration nozzle 18, a plurality of reagent containers, a sampling valve 12, and reactions chambers 13 through 17. The sample aspiration nozzle 18 aspirates sample from a sample container, and delivers the sample to the sampling valve 12. The sampling valve 12 divides the delivered sample into several aliquots of predetermined volume. The number of divisions differs depending on the mode of measurement (discrete mode); in the CBC mode the sample is divided into three aliquots to measure the number of red blood cells, the number of white blood cells, the number of platelets, and the hemoglobin concentration. In addition to the CBC measurement items, the sample is divided into four aliquots in the CBC-DIFF mode so as to also classify five types of white blood cells. Furthermore, In addition to the measurement items of the CBC+DIFF mode, the sample is divided into five aliquots in the CBC+DIFF+RET mode so as to also measure reticulocytes.

[0017] Similarly, in addition to the measurement items of the CBC+DIFF mode, the sample is divided into five aliquots in the CBC+DIFF+NRBC mode so as to also measure nucleated red blood cells. In addition to the measurement items of the CBC+DIFF+RET mode, the sample is divided into six aliquots in the CBC+DIFF+RET+NRBC mode so as to also measure nucleated red blood cells. The above mentioned measurement modes are blood measuring modes which measure whole blood. Finally, the sample is divided into two aliquots in the body fluid measuring mode for measuring body fluid.

[0018] Reagent (dilution solution) is introduced from a reagent container to the sampling valve, and the aliquots of the divided sample are delivered together with the reagent to the reaction chambers 13 through 17 and an HGB detection unit 43, which is described later. a predetermined amount of sample (aliquot) and a predetermined amount of reagent and a predetermined amount of stain collected by the sampling valve 12 are supplied to the reaction chamber 13 by a dosage pump which is not shown in the drawing, the sample and reagent are mixed to prepare a measurement sample for four classifications of white blood cells (DIFF).

[0019] The reagent "stomatolyzer 4DL" made by Sysmex Corporation may be used as the dilution solution. This reagent contains surface active agent and induces hemolysis of red blood cells. The reagent "stomatolyzer 4DS" made by Sysmex Corporation may be used as the stain. This stain contains ethylene glycol, low molecular alcohol, and polymethene colorant; a 50x dilute sample is ultimately prepared by staining the blood cell component after hemolysis by the dilution agent.

[0020] When the body fluid measurement mode has been selected, a measurement sample for the classification of white blood cells is prepared from a fluid sample under the conditions of the amount of the sample and reagent used for the four classifications of white blood cells are identical, the reagents are identical, and the amounts of the reagent are identical. In the white blood cell classification of the body fluid measurement mode, the white blood cells are classified, not in four types, but two types, as shall be described later.

[0021] A predetermined amount of sample collected by the sampling valve 12, a predetermined amount of hemolytic dilution agent, and a predetermined amount of stain solution are supplied to the reaction chamber 14 by a dosage pump which is not shown in the drawing, the sample and reagents are then mixed to prepare a measurement sample for measuring nucleated red blood cells (NRBC).

[0022] A predetermined amount of sample collected by the sampling valve 12, a predetermined amount of dilution agent, and a predetermined amount of stain solution are supplied to the reaction chamber 15 by a dosage pump which is not shown in the drawing, the sample and reagents are then mixed to prepare a measurement sample for measuring reticulocytes (RET).

[0023] A predetermined amount of sample collected by the sampling valve 12, and a predetermined amount of hemolytic dilution agent are supplied to the reaction chamber 16 by a dosage pump which is not shown in the drawing, the sample and reagents are then mixed to prepare a measurement sample for measuring white blood cells and basophils (WBC/BASO).

[0024] A predetermined amount of sample collected by the sampling valve 12, and a predetermined amount of dilution solution are supplied to the reaction chamber 17 by a dosage pump which is not shown in the drawing, the sample and reagents are then mixed to prepare a measurement sample for measuring red blood cells and platelets (RBC/PLT).

[0025] A predetermined amount of sample collected by the sampling valve 12, and a predetermined amount of hemolytic dilution agent are supplied to the HGB detection unit 43 which is described later.

[0026] The detection device 4 is provided with a white blood cell detection unit 41 for detecting white blood cells. The white blood cell detection unit 41 is also used to detect nucleated red blood cells and reticulocytes. In addition to the white blood cell detection unit, the detection device 4 is also provided with an RBC/PLT detection unit 42 for measuring the number of red blood cells and the number of platelets, and an HGB detection unit 43 for

measuring the amount of pigment in the blood.

[0027] The white blood cell detection unit 41 is configured as an optical detection unit, specifically, a detection unit which uses a flow cytometric method. Cytometry measures the optical properties and physical properties of cells and other biological particles, and flow cytometry measures these particles as they pass by in a narrow flow. FIG. 4 shows the optical system of the white blood cell detection unit 41. In the same drawing, the beam emitted from a laser diode 401 irradiates, via a collimator lens 402, the blood cells passing through the interior of a sheath flow cell 403. The intensity of the front scattered light, the intensity of the side scattered light, and the intensity of the side fluorescent light from the blood cells within the sheath flow cell irradiated by the light are detected by the white blood cell detection unit 41.

[0028] The scattered light is a phenomenon due to the change in the direction of travel of the light caused by particles such as blood cells and the like which are present as obstructions in the direction of travel of the light. Information on the characteristics of the particles related to the size and composition of the particles can be obtained by detecting this scattered light. The front scattered light emerges from the particles in approximately the same direction as the direction of travel of the irradiating light. Characteristic information related to the size of the particle (blood cell) can be obtained from the front scattered light. The side scattered light emerges from the particle in an approximate perpendicular direction relative to the direction of travel of the irradiating light. Characteristic information related to the interior of the particle can be obtained from the side scattered light. When a particle is irradiated by laser light, the side scattered light intensity is dependent on the complexity (that is, nucleus shape, size, density, and granularity) of the interior of the cell. therefore, the blood cells can be classified (discriminated) and the number of cells can be counted by using the characteristics of the side scattered light intensity. Although the front scattered light and side scattered light are described as the scattered light used in the present embodiment, the present invention is not limited to this configuration inasmuch as scattered light of any angle may also be used relative to the optical axis of the light emitted from a light source that passes through the sheath flow cell insofar as scattered light signals are obtained which represent the characteristics of the particles necessary for analysis.

[0029] When fluorescent material such as a stained blood cell is irradiated by light, light is given off by the particle at a wavelength which is longer than the wavelength of the irradiating light. The intensity of the fluorescent light is increased by the stain, and characteristics information can be obtained relating to the degree of staining of the blood cell by measuring the fluorescent light intensity. The classification and other measurements of the white blood cells can then be performed by the difference in the (side) fluorescent light intensity.

[0030] As shown in FIG. 4, the front scattered light from the blood cell (white blood cells and nucleated red blood cells) which pass through the sheath flow cell 403 is received by a photodiode (front scattered light receiving unit) 406 through a collective lens 404 and pinhole 405. The side scattered light is received by a photo multiplexer (side scattered light receiving unit) 411 through a collective lens 407, dichroic mirror 408, optical filter 409, and pinhole 410. The side fluorescent light is received by a photo multiplexer (side fluorescent light receiving unit) 412 through the collective lens 407 and dichroic mirror 408. The photoreception signals output from the light receiving units 406, 411, and 412 are subjected to analog processing such as amplification and waveform processing and the like by an analog processing unit 5 which is configured by amps 51, 52, 53 and the like, and the analog-processed photoreception signals are provided to the microcomputer 6.

[0031] The configuration of the RBC/PLT detection unit 42 is described below. FIG. 5 is a schematic view briefly showing the structure of the RBC/PLT detection unit 42. The RBC/PLT detection unit 42 is capable of measuring the numbers of red blood cells and platelets by a sheath flow-DC detection method. The RBC/PLT detection unit 42 has a sheath flow cell 42a as shown in FIG. 5. The sheath flow cell 42a is provided with a sample nozzle 42b which is open toward the top so that sample can be supplied from the reaction chamber 17 to the sample nozzle 42b. The sheath flow cell 42a has a tapered chamber 42c which narrows toward the top, and the sample nozzle 42b is disposed in the center part within the chamber 42c. An aperture 42d is provided at the top end of the chamber 42c, and this aperture 42d is aligned with the center position of the sample nozzle 42b. Measurement sample supplied from the sample supplying unit is sent upward from the tip of the sample nozzle 42b, and front sheath fluid is simultaneously supplied to the chamber 42c and flows upward toward the aperture 42d. The flow of the measurement sample, which is encapsulated in the front sheath fluid, is narrowly constricted by the tapered chamber 42c and the blood cells within the measurement sample pass one by one through the aperture 42d. Electrodes are provided at the aperture 42d, and a direct current is supplied between these electrodes. The change in the resistance of the direct current is detected at the aperture 42d when the measurement sample flows through the aperture 42d, and the electrical signal of the change in resistance is output to the controller 25. Since the resistance of the direct current increases when blood cells pass through the aperture 42d, the electrical signals reflect information of the passage of the blood cells through the aperture 42d so that the numbers of red blood cells and platelets can be counted by subjecting these electrical signals to signal processing.

[0032] A recovery tube 42e, which extends vertically, is provided above the aperture 42d. The recovery tube 42e is disposed within a chamber 42f which is connected to the chamber 42c through the aperture 42d. The inner wall of the chamber 42f is separated from the bottom end

of the recovery tube 42e. The chamber 42f is configured to supply a back sheath, and this back sheath flows downward through the chamber 42f in a region outside the recovery tube 42e. The back sheath which flows outside the recovery tube 42e arrives at the bottom part of the chamber 42f, and thereafter flows between the inner wall of the chamber 42f and the bottom end of the recovery tube 42e so as to flow into the interior of the recovery tube 42e. The blood cells which has passed through the aperture 42d are therefore prevented from refluxing, thus preventing erroneous detection of the blood cells.

[0033] The configuration of the HGB detection unit 43 is described below. The HGB detection unit 43 is capable of measuring the amount of hemoglobin (HGB) by an SLS hemoglobin method. FIG. 6 is a perspective view of the structure of the HGB detection unit 43. The HGB detection unit 43 has a cell 43a for accommodating a diluted sample, a light-emitting diode 43b for emitting light toward the cell 43a, and a photoreceptor element 43c for receiving the transmission light that has passed through the cell 43a. A fixed amount of blood is diluted with dilution fluid and a predetermined hemolytic agent at a predetermined dilution ratio by the sampling valve 12 to prepare a dilute sample. The hemolytic agent has properties which transform the hemoglobin in the blood to SLS-hemoglobin. The dilute sample is supplied to the cell 43a and accommodated therein. In this condition, the light-emitting diode 43b emits light that passes through the cell 43a and is received by the photoreceptor element 43c which is disposed opposite the light-emitting diode 43b with the cell 43a interposed therebetween. Since the light-emitting diode 43b emits light having a wavelength that is highly absorbed by the SLS-hemoglobin, and the cell 43a is configured of plastic material which has a high light transmittancy, the photoreceptor element 43c only receives the transmission light absorbed by the dilute sample of the light emitted from the light-emitting diode 43b. The photoreceptor element 43c outputs electrical signals which correspond to the amount of received light (optical density) to the microcomputer 6, and the microcomputer 6 compares the optical density with the optical density of the dilution solution which was measured previously, then calculates the hemoglobin value.

[0034] The microcomputer 6 is provided with an A/D converter 61 for converting the analog signals received from the analog processing unit 5 to digital signals. The output of the A/D converter 61 is sent to a calculation unit 62 of the microcomputer 6, and calculations are performed for predetermined processing of the photoreception signals in the calculation unit 62. The calculation unit 62 prepares distribution data (two-dimensional scattergrams (unclassified) and unidimensional histograms) based on the output of the detection device 4.

[0035] The microcomputer 6 is provided with a controller 63 configured by a memory for the control processor and the operation of the control processor, and a data analyzing unit 64 configured by a memory for the analysis processor and the operation of the analysis processor. The controller 63 controls the device 8 configured by a sampler (not shown in the drawing) for automatically supplying blood collection tubes, and a fluid system and the like for preparing and measuring samples, as well as performing other controls. The data analyzing unit 64 executes analysis processing such as clustering and the like on the distribution data. The analysis results are sent to an external data processing device 3 through an interface 65, and the data processing device 3 processes the data for screen display, storage and the like.

[0036] The microcomputer 6 is further provided with an interface 66 which is interposed between the microcomputer 6 and the display and operating unit 7, and an interface 67 which is interposed between the microcomputer 6 and the device 8. The calculation unit 62, controller 63, and interfaces 66 and 67 are connected through a bus 68, and the controller 63 and the data analyzing unit 64 are connected through a bus 69. The display and operating unit 7 includes a start switch by which the operator specifies to start a measurement, and a touch panel type liquid crystal display for displaying various types of setting values and analysis results, and receiving input from the operator.

[0037] The operation of the hematological analyzer 1 of the present embodiment is described below. FIG. 7 is a flow chart showing the flow of the operation of the hematological analyzer of the present embodiment. The hematological analyzer 1 starts when a user turns on the power source of the hematological analyzer 1 (step S1). The hematological analyzer 1 first executes a self check during startup (step S2). In the self check, the microcomputer 6 tests and checks the operation of all operating device of the hematological analyzer 1, and performs a blank check operation which measures a blank sample that does not contain a real sample. Next, the microcomputer 6 sets an initial measurement mode (step S3). The CBC+DIFF mode is the initial setting. Specifically, in the process of step S3, parameters (operating conditions) for performing blood measurements are set, for example, which reaction chamber to use and the set time for the measurement. The blood measurement mode is thus set as the initial operating mode in the hematological analyzer 1 of the present embodiment. The hematological analyzer 1 therefore remains in a standby state waiting to receive a measurement start instruction. The microcomputer 6 displays a screen on the liquid crystal display which alerts the operator to the standby state (step S4).

[0038] In the standby state, the operator can change the measurement mode by operating the display and operation unit 7. FIG. 8 is a schematic view of an input screen for setting the measurement mode. This screen is provided with discrete display regions including the sample number 120, type of sample uptake mode 121, type of discrete test (measurement mode) 122, and type of sample 123. The three sample uptake modes include a manual mode for aspirating a sample after the operator has manually inserted a sample container in the sample aspiration nozzle 18, a capillary mode for aspirating a

measurement sample via the sample aspiration nozzle 18 after the operator has previously prepared the measurement sample by mixing a sample and reagent, and a closed mode for supplying a sample by automatically transporting a sample container using a conveyer device. The types of samples include NORMAL, which are normal blood samples; HPC, which are hematopoietic progenitor cell samples; and BODY FLUID, which are other fluids of the body. The operator can specify the sample take-up mode, measurement mode, and type of sample. When the blood measurement mode has been specified, the NORMAL sample type is specified, and an optional sample take-up mode and measurement mode are specified. When specifying the BODY FLUID measurement mode, the operator specifies MANUAL mode as the take-up mode, [CBC+DIFF], [CBC+DIFF+RET], [CBC+DIFF+NRBC], or [CBC+DIFFNRBC+RET] as the DISCRETE test, and [BODY FLUID] as the type of sample. In step S4, the operator specifies the desired mode. The operator presses the start switch to start the measurement when blood measurement is performed without changing the initially set measurement mode(step S5: N). The microcomputer 6 receives the instruction to start the measurement (step S6), and the blood sample is aspirated by the sample aspiration nozzle (step S7).

[0039] After the blood sample has been aspirated, the sample is introduced to the previously mentioned sampling valve 18, and the necessary sample preparation is performed for the measurement according to the type discrete test of the measurement mode(step S14). The measurement operation is then executed for this measurement sample (step S16). When [7] is set as the type of discrete test, for example, HGB, WBC/BASO, DIFF, RET, NRBC, and RBC/PLT measurement samples are prepared. Thereafter, the WBC/BASO, DIFF, RET, and NRBC measurement samples are measured by the white blood cell detection unit 41, the RBC/PLT measurement sample is measured by the RBC/PLT detection unit 42, and the HGB measurement sample is measured by the HGB detection unit 43. At this time, the WBC/BASO, DIFF, RET, and NRBC measurement samples are introduced to the white blood cell detection unit 41 in the order NRBC, WBC/BASO, DIFF, RET and sequentially measured since only a single white blood cell detection unit 41 is provided. In this measurement operation, the calculation unit 62 creates particle distribution maps (scattergram, histogram). The scattergram created from the optical information obtained by the DIFF measurement is described below. The calculation unit 62 generates a two-dimensional scattergram (particle distribution map) using, as characteristic parameters, the side scattered light and side fluorescent light among the photoreception signals output from the white blood cell detection unit 41 in the DIFF measurement. This scattergram (referred to as "DIFF scattergram" hereinafter) plots the side scattered light intensity on the X axis and the side fluorescent light on the Y axis; red blood cell ghost clusters, lymphocyte clusters, monocyte clusters, neutrophil+basophil clusters, and eosinophil clusters normally appear. These clusters are recognized by processing performed on the DIFF scattergram by the data analyzing unit 64.

[0040] Analysis processing is then performed based on the particle distribution maps obtained by the measurement(step S18). In the analysis processing, the data analyzing unit 64 of the microcomputer 6 classifies the four white blood cell clusters (lymphocyte cluster, monocyte cluster, neutrophil+basophil cluster, and eosinophil cluster), and the red blood cell ghost cluster as shown in FIG. 12 from the DIFF scattergram prepared by the calculation unit 62 when the DIFF measurement samples were measured by the white blood cell detection unit 41. In the analysis process of the present embodiment, each particle plotted on the scattergram and the degree of attribution of particles to each cluster at a distance from the center of gravity of each cluster is obtained. Then, each particle is attributed to a cluster according to the degree of attribution. The particle classification method is disclosed in detail in US Patent No. 5,555,196. The basophil cluster, and white blood cell clusters other than basophils, and the red blood cell ghost cluster are classified on the scattergram obtained by the WBC/BASO measurement. White blood cells are classified in five groups based on the results of the four classifications and numbers of white blood cells (refer to FIG. 12) by the analysis processing of the DIFF scattergram, and the results of the two classification and numbers of white blood cells by the analysis processing of the WBC/BASO scattergram. Specifically, the data analysis unit 64 subtracts the basophil cell count obtained by the analyzing the WBC/BASO scattergram from the neutrophil+basophil cell count obtained by analyzing the DIFF scattergram, to obtain the neutrophil cell count and the basophil cell count. Thus, five classifications of white blood cells are obtained as well as the number of blood cells in each classification. In addition, the trough is detected in the curve in the unidimensional histogram created based on the characteristic information from the detection unit 42, and the particles are classified as red blood cells and platelets in the RBC/PLT measurement. The analysis results thus obtained are output to the display unit 302 of the data processing unit 3 (step S20).

[0041] When input specifying the measurement mode is received as described above in step S5, the microcomputer 6 sets the parameters (operating conditions) for the body fluid measurement, for example, the reaction chamber to use and the set time of the measurement and the like (step S8). In the present embodiment, the measurement time is three times the time for blood measurement, as will be described later.

[0042] The measuring unit 2 starts the pre sequence (step S10) when the measurement mode has been switched from the previous measurement mode (in this instance, the blood measurement mode) to the body fluid measurement mode (step S9). The pre sequence is a process of preparing for the body fluid measurement. Since samples which have a low concentration of blood

cell component are measured in the body fluid measurement, the setting is switched from the blood measurement mode ([1:NORMAL] is displayed in FIG. 8) to the body fluid measurement mode, and the lack of background influence is confirmed in the body fluid measurement results.

[0043] The pre sequence includes a blank check operation. The blank check determination standard of the pre sequence is set at a fraction and is more strict than the determination standard of the blank check (for example, the blank check performed after power on and automatic wash) performed in the blood measurement mode. When the setting is changed from the body fluid measurement mode to the blood measurement mode, this pre sequence is not performed since there is no background influence (carry over effect) on the normal blood measurement results. Furthermore, when body fluid samples are measured in a repeated body fluid measurement mode, this pre sequence is not performed since there is normally no background influence. There is concern, however, that the next sample measurement may be affected when the body fluid sample analysis results exceed a predetermined value due to an extremely high number of particles in the body fluid since the measurement results are high, and therefore the operator is alerted of this concern that the analysis results of the next sample may be affected. Then, the blank check measurement is performed. A configuration is desirable in which a message "please press VERIFY" is output to the screen, and the blank check is performed when the operator presses the VERIFY button. In this case, a configuration is possible in which a CANCEL button may be provided on the screen to transition to the standby screen without performing a blank check when the operator presses the CANCEL button. It is also desirable that a flag indicate the low reliability of the measurement results when a blank check is not performed. Wasted reagent and time can thus be avoided by performing an additional blank check only when needed.

[0044] FIG. 9 is a flow chart showing the sequence of the pre sequence process performed when the measurement mode is changed from the blood measurement mode to the body fluid measurement mode. The hematological analyzer 1 performs the pre sequence by measuring a blank sample using the measuring unit 2 (step S31), comparing the measurement result with predetermined tolerance values and determining whether or not the measurement results are less than the tolerance values using the microcomputer 6 (step S32). When the measurement results are less than the tolerance values, the microcomputer 6 ends the pre sequence and the process returns. When the measurement results are not less than the tolerance value, the microcomputer 6 determines whether or not the blank check was executed the set number of times (for example, three times) (step S33), and when the number of executions of the blank check is less than a predetermined number, the process returns to step S31 and the blank check is performed again for the predetermined number of times. When the measurement results of the blank check performed a predetermined number of times are not less than the tolerance values, a screen is displayed with includes a VERIFY button, BLANK CHECK button, and AUTOMATIC WASH button and the blank check measurement results are displayed on the display and operation unit 7 (step S34). When the operator has pressed the VERIFY button (step S35), the microcomputer 6 ends the pre sequence and the process returns. When the BLANK CHECK button has been pressed (step S36), the process returns to step S31 and the blank check is performed again; when the AUOMATIC WASH button has been pressed (step S37), automatic washing is performed using a special washing solution (strep S38), and thereafter the process returns to step S31 and the blank check is performed again.

[0045] When the pre sequence ends as described above, the hematological analyzer 1 enters the standby state (step S11). When the operator presses the start switch and starts the body fluid measurement, the sample aspiration nozzle 18 of the measuring unit 2 is immersed in the sample container in the same manner as for the manual measurement of the blood sample. When the instruction to start measurement is received by the microcomputer 6 (step S12), the body fluid aspiration begins (step S13).

[0046] After the body fluid sample has been aspirated, the body fluid sample is introduced to the sampling valve 91 in the same manner as the blood sample. Then, the RBC/PLT measurement sample is prepared by the reaction chamber 13 (step S15). Subsequently, the DIFF measurement sample is measured by the white blood cell detection unit 41, and the RBC/PLT measurement sample is measured by the RBC/PLT detection unit 42 (step S17). Since only the DIFF measurement sample is measured by the white blood cell detection unit 41 in the body fluid measurement mode, the measurement is completed in a shorter time than the blood measurement even though the measurement time is longer than the measurement time in the blood measurement mode. the analysis accuracy of the low particle concentration body fluid sample can therefore be improved by increasing the measurement time of the body fluid measurement to be longer than the measurement time of the blood measurement. Although the measurement accuracy can be improved due to the increased number of particles counted by lengthening the measurement time, a two to six fold increase in the measurement time is suitable because the sample processing ability is reduced when the measurement time is excessively long, and there is a limit to the performance of the syringe pump which delivers the measurement sample to the white blood cell detection unit 41. In the present embodiment, the measurement time in the body fluid measurement mode is set at three times the measurement time of the blood measurement mode.

[0047] The RBC/PLT measurement sample is intro-

duced to the electrical resistance detection unit 41 in the same manner for all measurement modes, and measurement is performed under a fixed flow speed condition. The analysis processing is performed thereafter based on the characteristic information obtained by the measurements (step S19), and the analysis results are output to the display unit 302 of the data processing unit 3 (step S21). In the analysis processing of the blood measurement mode, the DIFF scattergram and the like are analyzed, and information is calculated for five types of white blood cell subclasses (NEUT: neutrophil, LYMPH: lymphocyte, MONO: monocyte, EO: eosinophil, and BASO: basophil), whereas in the analysis processing of the body fluid measurement mode, two subclasses (MN: mononuclear cell, PMN: polymorphonuclear cell) are classified in a partially integrated form because there are a lesser number of blood cells and these cells are sometimes damaged. The lymphocytes and monocytes belong to mononuclear cells, and neutrophils, eosinophils, and basophils belong to polymorphonuclear cells. Since the classification algorithm is the same as the algorithm described for the analysis processing in the blood measurement mode, further description is omitted.

[0048] Next, the analysis results obtained in step S19 are compared to the tolerance value (predetermined threshold value) (step S22). The tolerance value is the same value as the tolerance value used in the blank check of the pre sequence performed in step S10. When the analysis result is greater than the tolerance value (step S22: Y), the verification screen 151 at the start of the blank check is displayed, as shown in FIG. 17. A message is displayed on the verification screen 151 indicating there is concern that the measurement of the next sample may be influenced due to the high measurement result. Then, the blank check measurement is performed. A message display area 152 for displaying the message "please press the VERIFY button", a VERIFY button 153, and a CANCEL button 154 are displayed. Next, determinations are made as to whether or not the user has pressed the VERIFY button 153 or the CANCEL button 154 (step S24), and the blank check is executed when the VERIFY button has been pressed (VERIFY in step S24) (step S25). The process returns to step S5 without performing the blank check when the analysis result obtained in step S19 is less than the tolerance value (step S22: N), and the when the CANCEL button has been pressed (CANCEL in step S24).

[0049] Anomalous particles (macrophages, mesothelial cells, tumor cells and the like) other than blood cells may be present in the body fluid sample. Although it is rare for such anomalous cells to be present in cerebrospinal fluid, such cells appear comparatively frequently in abdominal and thoracic fluids. The influence of these anomalous particles must be eliminated in order to obtain a high precision classification of blood cells within the body fluid regardless of the type of body fluid. White blood cells in body fluid can be measured with greater precision based on the new knowledge than anomalous particles appear in the top part of the DIFF scattergram produced by this blood cell analyzer of the present invention. This aspect was not considered in the previously mentioned conventional art.

[0050] FIG. 10 is a schematic view of a scattergram obtained by measuring and analyzing a DIFF measurement sample prepared from body fluid and white blood cell measurement reagent in the body fluid measurement mode of the blood cell analyzer 1 of the present embodiment. The vertical axis of the scattergram represents the side fluorescent light intensity (the fluorescent light intensity at the top is greatest), and the horizontal axis represents the side scattered light intensity (the scattered light intensity at the right side is greatest). A red blood cell ghost Gc caused by hemolysis is distributed in the region LF in which the fluorescent light intensity is weakest in the scattergram, anomalous particles such as mesothelial cells and the like is distributed in the region HF in which the fluorescent light intensity is greatest, and mononuclear white blood cells Mc and polynuclear white blood cells Pc are distributed in the intermediate region MF. In the analysis of the scattergram, the particle component distributed in the region MF is analyzed as white blood cells after excluding region LF and region HF, and the particles are classified and counted in two groups. Lymphocytes and monocytes are included in the mononuclear white blood cells Mc, and neutrophils, basophils, and eosinophils are included in the polynuclear white blood cells Pc.

[0051] Since fewer and damaged blood cells are contained in body fluid, white blood cells are classified and counted as mononuclear white blood cells and polynuclear white blood cells when analyzing white blood cells in body fluid.

[0052] Anomalous particles (nucleated cells such as tumor cells, macrophages, mesothelial cells) other than blood cells may also be present in body fluid. Although it is rare for such anomalous cells to be present in cerebrospinal fluid, such cells appear comparatively frequently in abdominal and thoracic fluids. In the scattergram of FIG. 10, such nucleated cells other than white blood cells are distributed in region HF. In the present embodiment, it is possible to determine accurate white blood cells counts even in body fluid which contains such nucleated cells other than white blood cells since nucleated cells other than white blood cells can be identified. The degree of occurrence of anomalous cells can be determined by counting the cells which appear in region HF. In the present embodiment, cells are demarcated in the regions LF, MF, and HF by threshold values for demarcating each region; these threshold values may also be changed manually.

[0053] FIG. 11 compares the analysis results of the blood cell analyzer 1 of the present embodiment and the count results of a reference method to show the validity of the scattergram analysis method described above. The sample material is thoracic fluid; in the drawing, "this method" refers to the white blood cell count (WBC) and

anomalous particle count (Others) calculated by the blood cell analyzer 1 of the present embodiment, and "Ref" refers to the calculation result by the reference methods (Fuchs Rosenthal calculation method and site-spin method). Examples 1, 2, and 3 are the results of analysis of thoracic fluid in which anomalous particles were plentiful, and the correlation between the reference methods and the analysis results of the blood cell analyzer 1 of the present invention can be readily understood.

[0054] FIG. 13 shows a screen 200 which is displayed on the display unit 302 of the data processing unit 3, showing the analysis results of the DIFF measurement sample prepared from blood. A sample number display region which displays a sample number 101 is provided at the top of the screen 200, and an attribute display region which displays patient attributes is provided adjacently. The attribute display region specifically includes a patient ID, patient name, date of birth, sex, hospital department/ward, attending physician, date of measurement, time of measurement, comments and the like. A measurement result display region which displays the measurement results is provided at the bottom of the attribute display region. The measurement result display region includes several pages, and these pages can be displayed by selecting a plurality of tabs 102. Tabs have a plurality of arrangements matching the main screen, graph screen, and measurement items. FIG. 12 is a screen which is displayed when the graph screen tab has been selected. A graph display region 104 for displaying graphs and a measurement value display region 103 for displaying the measurement result values are provided in the left half of the measurement value display region , and a distribution map display region for displaying the measurement result distribution map 105 is provided in the right half. WBC, RBC, ..., NEUT#,..., BASO#,..., NEUT#,..., BASO% and the like, data, and units are displayed in the measurement value display region, and flagging results representing sample anomalies and disease suspicions which are clinically useful information relating to WBC, PLT, RBC or RET are displayed in the flag display region 104.

[0055] Six distribution maps are displayed in the distribution map display region 105. The scattergram on the upper left side is a DIFF scattergram. The WBC/BASO scattergram is shown at the top right, the immature cell (IMI) scattergram is shown at mid left, and the RET scattergram is shown at mid right. The RBC scattergram is shown at the bottom left, and the PLT scattergram is shown at the bottom right.

[0056] FIG. 14 shows a screen 110 displayed in the display area 302 of the data processing unit 3 as the measurement results of the DIFF measurement sample prepared from body fluid. A sample number display region 111 for displaying a sample number is provided at the top of the screen 110, and a patient attribute display region is provided adjacently. An [F], which indicates measurement has been conducted in the body fluid measurement mode, is displayed at the left end of the sample number display region 111. Thus, it can be clearly recognized that the analysis results are for body fluid measurement results. The measurement result display region includes a plurality of pages which are selectable by tab 112. In this example, the tab for body fluid measurement is selected.

[0057] The measurement value display region 113 includes the name of the measurement items for body fluid measurement rather than the measurement results of the blood measurement mode; WBC-BF (WBC count), RBC-BF (RBC count), MN# (mononuclear cell count (lymphocytes + monocytes)), PMN# (polymorphonuclear cell count (neutrophils + basophils + eosinophils)), MN% (ratio of mononuclear cells among white blood cells), PMN% (ratio of polymorphonuclear cells among white blood cells), measurement values, and units are associated and displayed. A flag display region 114 is provided in the body fluid measurement similar to the blood measurement. Two distribution maps 115 are displayed in the distribution map display region, and the top scattergram is a DIFF scattergram. The bottom scattergram is an RBC scattergram.

[0058] FIG. 15 shows an example in which the Research BF tab 112 is selected in the screen 110 of FIG. 14. This screen displays the same items as screen 110 with the exception that a research parameter display region 116 is also displayed. The research parameter display region 116 displays number of particles in region HF [HF-BF#], the ratio of the number of particles in the region HF relative to the number of particles in the region including both region HF and region MF [HF-BF%], and the number of particles in the region including both region HF and region MF [TC-BF#] in FIG. 10. [HF-BF%] is the percentage of HF-BF relative to TC-BF.

[0059] FIG. 16 shows a screen 120 showing a list of stored samples which is displayed on the display unit 302 of the data processing unit 3. Reference number 130 refers to a patient attribute display region. Provided above this region is a measurement result display region which displays the measurement result selected by a tab. A row 131 on the left end of the measurement result display region is used to indicate whether the validation operation has been performed or not for the measurement result. A "V" symbol indicates validation has been performed. A row 132 on the right indicates the measurement mode. An "F" symbol indicates the measurement results are for the body fluid mode. Although there are high value samples that require blank checking in the body fluid mode, and inverted "F" symbol can be displayed to indicate the blank check has not been performed (that is, CANCEL was selected in step S24).

[0060] Although the structure and functions of the blood cell analyzer of the present invention have been described as being pre-established in the blood cell analyzer, the same functions may be realized by a computer program so that the functions of the present invention can be realized in a conventional blood cell analyzer by installing the computer program in a conventional blood

cell analyzer.

[0061] Although the amount of sample, type of reagent, and amount of reagent are the same when preparing measurement samples for the white blood cell classification measurement in the blood measurement mode and the white blood cell classification measurement in the body fluid measurement mode in the present embodiment, the present invention is not limited to this configuration inasmuch as the amount of sample and the amount of reagent used to prepare a measurement sample for white blood cell classification in the body fluid measurement mode may be greater than the amount of sample and the amount of reagent used to prepare a measurement sample for white blood cell classification in the blood measurement mode. Since the measurement time is greater and the amount of measurement sample needed for measurement is greater for white blood cell classification in the body fluid measurement mode than in the blood measurement mode, it is thereby possible to prepare suitable amounts of measurement sample for white blood cell classification in the blood measurement mode and for white blood cell classification in the body fluid measurement mode. Moreover, the type of reagent used fro white blood cell classification in the blood measurement mode may differ from the type of reagent used for white blood cell classification in the body fluid measurement mode.

[0062] Although white blood cell classification is performed in the body fluid measurement mode using scattered light and fluorescent light in the present embodiment, the present invention is not limited to this configuration inasmuch as white blood cell classification may also be performed in the body fluid measurement mode using, for example, scattered light and absorbed light. The measurement of absorbed light may be accomplished by preparing a measurement sample by mixing a staining reagent to stain the white blood cells, and other reagent together with the sample, supplying this measurement sample to a flow cell to form a sample flow within the flow cell, irradiating this sample flow with light, and receiving the light emitted from the sample flow via a photoreceptor element such as a photodiode or the like. The light is absorbed by the white blood cells when the white blood cells pass through the flow cell, and the degree of that absorption can be grasped as the amount of light received by the photoreceptor element. Such measurement of absorbed light is disclosed in US Patent Nos. 5,122,453, and 5,138,181. furthermore, electrical resistance may be measured rather than scattered light, in which case white blood cells can be classified by the electrical resistance and absorbed light.

Claims

1. A hematological analyzer for measuring blood, comprising:

   a measurement mode setting means for setting a body fluid measurement mode;

   a measurement starting means for receiving a measurement start instruction;

   an optical information obtaining means for irradiating a measurement sample with light and obtaining optical information from cells contained in the measurement sample; and

   an analyzing means for classifying at least white blood cells and nucleated cells other than white blood cells contained in the measurement sample, and counting the white blood cells and nucleated cells other than white blood cells based on the optical information obtained from the cells in the measurement sample prepared from a body fluid sample and white blood cell measuring reagent when the body fluid measurement mode has been set and the measurement starting means has received the measurement start instruction.

2. The hematological analyzer of claim 1, wherein the analyzing means classifies the white blood cells as polynuclear white blood cells and mononuclear white blood cells, and counts the respective numbers of the polynuclear white blood cells and the mononuclear white blood cells.

3. The hematological analyzer of claim 2, wherein the analyzing means determines ratio of the polynuclear white blood cells among the white blood cells, or ratio of the mononuclear white blood cells among the white blood cells.

4. The hematological analyzer of any one among claims 1 through 3, wherein the analyzing means determines total number of nucleated cells from the number of white blood cells and the number of nucleated cells other than the white blood cells, and determines ratio of the nucleated cells other than the white blood cells relative to the total number of nucleated cells.

5. The hematological analyzer of any one among claims 1 through 4, wherein the analyzing means further classifies cells contained in the measurement sample as red blood cell ghosts.

6. The hematological analyzer of any one among claims 1 through 5, wherein the analyzing means classifies the white blood cells contained in the measurement sample into a plurality of subclasses based on the optical information obtained from the cells in the measurement sample prepared from a blood sample and the white blood cell measuring reagent when the body fluid measurement mode has not been set and the measurement starting means has received the measurement start instruction.

7. The hematological analyzer of any one among claims 1 through 6, further comprising an output means for outputting a display screen for displaying an analysis result of the analyzing means.

8. The hematological analyzer of any one among claims 1 through 7, wherein the optical information is information selected from a group consisting of scattered light information from cells, fluorescent light information from cells, light absorption information from cells, and combinations thereof.

9. The hematological analyzer of any among claims 1 through 8, wherein the body fluid sample is selected from a group consisting of cerebrospinal fluid, thoracic fluid, abdominal fluid, fluid of the cardiac sac, synovial fluid, dialysate from peritoneal dialysis, and intraperitoneal rinse.

10. The hematological analyzer of any among claims 1 through 9, wherein the nucleated cells are selected from a group consisting of macrophages, mesothelial cells, tumor cells, red blood cell ghosts, and combinations thereof.

11. A hematological analyzer comprising:

a measurement mode setting means for setting a body fluid measurement mode;
a measurement starting means for receiving a measurement start instruction;
a sample aspirating means for aspirating a body fluid sample;
a measurement sample preparing means for preparing a measurement sample using a white blood cell measuring reagent and the body fluid sample aspirated by the sample aspirating means;
an optical information obtaining means for irradiating the measurement sample with light and obtaining optical information from cells contained in the measurement sample; and
analyzing means for classifying the cells contained in the measurement sample based on the obtained optical information, and counting the classified cells;

wherein the measurement sample preparing means prepares the measurement sample from the white blood cell measuring reagent and the body fluid sample aspirated by the sample aspirating means when the body fluid measurement mode has been set by the measurement mode setting means and the measurement start instruction has been received by the measurement starting means, and the analyzing means classifies the cells contained in the measurement sample as white blood cells and nucleated cells other than the white blood cells based on the optical information, and counts the white blood cells and the nucleated cells other than white blood cells.

12. The hematological analyzer of claim 11, wherein the measurement sample preparing means prepares the measurement sample from the white blood cell measuring reagent and a blood sample aspirated by the sample aspirating means when the body fluid measurement mode has not been set and the measurement start instruction has been received by the measurement starting means, and the analyzing means classifies the white blood cells contained in the measurement sample into a plurality of subclasses based on the optical information.

13. A method for analyzing body fluid comprising:

(a) a step of setting a measurement mode to a body fluid measurement mode;
(b) a step of receiving a measurement start instruction after the body fluid measurement mode has been set;
(c) a step of irradiating a measurement sample prepared from a white blood cell measuring reagent and a body fluid sample and obtaining optical information from cells contained in the measurement sample after receiving the measurement start instruction; and
(d) a step of classifying the cells contained in the measurement sample at least as white blood cells and nucleated cells other than the white blood cells based on the obtained optical information, and counting the number of the white blood cells and the number of the nucleated cells other than white blood cells.

14. The method for analyzing body fluid of claim 13, further comprising:

(e) a step of aspirating the body fluid sample and preparing the measurement sample from the white blood cell measuring reagent and the aspirated body fluid sample after the measurement start instruction has been received.

15. The method for analyzing body fluid of claim 13 or 14, wherein step (d) includes a step of classifying white blood cells as polynuclear white blood cells and mononuclear white blood cells, and counting the respective numbers of the polynuclear white blood cells and the mononuclear white blood cells.

16. The method for analyzing body fluid of claim 15, wherein step (d) includes a step of determining ratio of the polynuclear white blood cells among white blood cells, or determining ratio of the mononuclear white blood cells among the white blood cells.

17. A computer program product, comprising:

a computer readable medium; and
instructions, on the computer readable medium, adapted to enable a general purpose computer to perform operations, comprising

(a) a step of setting a measurement mode to a body fluid measurement mode;
(b) a step of receiving a measurement start instruction after the body fluid measurement mode has been set;
(c) a step of irradiating a measurement sample prepared from a white blood cell measuring reagent and a body fluid sample and obtaining optical information from the cell contained in the measurement sample after the measurement start instruction has been received; and
(d) a step of classifying the cells contained in the measurement sample at least as white blood cells and nucleated cells other than white blood cells based on the obtained optical information, and counting the number of the white blood cells and the number of the nucleated cells other than white blood cells.

18. The computer program product of claim 17, wherein step (d) includes a step of classifying the white blood cells as polynuclear white blood cells and mononuclear white blood cells, and counting the respective numbers of the polynuclear white blood cells and the mononuclear white blood cells.

19. The computer program product of claim 18, wherein step (d) includes a step of determining ratio of the polynuclear white blood cells among the white blood cells, or determining ratio of the mononuclear white blood cells among the white blood cells.

20. The computer program product of any one among claims 17 through 19, wherein step (d) includes a further step of classifying cells contained in the measurement sample as red blood cell ghosts.

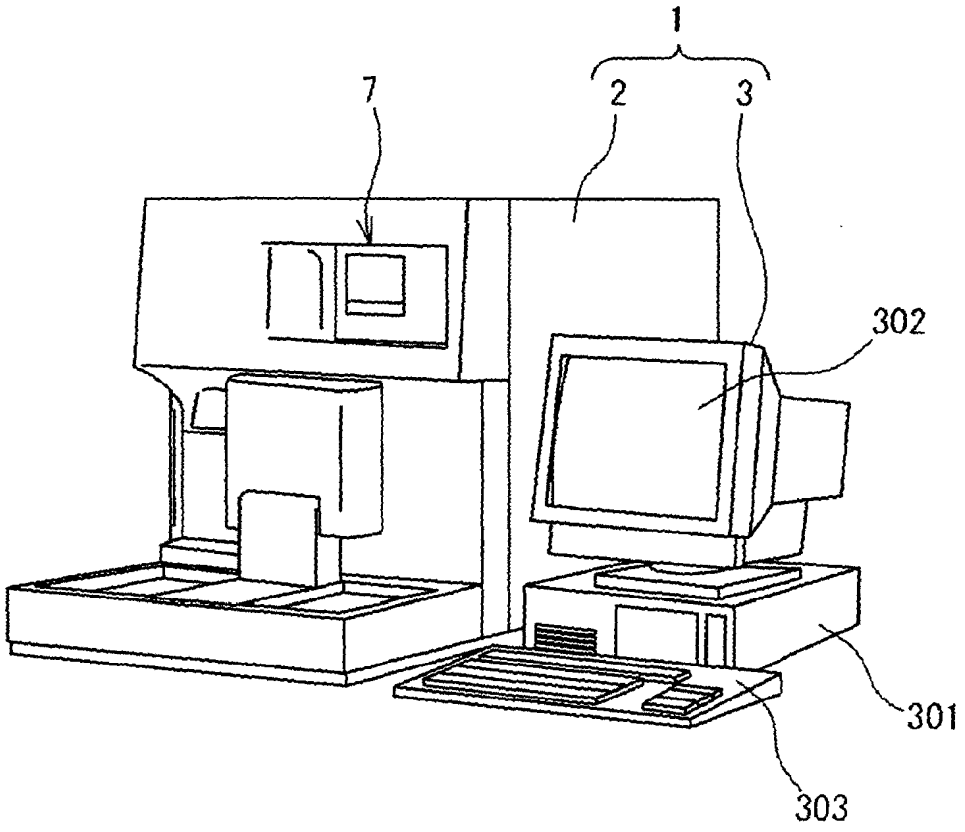


FIG.1

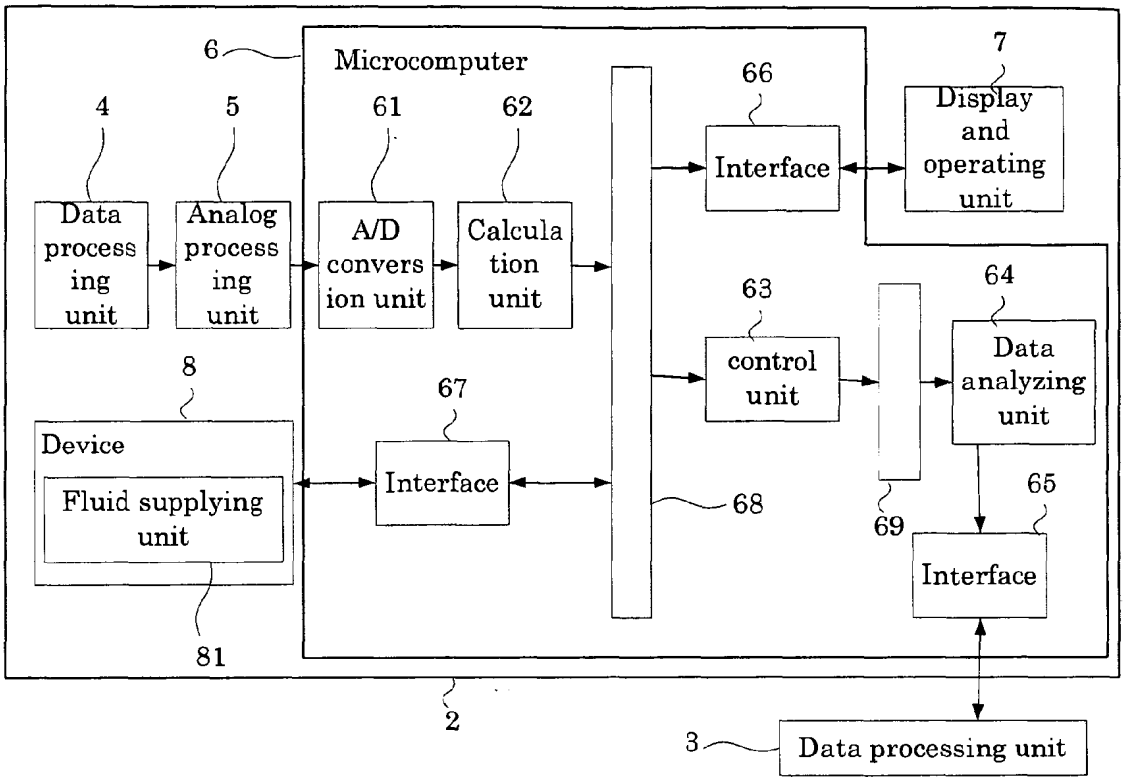

6
Microcomputer
7
4
5
61
62
66
Display and operating unit
Data processing unit
Analog processing unit
A/D conversion unit
Calculation unit
Interface
63
64
control unit
Data analyzing unit
8
67
Device
Fluid supplying unit
Interface
68
69
65
Interface
81
2
3
Data processing unit

FIG.2

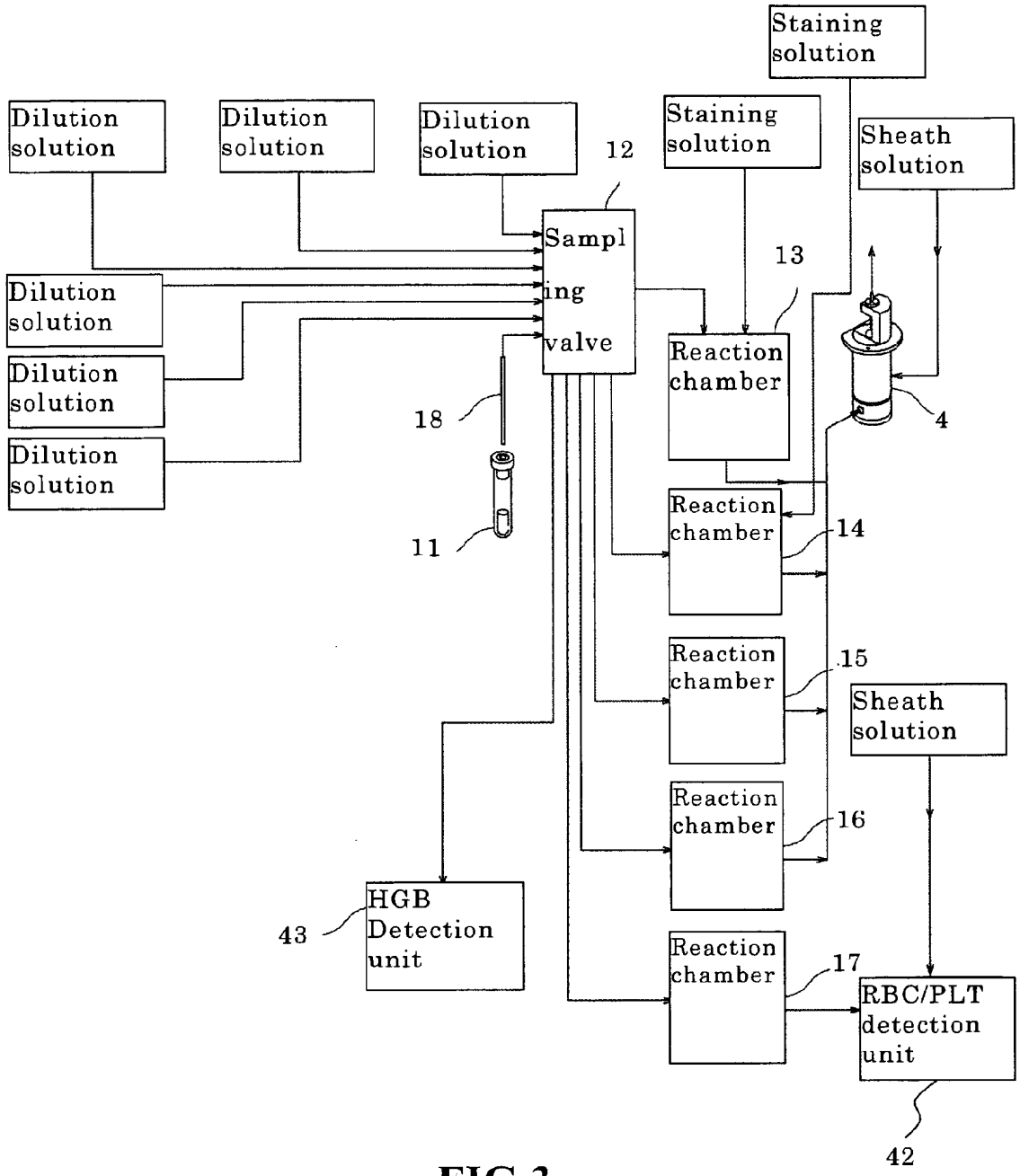

Staining solution
Dilution solution
Dilution solution
Dilution solution
Staining solution
Sheath solution
12
Sampl ing valve
13
Dilution solution
Dilution solution
Dilution solution
Reaction chamber
4
18
11
Reaction chamber
14
Reaction chamber
15
Sheath solution
Reaction chamber
16
HGB Detection unit
43
Reaction chamber
17
RBC/PLT detection unit
42

FIG.3

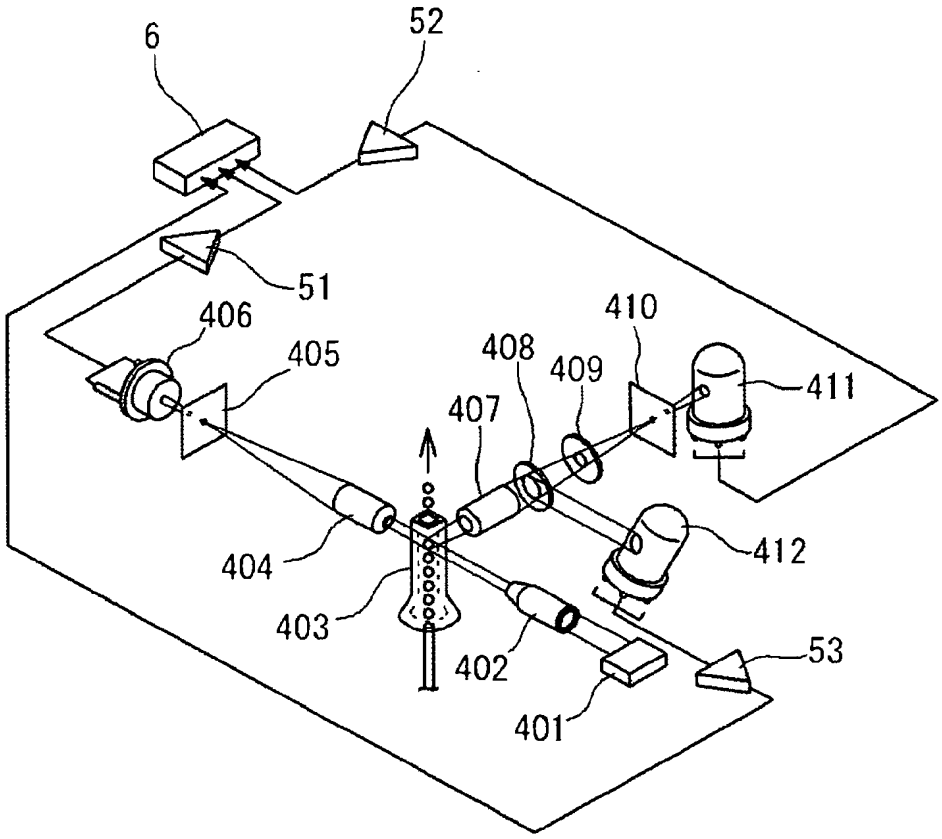


FIG.4

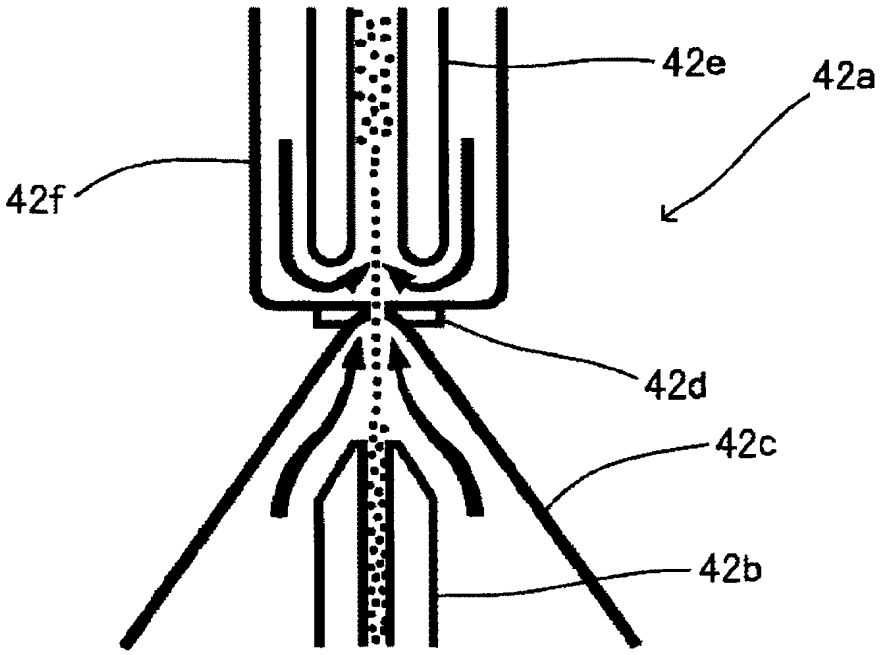


FIG.5

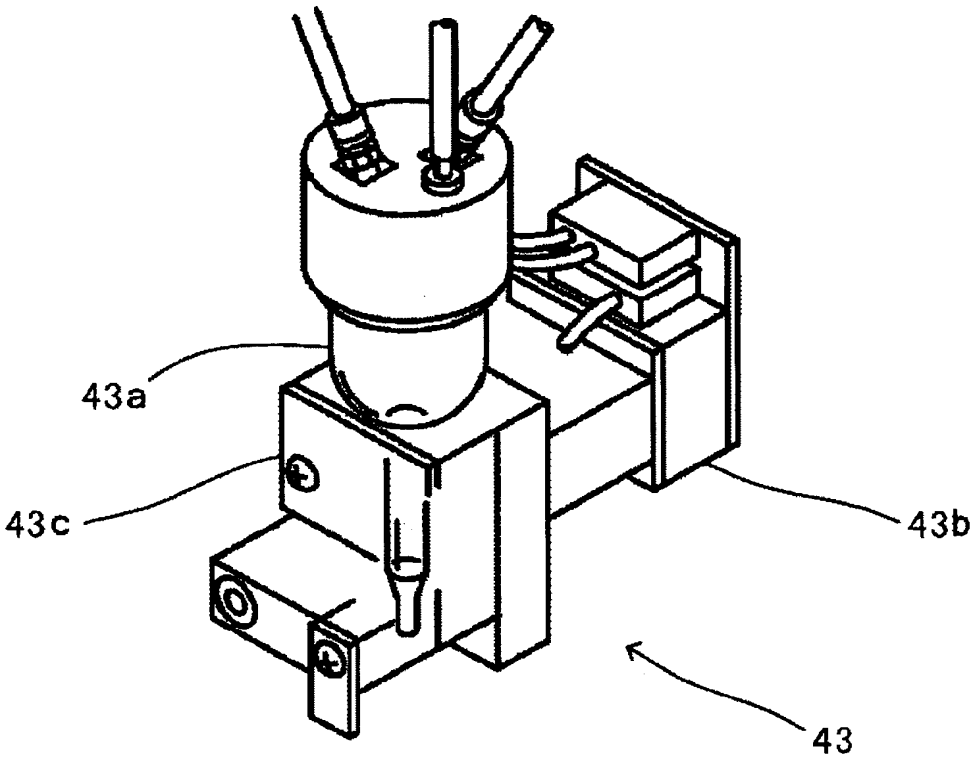


FIG.6

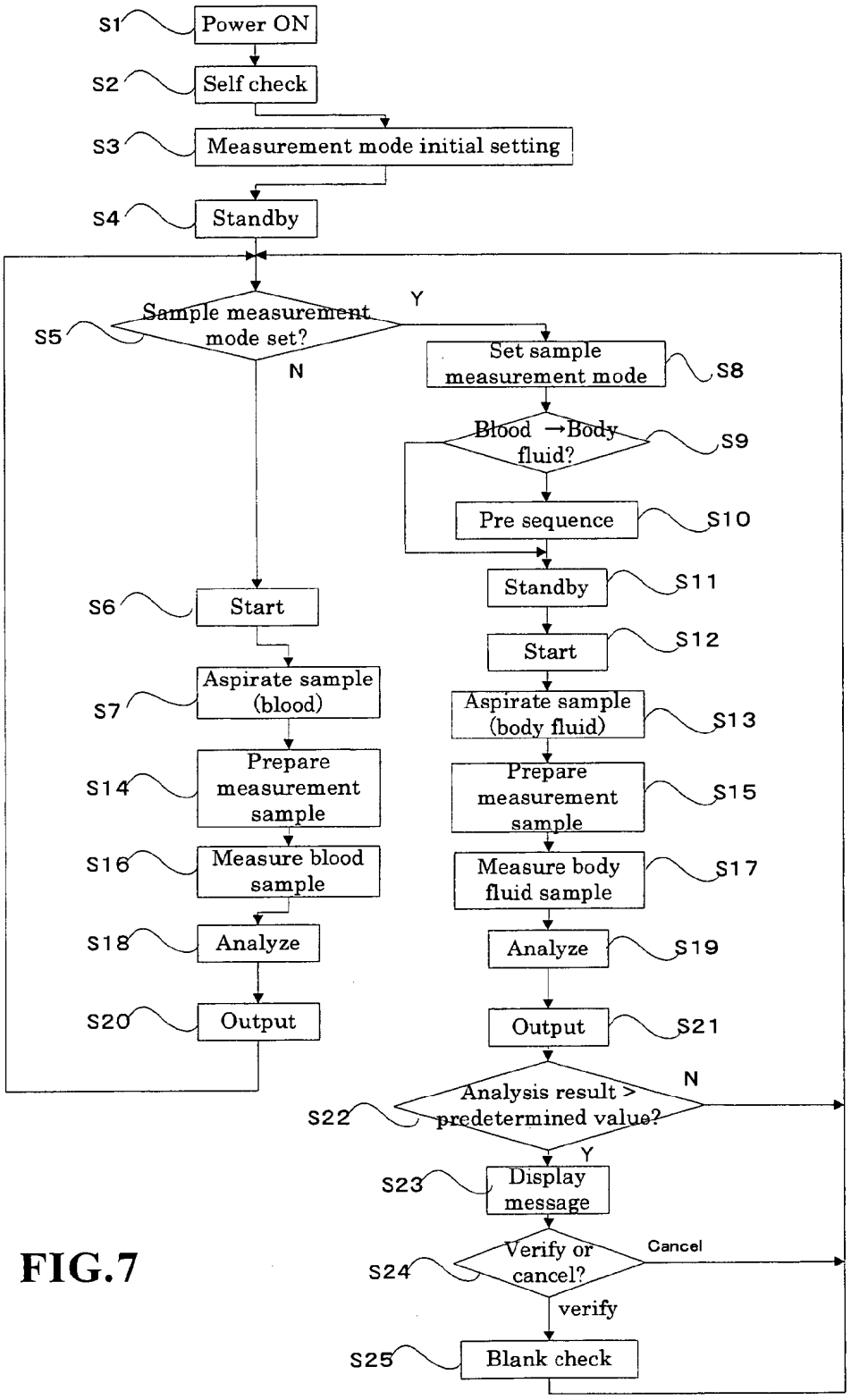

S1
Power ON
S2
Self check
S3
Measurement mode initial setting
S4
Standby
S5
Sample measurement mode set?
Y
N
S6
Start
S7
Aspirate sample (blood)
S14
Prepare measurement sample
S16
Measure blood sample
S18
Analyze
S20
Output
S8
Set sample measurement mode
S9
Blood →Body fluid?
S10
Pre sequence
S11
Standby
S12
Start
S13
Aspirate sample (body fluid)
S15
Prepare measurement sample
S17
Measure body fluid sample
S19
Analyze
S21
Output
S22
Analysis result > predetermined value?
N
Y
S23
Display message
S24
Verify or cancel?
Cancel
verify
S25
Blank check

FIG.7

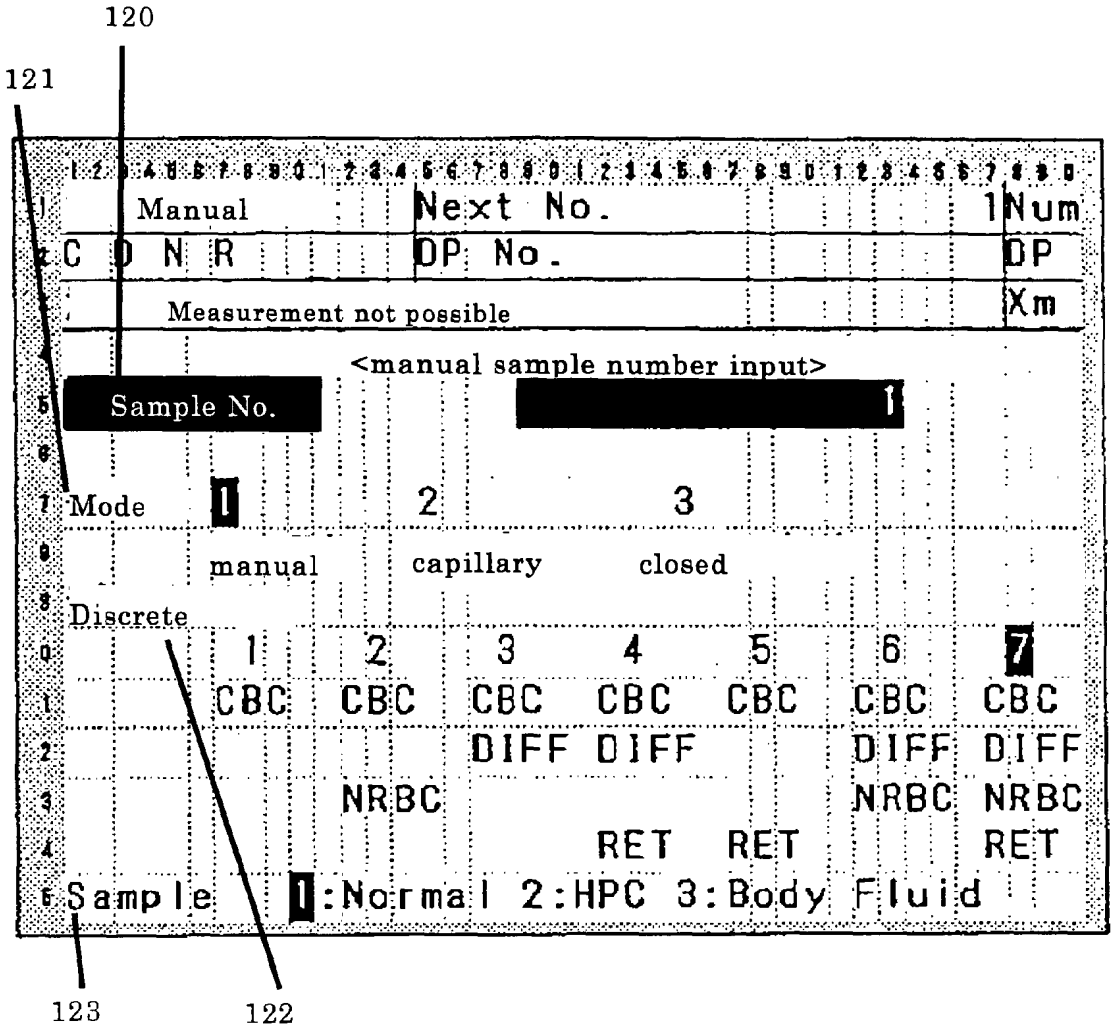

120
121
Manual
Next No.
Num
C O N R
DP No.
DP
Measurement not possible
Xm
<manual sample number input>
Sample No.
Mode
manual
capillary
closed
Discrete
CBC
DIFF
NRBC
RET
Sample
:Normal 2:HPC 3:Body Fluid
123
122

FIG.8

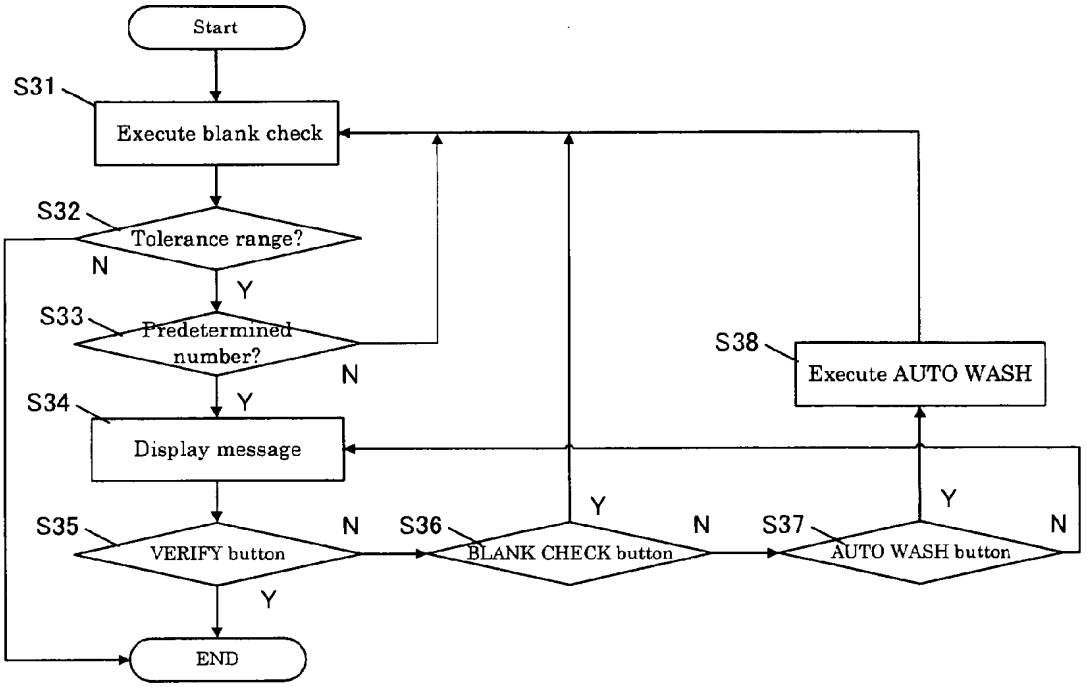

Start
S31
Execute blank check
S32
Tolerance range?
N
Y
S33
Predetermined number?
N
Y
S34
Display message
S35
VERIFY button
N
Y
S36
BLANK CHECK button
N
Y
S37
AUTO WASH button
N
Y
S38
Execute AUTO WASH
END

FIG.9

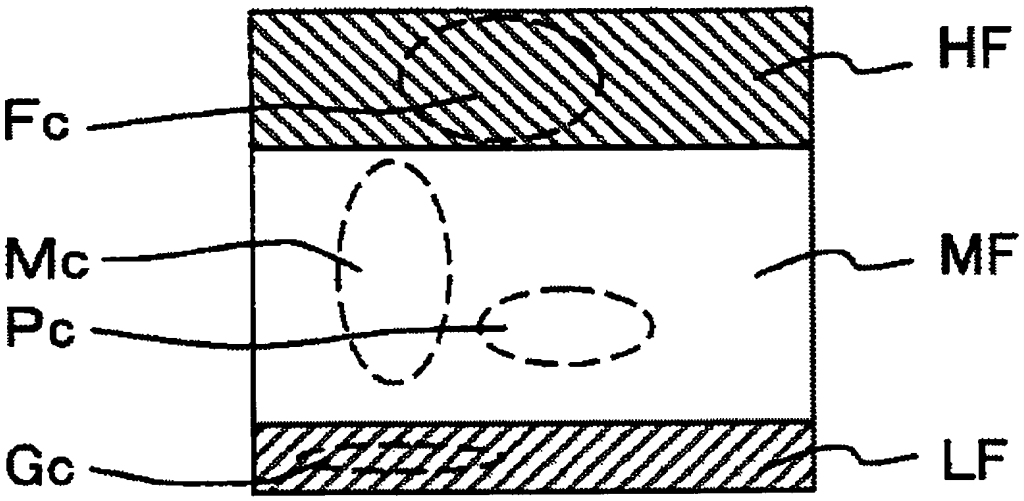


FIG.10

Example 1



	Ref	This Method
WBC	4580	4364
Others	1420	1387

Example 2



	Ref	This Method
WBC	1370	1160
Others	670	535

Example 3



	Ref	This Method
WBC	1360	1391
Others	70	74

FIG.11

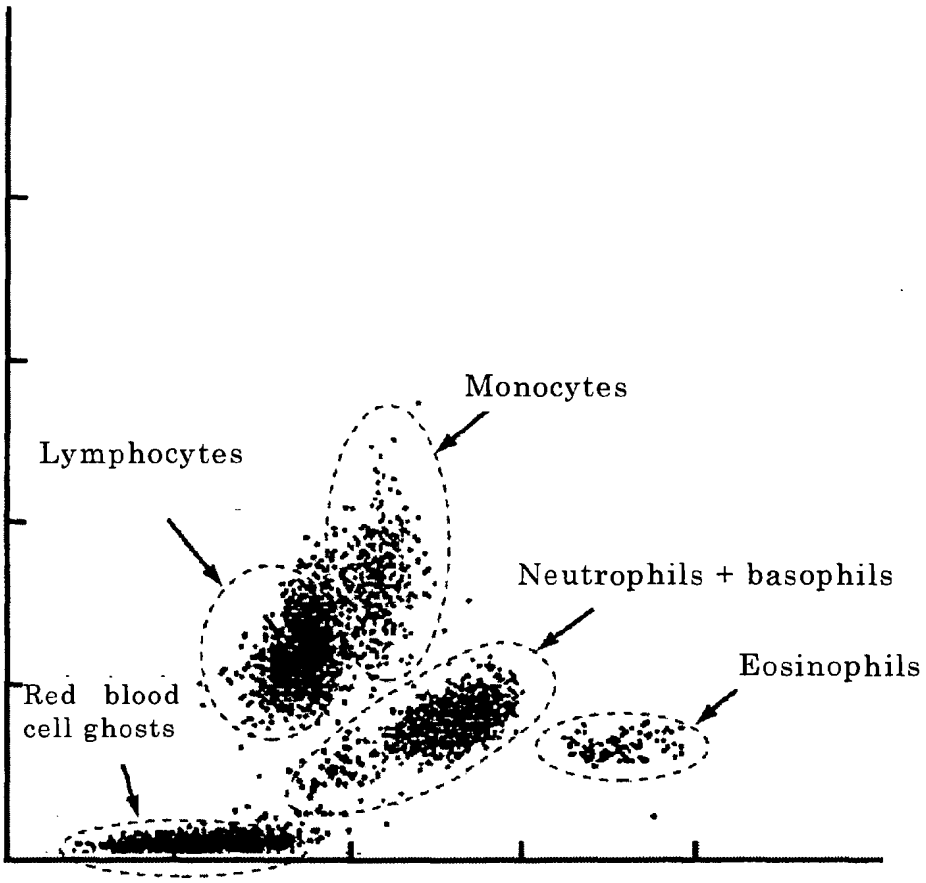

Monocytes
Lymphocytes
Neutrophils + basophils
Eosinophils
Red blood cell ghosts

FIG.12

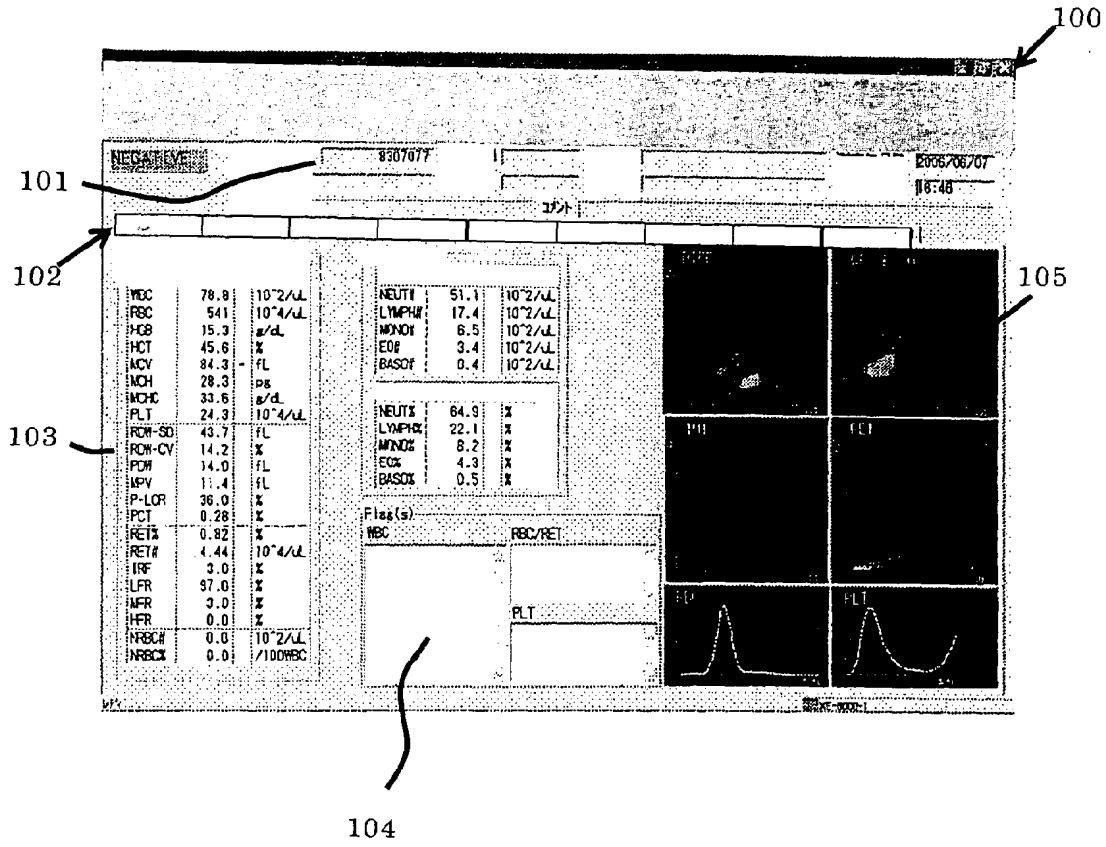

100
101
102
103
104
105
NEGATIVE
2006/06/07
18:46
WBC 78.8 10^2/uL
RBC 541 10^4/uL
HGB 15.3 g/dL
HCT 45.6 %
MCV 84.3 fL
MCH 28.3 pg
MCHC 33.6 g/dL
PLT 24.3 10^4/uL
RDW-SD 43.7 fL
RDW-CV 14.2 %
PDW 14.0 fL
MPV 11.4 fL
P-LCR 36.0 %
PCT 0.28 %
RET% 0.82 %
RET# 4.44 10^4/uL
IRF 3.0 %
LFR 97.0 %
MFR 3.0 %
HFR 0.0 %
NRBC# 0.0 10^2/uL
NRBC% 0.0 /100WBC
NEUT# 51.1 10^2/uL
LYMPH# 17.4 10^2/uL
MONO# 6.5 10^2/uL
EO# 3.4 10^2/uL
BASO# 0.4 10^2/uL
NEUT% 64.9 %
LYMPH% 22.1 %
MONO% 8.2 %
EO% 4.3 %
BASO% 0.5 %
Flag(s)
WBC
RBC/RET
PLT

FIG.13

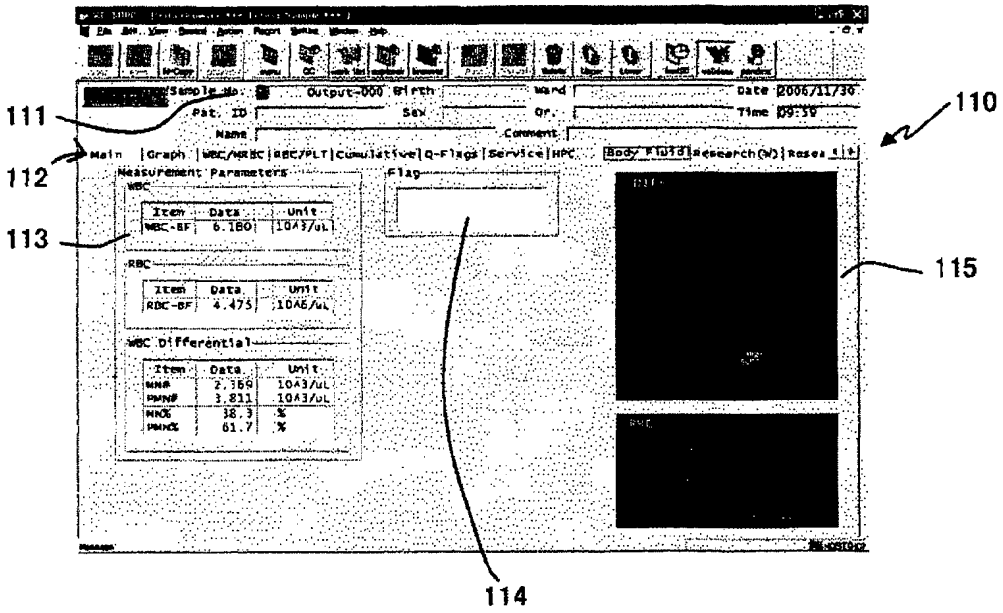

111
112
113
110
115
114
Sample No.
Output-000
Pat. ID
Sex
Name
Comment
Ward
Date 2006/11/30
Time 09:39
Main
Graph
WBC/HRBC
RBC/PLT
Cumulative
Q-Flags
Service
HPC
Body Fluid
Research(W)
Measurement Parameters
WBC
Item
Data
Unit
WBC-BF
6.180
10A3/uL
RBC
RBC-BF
4.475
10A6/uL
WBC Differential
NEUT
2.359
10A3/uL
LYMP
3.811
10A3/uL
NEUT%
38.3
%
LYMP%
61.7
%
Flag

FIG.14

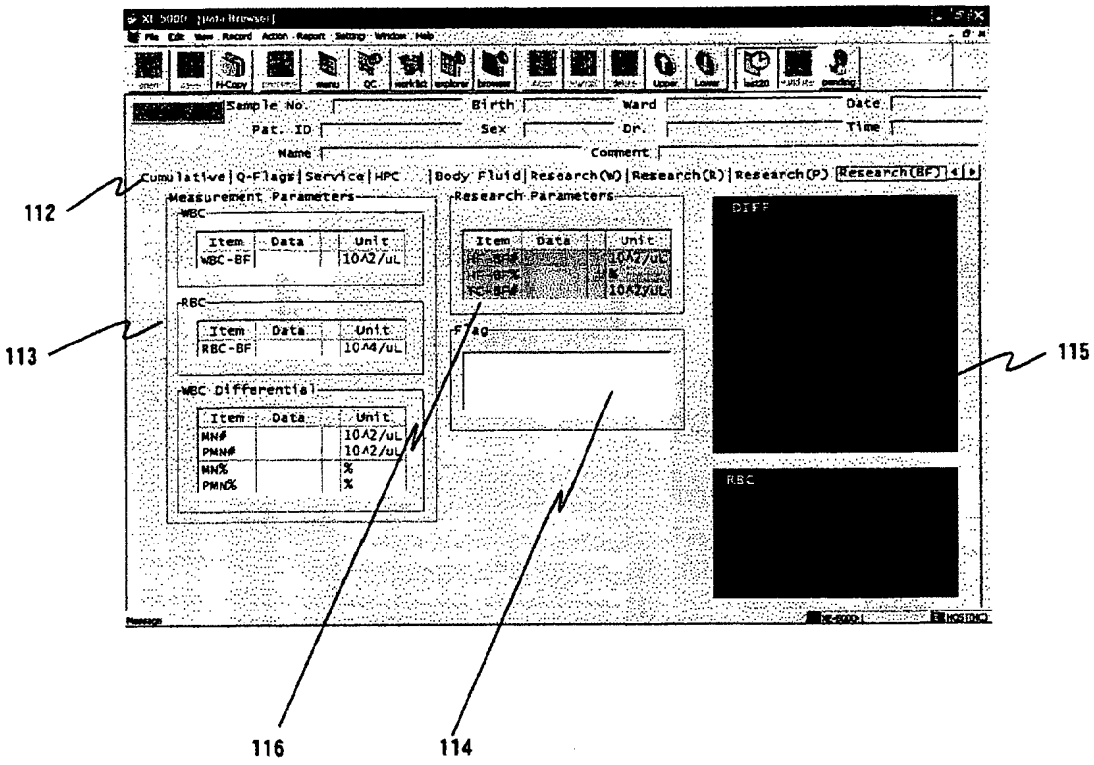

112
113
115
116
114
Sample No.
Birth
Ward
Date
Pat. ID
Sex
Dr.
Time
Name
Comment
Measurement Parameters
Research Parameters
WBC
RBC
WBC Differential
Flag
DIFF
RBC
Item
Data
Unit

FIG.15

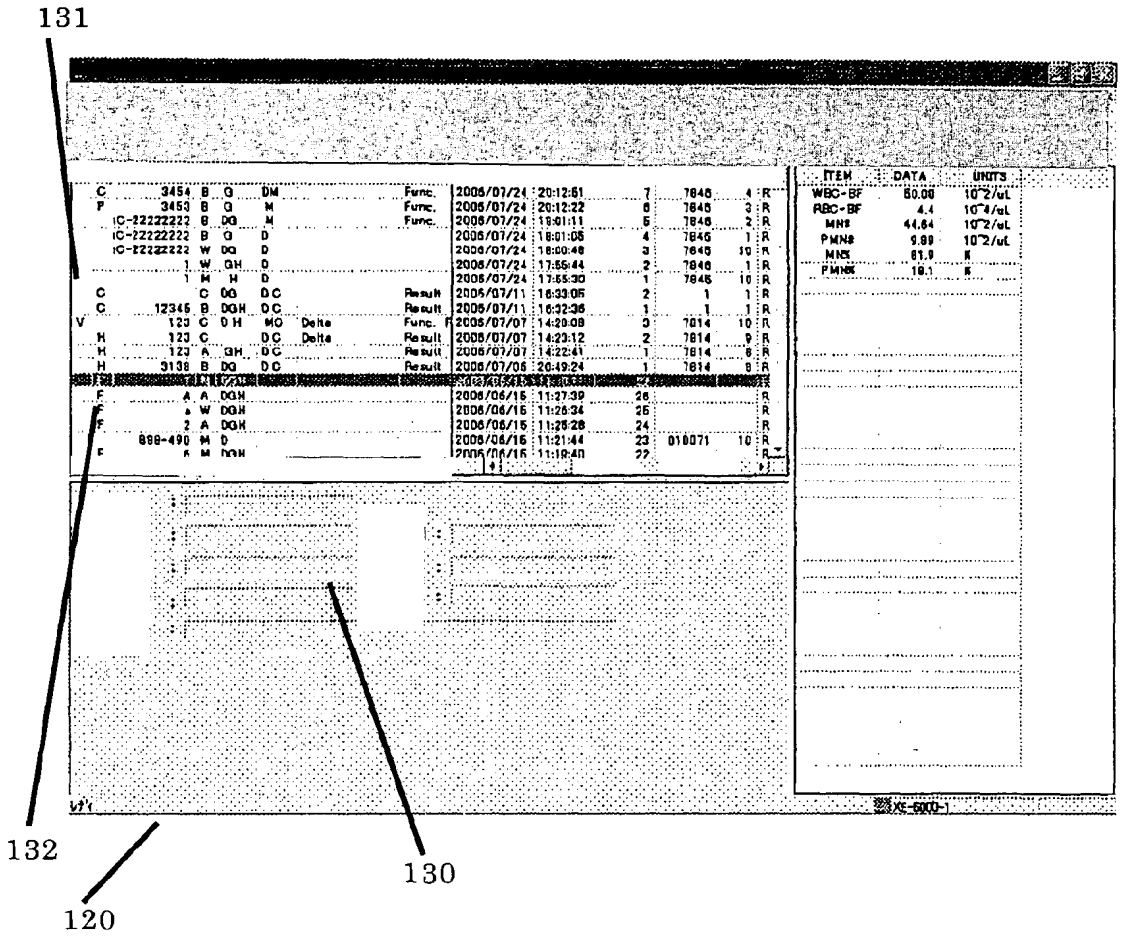


FIG.16

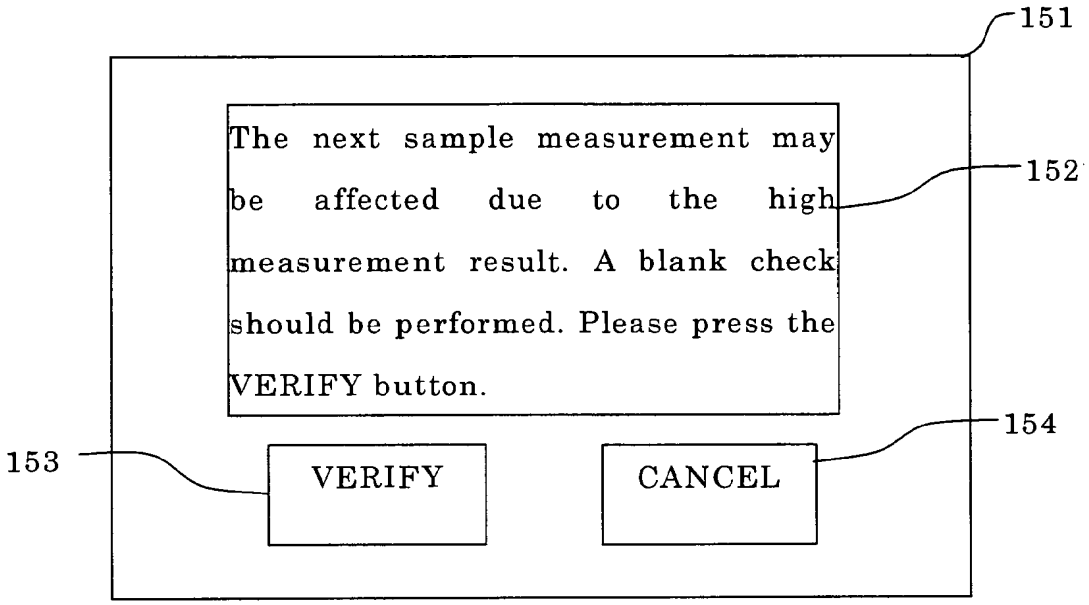

151
The next sample measurement may be affected due to the high measurement result. A blank check should be performed. Please press the VERIFY button.
152
153
VERIFY
CANCEL
154

FIG.17

REFERENCES CITED IN THE DESCRIPTION

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- US 5122453 A **[0062]**
- US 5138181 A **[0062]**

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 381 475 B1

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H01L 27/146 *(2006.01)*

(21) Application number: **11163603.1**

(22) Date of filing: **22.04.2011**

(54) Method of managing a silicon photomultiplier device

Verfahren zur Verwaltung eines Silizium-Fotovervielfacherbauelement

Méthode de fonctionnement d'un dispositif photomultiplicateur en silicium

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **22.04.2010 IT VI20100110**

(43) Date of publication of application:
26.10.2011 Bulletin 2011/43

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US-A1- 2008 308 928

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EP 2 381 475 B1

Description

[0001] The invention concerns a method for managing an improved silicon photomultiplier device (SiPM) particularly suited to detect one or more photons incident on the sensitive surface of the above mentioned device.

[0002] It is known that there are many fields of application in which it is necessary to detect very low intensity light.

[0003] In the medical sector, for example, said need is present in the equipment applying the technique known as positron emission tomography (PET), through which it is possible to obtain bio-images that allow the creation of maps of the functional processes that take place inside the body.

[0004] In particular, according to said technique, a tracer isotope is administered to the patient subjected to the examination and after a decay and annihilation process carried out by means of electrons said isotope causes two photons to be emitted in opposite directions.

[0005] Said photons, when they reach a scintillator material placed in the scanner device, produce a light flash that is detected by said photomultiplier devices.

[0006] In these specific cases, as well as in other applications, the purpose is to "count" the number of photons and usually also to determine, as precisely as possible, the instant of time of arrival of the high-energy particle.

[0007] In order to allow this data to be detected, it is therefore necessary to use high-resolution photomultiplier devices having limited or no problems related to sensitivity to intrinsic and/or external interferences.

[0008] From this last point of view, photomultiplier tubes or "PMT" have been known and used for years as detecting devices, said photomultiplier tubes being vacuum devices characterized by high gain (number of electrons produced per photon) and high response speed.

[0009] However, said devices pose a series of drawbacks that are described below. A first drawback lies in that said devices have a given, not negligible size that does not facilitate their integration in a complex system.

[0010] Furthermore, from the structural point of view said photomultiplier tubes are disadvantageously rather fragile.

[0011] Finally, a further drawback lies in that said devices are very sensitive to external magnetic fields that can deviate the path of the electrons inside them and thus affect the precision of the measurement.

[0012] In order to overcome this series of drawbacks, silicon devices have been recently developed, which in technical jargon are called "silicon photomultipliers" or "SiPMs" and hereinafter are simply called photomultiplier devices, if not indicated otherwise, comprising a plurality of photodiodes (usually arranged in a matrix), made on a single semiconductor substrate and operating in Geiger mode.

[0013] The surface of said photomultiplier devices is thus sensitive to the photons incident on it and in particular each photodiode responds independently of the others, emitting an elementary charge packet where a photon is detected.

[0014] In general, as previously mentioned, silicon photomultiplier devices present higher compactness, sturdiness, integrability in the system and insensitivity to magnetic fields compared to photomultiplier tubes (PMT).

[0015] It is also known that at present there are two types of silicon photomultiplier devices, depending on how the signals of the individual photodiodes are treated.

[0016] A first type consists of digital photomultiplier devices, in which the signals of the individual photodiodes are digitized locally and sent to a logic unit positioned on the same surface on which there are said photodiodes.

[0017] Said logic unit outputs the data concerning the detection of incident photons. The second type of photomultiplier devices is analog and its output is a single analog signal proportional to the number of activated photodiodes (on which incidence of one or more photons has taken place).

[0018] For this purpose, the above mentioned photodiodes of this last type of photomultiplier devices are connected in parallel to one another and each one of them has an integrated series resistor of a few hundred kiloohm serving as quenching circuit for the same photodiode.

[0019] The two above mentioned types of silicon photomultiplier devices present mutual advantages and disadvantages with respect to each other, which are described here below.

[0020] Advantageously, the photomultiplier devices of the digital type, output signals that are immune to external interferences from each photodiode, contrary to what may happen with the analog photomultiplier devices in which the resulting analog signal can be subjected to distortions.

[0021] Finally, a further advantage offered by digital photomultipliers compared to analog multipliers lies in that their temporal resolution is independent of the dimensions of the device.

[0022] On the other hand, analog photomultiplier devices are simpler to construct and to manage than digital photomultiplier devices.

[0023] Therefore, the photomultiplier devices of the analog type have lower construction costs than the digital ones and therefore in some situations their use is preferable even though their efficiency is lower.

[0024] Furthermore, the sensitive surface of analog photomultipliers is independent of the logic unit that manages the output signals of the photodiodes, as the connection between the two entities is carried out through discrete connection means (wire bonds or similar), differently from the digital photomultiplier devices in which the logic unit, as already mentioned above, is usually obtained on the same surface on which the photodiodes are provided. Consequently and advantageously, with analog photomultiplier devices it is possible to intervene

on the logic unit independently of the sensitive surface and vice versa.

[0025] Furthermore, the fact that digital photomultiplier devices have the logic unit on the surface where the photodiodes are provided determines the presence of a "dead area" insensitive to photon incidence that, in turn, determines a reduced resolution for the entire device.

[0026] However, said photomultiplier devices of the analog type have an important acknowledged limitation related to their management.

[0027] In fact, if one or more of the photodiodes connected to one another in parallel and present on the sensitive surface of said analog photomultiplier devices are not operating or are noisy, they may affect the output signal of the photomultiplier device.

[0028] Disadvantageously, the only way to resolve said malfunction of one or more photodiodes is to replace the entire photomultiplier device.

[0029] It is consequently clear that in this situation a large amount of money is wasted as, in the worst case, for a single not operating or noisy photodiode it is necessary to replace an entire photomultiplier device.

[0030] The compared analysis of the two types of silicon photomultiplier devices described above makes it possible to understand the drawbacks of the known art.

[0031] The present invention aims to overcome the drawbacks listed above.

[0032] In particular, it is the object of the invention to propose a method for managing a silicon photomultiplier device that makes it possible to inhibit in a simple manner a sector or, in the limit case, an individual noisy or non operating photodiode that determine the reduction of the overall efficiency.

[0033] In the same time, it is a further object of the invention to propose a method for managing a photomultiplier device whose sensitive surface is independent of the logic unit, so as to allow separate intervention on the two entities.

[0034] It is another object of the invention to propose a method for managing a photomultiplier device whose sensitive surface has reduced dimensions, though maintaining high resolution.

[0035] Furthermore, it is the object of the present invention to propose a method for managing a photomultiplier device whose efficiency and temporal resolution can be compared to those of digital photomultipliers.

[0036] The objects described above are achieved by a method for managing a photomultiplier device having the characteristics illustrated in the main claim. Further characteristics of the method of the invention concerning the management of a photomultiplier device that is the subject of the invention are described in the dependent claims.

[0037] Advantageously, the photomultiplier device to which the management method of the invention is applied makes it possible to connect the sensitive surface to different types of logic units depending on the use needs.

[0038] Consequently, a further advantage is represented by the possibility to construct the photomultiplier device to which the management method of the invention is applied so that it can be modulated with different levels of efficiency from the functional point of view, determining different production costs and selling prices.

[0039] US 2004/0129991 describes a front side illuminated photodiode.

[0040] The objects and advantages described above will be highlighted in greater detail in the description of a preferred embodiment of the invention that is supplied as an indicative, non-limiting example, with reference to the enclosed drawings, wherein:

- Figure 1 shows an axonometric plan view of the photomultiplier device to which the management method of the invention is applied;
- Figure 2 shows an axonometric view of a detail of the photomultiplier device of Figure 1 to which the management method of the invention is applied;
- Figure 3 shows an axonometric view from below of the photomultiplier device to which the management method of the invention is applied;
- Figure 4 shows the front view of the semiconductor substrate from the sensitive surface side;
- Figure 5 shows the front view of the semiconductor substrate from the side of the extraction points opposite the sensitive surface;
- Figure 6 shows the front view of the photomultiplier device from the logic unit side;
- Figure 7 shows a cross section side view along a transversal plane of the photomultiplier device that is the subject of the invention.

[0041] The silicon photomultiplier device (SiPM) for detecting one or more photons, to which the management method of the invention is applied, is represented as a whole in Figures 1, 3, 6 and 7, where it is indicated by **1.**

[0042] It can be observed in Figure 1 that the above mentioned photomultiplier device **1** comprises a sensitive surface **3** created on a first side **4** of a semiconductor substrate **2**.

[0043] In particular, the sensitive surface **3** comprises a plurality of Geiger-mode avalanche photodiodes **5** electronically insulated from one another.

[0044] As can be observed in Figure 2, according to the preferred embodiment of the photomultiplier device to which the management method of the invention is applied described herein, each photodiode **5** is connected in series to a resistor **12** usually made of polysilicon.

[0045] Said resistors **12**, as is well known, make it possible to dampen the avalanche effect (passive quenching circuit) that is generated in the corresponding photodiodes **5** at the moment when one or more photons result to be incident on the same photodiodes.

[0046] It cannot be excluded that in different construction forms said resistor **12** may not be present, as in the special case described further on in this document. Furthermore, in the preferred embodiment of the invention

described herein and represented in the figures, the plurality of photodiodes **5** is arranged in a matrix.

[0047] Nonetheless, in different construction forms the above mentioned photodiodes **5** can be arranged with a different configuration with respect to one another. Regarding the sensitive surface **3**, it is defined by a plurality of sectors **6**, each one of which comprises one or more photodiodes **51** belonging to the above mentioned plurality of photodiodes **5**, connected to one another in parallel, as shown in Figures 1 and 4 and in the detail of Figure 2.

[0048] The number of photodiodes **51** belonging to a single sector **6** is preferably but not necessarily less than twenty.

[0049] According to the preferred embodiment of the photomultiplier device to which the management method of the invention is applied described herein, each sector **6** comprises ten photodiodes **51** connected in parallel.

[0050] In a particular embodiment of the invention, each sector **6** may comprise a single photodiode **51** and in this case the resistor **12** may be absent, as previously mentioned.

[0051] It cannot be excluded, however, that in different embodiments the number of photodiodes **51** per sector **6** may exceed twenty.

[0052] Regarding the parallel connection of the photodiodes **51** belonging to each sector **6**, this is obtained through conductor means **7**.

[0053] As shown in Figures 2 and 4, in particular, said conductor means **7** comprise a metal strip **71** provided on the sensitive surface **3**.

[0054] In alternative embodiments of the invention it is not excluded that the connection of the above mentioned photodiodes **51** of a single sector **6** may be carried out with a different type of means or techniques, provided that they belong to the known art.

[0055] Regarding the sectors **6**, they are preferably but not necessarily present in a number lower than or equal to one hundred.

[0056] Also in this case, it cannot be excluded that the number of sectors **6** in which the sensitive surface **3** can be divided may exceed one hundred.

[0057] The parallel connection of the photodiodes **51** belonging to a single sector **6** makes it possible to define as output of the latter a resulting analog signal that is carried, through the same conductor means **7**, to an extraction point **81** belonging to a plurality of extraction points **8** electronically insulated from one another.

[0058] In their turn, as will be better explained below, the above mentioned extraction points **8** allow the pickup and successive processing, in an independent manner, of the individual analog signals defined by each sector **6** by a logic unit **9** illustrated in Figure 3.

[0059] According to the invention, the method for managing the photomultiplier device **1** described up to now makes it possible to inhibit one or more of said sectors **6**, in the case where one or more photodiodes **51** belonging to said sectors **6** are not operating or are noisy.

[0060] In particular, according to the preferred embodiment of the photomultiplier device **1** to which the management method of the invention is applied, as will be explained in detail below, said logic unit **9** is of the digital type.

[0061] In this case, said operation for inhibiting one or more sectors requires that the same malfunctioning sectors are disabled at a logic level.

[0062] In a different embodiment of the photomultiplier device **1** to which the management method of the invention is applied, as is described in detail here below, the logic unit **9** may be of the analog type.

[0063] In this case, said inhibition operation requires that the physical connection between the extraction points **8** corresponding to the malfunctioning sectors **6** and said logic unit **9** be disconnected.

[0064] According to the preferred embodiment of the photomultiplier device to which the management method of the invention is applied described herein and illustrated in Figures **5** and **6**, said extraction points **8** are arranged on the second side **10** of the semiconductor substrate **2** opposite the first side **4** where the sensitive surface **3** is provided, and are therefore accessible from the same side **10**.

[0065] Said arrangement on the second side **10** of the extraction points **8** is made possible by means of conductor columns **11** carried out so that they pass through the semiconductor substrate **2**, each one of which intercepts the conductor means **7** of a specific sector **6** on said first side **4** and carries the analog signal to the opposite side, as shown in Figure 7.

[0066] With this configuration, advantageously, the whole sensitive surface **3** substantially presents the plurality of photodiodes **5** and no further elements, like for example the extraction points **8**, making it possible to increase the number of the same photodiodes **5** and consequently to increase the resolution of the entire photomultiplier device **1**, as shown in Figure 4.

[0067] It cannot be excluded that in different embodiments, neither described nor illustrated herein, the extraction points **8** may be located on the same sensitive surface **3**, negatively affecting, however, the resolution of the photomultiplier device **1** of the invention.

[0068] Regarding the photomultiplier device **1** to which the management method of the invention is applied, in the preferred embodiment described herein and illustrated in Figures **1**, **3** and **7**, it comprises a substantially plate-like dielectric support element **13** that is coupled with the second side **10** of said semiconductor substrate **2** at the level of a first face **14**.

[0069] In particular, therefore, in the preferred embodiment of the invention the extraction points **8** of the analog signals face towards said dielectric support element **13**.

[0070] Obviously, in order to make said extraction points **8** accessible from the outside, one or more through openings **15** are created in the dielectric support element **13**, as shown in Figures **3**, **6** and **7**.

[0071] Thanks to this configuration, with the preferred

embodiment of the invention it is possible to arrange the logic unit **9** on the second face **16** of the dielectric support element **13**, opposite said first face **14**.

[0072] In its turn, the logic unit **9** comprises a plurality of inputs **17**, each one of which is suited to be connected to one of the extraction points 8 accessible through said one or more openings **15**.

[0073] In particular, according to the preferred embodiment of the photomultiplier device **1** to which the management method of the invention is applied, as shown in Figure **6**, the electronic connection between each input **17** of the logic unit **9** and the corresponding extraction point **8** is carried out through wire bonds **18**.

[0074] In different embodiments of the invention, said connection may be carried out with different techniques or means, provided that they belong to the known art. Regarding the dielectric support element **13**, in the preferred embodiment of the photomultiplier device **1** to which the management method of the invention is applied described herein, it is a printed circuit board (PCB) **131**.

[0075] In different embodiments of the invention, neither described nor illustrated herein, said dielectric support element **13** may be made with a ceramic material or a different material, provided that it is of the dielectric type.

[0076] As already mentioned, in the preferred embodiment of the invention the logic unit **9** is arranged on the second face **16** of the dielectric support element **13** opposing the semiconductor substrate **2**.

[0077] It cannot be excluded, however, that in different embodiments said logic unit **9** may be external to the photomultiplier device **1** and connected to the plurality of extraction points **8** through discrete connection means (wires).

[0078] As already mentioned, according to the preferred embodiment of the photomultiplier device **1** to which the management method of the invention is applied, the logic unit **9** comprises an integrated circuit **91** specifically designed (ASIC) with a digital processing logic, so as to output multiple data concerning, for example, the number and time of arrival of the incident photons.

[0079] Furthermore, said type of integrated circuit **91** can be used as a control unit by acting on the power supply voltage of the individual photodiodes 5 in order to cope with possible fluctuations due to temperature changes or to non conformities between the devices.

[0080] Furthermore, said type of logic unit **9**, as already explained, makes it possible to inhibit the individual sectors **6** that are not operating or are particularly noisy. In the limit case, neither described nor illustrated herein, of a single photodiode **51** per sector **6** the dielectric support element **13** may not be provided, while on the other hand the integrated circuit **91** and the semiconductor substrate 2 may be directly coupled with each other by connecting the extraction points 8 of the individual sectors **6** to the inputs **17** of the same integrated circuit 91 through a "bump-bonding" process.

[0081] In different embodiments it cannot be excluded that said integrated circuit **91** may comprise one or more amplifiers/adders, exclusively of the analog type, giving as an output a resulting analog signal proportional to the number of photodiodes **5** that reacted to the incidence of the photons.

[0082] In this way the signals coming from the sensitive surface **3** are managed by a considerably simplified logic unit **9**, thus granting an advantage from both the productive and the economic point of view compared to the logic units of the digital type described above.

[0083] In greater detail, as already explained, in the case of the logic unit **9** of the analog type, it is simply omitted to connect any non operating or excessively noisy sectors **6** to the corresponding input **17** of the logic unit **9**.

[0084] The identification of said non operating or excessively noisy sectors 6 can be carried out quickly through automatic current measurements on the entire semiconductor substrate **2**.

[0085] Furthermore, with the method of the invention concerning the management of the photomultiplier device **1** configured as described above, the amplification of the analog signal of the individual sectors **6** grants some advantages also in terms of timing performance compared to the analog photomultiplier devices of the known art.

[0086] In fact, each amplifier works on a photodiode **5** with reduced parasitic capacity and therefore it is easier to have signal to noise ratios (SNR) suited to the desired timing performance.

[0087] Furthermore, the above mentioned logic unit **9**, in alternative embodiments not illustrated in the figures, may comprise discrete components.

[0088] In particular, said discrete components may, as in the case of the integrated circuit **91**, implement a digital logic, giving as output, in fact, a digital signal or an analog logic and clearly generating an analog signal.

[0089] In general, independently of the type of logic unit **9** used, it makes it possible to implement different and multiple functions with no need to reduce the sensitive surface **3**, as the logic unit **9** first of all is independent of said sensitive surface **3** and furthermore is located on the opposite side with respect to the dielectric support element **13**, as already explained.

[0090] Furthermore, thanks to the connection of the same logic unit **9** to the second face **16** of the dielectric support element **13**, it is easier to integrate the photomultiplier device **1** in a modular system.

[0091] On the basis of the above, it is clear that the method of the invention concerning the management of a photomultiplier device that is the subject of the invention achieves all the set objects.

[0092] In particular, the invention achieves the object to propose a method for managing a silicon photomultiplier device that makes it possible to inhibit in a simple manner a sector or, in the limit case, an individual noisy or non operating photodiode that determine the reduction of the overall efficiency.

[0093] In the same time, the invention also achieves the object to propose a method for managing a photomultiplier device whose the sensitive surface is independent of the logic unit, so as to allow separate intervention on the two entities.

[0094] Another object achieved is to propose a method for managing a photomultiplier device whose sensitive surface has reduced dimensions, though maintaining high resolution.

[0095] Finally, the invention also achieves the object to propose a method for managing a photomultiplier device whose efficiency and temporal resolution can be compared to those of digital photomultipliers.

[0096] On implementation, the method of the invention concerning the management of a photomultiplier device may undergo further changes that, though not illustrated or described herein, shall nonetheless be covered by the present patent, provided that they come within the scope of the claims that follow. Where technical features mentioned in any claim are followed by reference signs, those reference sings have been included for the sole purpose of increasing the intelligibility of the claims and accordingly such reference signs do not have any limiting effect on the protection of each element identified by way of example by such reference signs.

Claims

1. Method for managing a silicon photomultiplier device (SiPM) (1) for detecting one or more photons, of the type comprising a surface (3) that is sensitive to said photons, provided on a first side (4) of a semiconductor substrate (2) and defined by a plurality of sectors (6) each one of which comprises a plurality of photodiodes (5) operating in Geiger mode and connected to each other in parallel through conductor means (7), so as to define an analog output signal, said semiconductor substrate (2) comprising a plurality of extraction points (8) electronically insulated from one another, each one of which is suited to be connected to a corresponding sector (6) through conductor means (7) so that said analog signals can be picked up and processed in an independent manner by a logic unit (9), **characterized in that** one or more of said sectors (6) are inhibited in the case where at least one of said photodiodes (5) belonging to said one or more sectors (6) is not operating or is noisy.

2. Method according to claim 1), wherein said operation for inhibiting said one or more sectors (6) requires that said one or more sectors (6) are disabled at a logic level, in the case where said logic unit (9) is of the digital type.

3. Method according to claim 1), wherein said operation for inhibiting said one or more sectors (6) requires that the physical connections between said one or more extraction points (8) corresponding to said one or more sectors (6) and said logic unit (9) are disconnected, in the case where said logic unit (9) is of the analog type.

4. Method according to any of the preceding claims, wherein said logic unit (9) outputs at least one resulting signal proportional to the number of said photons.

5. Method according to claim 4), wherein said logic unit (9) outputs also data concerning the detection time for said photons.

6. Method according to any of the preceding claims, wherein said extraction points (8) are located on the second side (10) of said semiconductor substrate (2) opposite said first side (4) where said sensitive surface (3) is arranged through the connection with conductor columns (11) carried out so that they pass through said semiconductor substrate (2).

7. Method according to any of the preceding claims, wherein said conductor means (7) comprise a metal strip (71) provided on said sensitive surface (3) for connecting in parallel said photodiodes (5) belonging to each one of said sectors (6).

8. Method according to any of the preceding claims, wherein said photomultiplier device (1) comprises a substantially plate-like dielectric support element (13) coupled, at the level of a first one (14) of its faces, to said second side (10) of said semiconductor substrate (2), and, at the height of its second face (16) opposite said first face (14), to said logic unit (9) having a plurality of inputs (17), each one of which is suited to be connected to one of said extraction points (8).

9. Method according to claim 8), wherein said photomultiplier device (1) comprises a plurality of wire bonds (18) arranged so that they pass through one or more openings (15) defined in said dielectric support element (13) and suited to connect each one of said inputs (17) to a corresponding extraction point (8).

10. Method according to claim 8) or 9), wherein said dielectric support element (13) is a printed circuit board (PCB) (131).

11. Method according to any of the preceding claims, wherein each one of said sectors (6) comprises said photodiodes (5) in a number under twenty.

12. Method according to any of the preceding claims, wherein said photomultiplier device (1) comprises a polysilicon resistor (12) connected in series between

each one of said photodiodes (5) and the corresponding conductor means (7).

13. Method according to any of the preceding claims, wherein said logic unit (9) is an integrated circuit (91).

14. Method according to any of the preceding claims, wherein said logic unit (9) is of the digital type.

15. Method according to any of the claims from 1) to 13), wherein said logic unit (9) comprises one or more amplifiers/adders of the analog type.

Patentansprüche

1. Methode zur Steuerung einer Silicon-Photomultiplier-Vorrichtung (SiPM) (1) zur Erkennung eines oder mehrerer Photonen, des Typs, der eine Oberfläche (3) umfasst, die gegenüber den besagten Photonen empfindlich, an einer ersten Seite (4) mit einem Halbleitersubstrat (2) versehen und durch eine Vielzahl von Abschnitten (6) definiert ist, von denen ein jeder eine Vielzahl von Photodioden (5) umfasst, die im Geiger-Modus arbeiten und über Leitmittel (7) parallel miteinander verbunden sind, so dass sie ein Analog-Ausgangssignal definieren, wobei das besagte Halbleitersubstrat (2) eine Vielzahl elektronisch voneinander isolierter Auszugspunkte (8) umfasst, von denen ein jeder geeignet ist, durch Leitmittel (7) mit einem entsprechenden Abschnitt (6) verbunden zu werden, so dass die besagten Analogsignale erfasst und auf unabhängige Weise durch eine Logikeinheit (9) verarbeitet werden können, **dadurch gekennzeichnet, dass** einer oder mehrere der besagten Abschnitte (6) gehemmt werden, wenn wenigstens eine der besagten, zu einem oder mehreren Abschnitten (6) gehörigen Photodioden (5) nicht arbeitet oder laut ist.

2. Methode gemäß Patentanspruch 1), wobei der besagte Vorgang zur Hemmung des/der besagten, einen oder mehreren Abschnitts/Abschnitte (6) die Deaktivierung des/der besagten, einen oder mehreren Abschnitts/ Abschnitte (6) auf einem logischen Niveau erfordert, wenn die besagte Logikeinheit (9) vom digitalen Typ ist.

3. Methode gemäß Patentanspruch 1), wobei der besagte Vorgang zur Hemmung des/der besagten, einen oder mehreren Abschnitte (6) die Abtrennung der physischen Verbindungen zwischen dem/den besagten einen oder mehreren, zu dem/den besagten einem oder mehreren Abschnitt/ Abschnitten (6) entsprechenden Auszugspunkt(en) (8) und der besagten Logikeinheit (9) erfordert, wenn die besagte Logikeinheit (9) vom analogen Typ ist.

4. Methode gemäß eines jeden der vorstehenden Patentansprüche, wobei die besagte Logikeinheit (9) wenigstens ein resultierendes Signal ausgibt, das proportional zur Anzahl der besagten Photonen ist.

5. Methode gemäß Patentanspruch 4), wobei die besagte Logikeinheit (9) auch Daten über die Dauer der Erfassung der besagten Photone ausgibt.

6. Methode gemäß eines jeden der vorstehenden Patentansprüche, wobei die besagten Auszugspunkte (8) an der zweiten, der besagten ersten Seite (4) gegenüberliegenden Seite (10) des besagten Halbleitersubstrats (2) positioniert sind, wo die besagte, empfindliche Oberfläche (3) unter Verbindung mit leitenden Säulen (11) angebracht ist, welche derart ausgeführt sind, dass sie durch das besagte Halbleitersubstrat (2) verlaufen.

7. Methode gemäß eines jeden der vorstehenden Patentansprüche, wobei die besagten Leitmittel (7) einen Metallstreifen (71) umfassen, der an der besagten, empfindlichen Oberfläche (3) für die Parallelverbindung der besagten, zu jedem der besagten Abschnitte (6) gehörigen Photodioden (5) vorhanden ist.

8. Methode gemäß eines jeden der vorstehenden Patentansprüche, wobei die besagte Photomultiplier-Vorrichtung (1) ein im Wesentlichen plattenartiges, dielektrisches Trägerelement (13) umfasst, das auf Ebene einer ersten (14) seiner Seiten an die besagte zweite Seite (10) des besagten Halbleitersubstrats (2) gekuppelt ist und auf Höhe seiner zweiten Seite (16), die der besagten ersten Seite (14) entgegengesetzt ist, an die besagte Logikeinheit (9), welche eine Vielzahl von Eingängen (17) aufweist, von denen jeder geeignet ist, an einen der besagten Auszugspunkte (8) angeschlossen zu werden.

9. Methode gemäß Patentanspruch 8), wobei die besagte Photomultiplier-Vorrichtung (1) eine Vielzahl von Bonddrähten (18) umfasst, die so angeordnet sind, dass sie durch eine oder mehrere Öffnungen (15) verlaufen, die in dem besagten dielektrischen Trägerelement (13) definiert sind und geeignet sind, jeden der besagten Eingänge (17) mit einem entsprechenden Auszugspunkt (8) zu verbinden.

10. Methode gemäß Patentanspruch 8) oder 9), wobei das besagte dielektrische Trägerelement (13) eine Platine (PCB) (131) ist.

11. Methode gemäß eines jeden der vorstehenden Patentansprüche, wobei jeder der besagten Abschnitte (6) eine Anzahl von weniger als zwanzig der besagten Photodioden (5) umfasst.

12. Methode gemäß eines jeden der vorstehenden Patentansprüche, wobei die besagte Photomultiplier-Vorrichtung (1) einen Polysilikonwiderstand (12) umfasst, der in Reihe zwischen jeder der besagten Photodioden (5) und den entsprechenden Leitmitteln (7) angeschlossen ist.

13. Methode gemäß eines jeden der vorstehenden Patentansprüche, wobei die besagte Logikeinheit (9) ein integrierter Schaltkreis (91) ist.

14. Methode gemäß eines jeden der vorstehenden Patentansprüche, wobei die besagte Logikeinheit (9) vom digitalen Typ ist.

15. Methode gemäß eines jeden der Patentansprüche von 1) bis 13), wobei die besagte Logikeinheit (9) einen oder mehrere Verstärker/Addierer vom analogen Typ umfasst.

Revendications

1. Méthode pour la gestion d'un dispositif photomultiplicateur silicium (SiPM) (1) pour la détection d'un ou plusieurs photons du type comprenant une surface (3) sensible auxdits photons, réalisée sur un premier côté (4) d'un substrat semi-conducteur (2) et définie par une pluralité de secteurs (6) chacun desquels comprend une pluralité de photodiodes (5) fonctionnant en mode Geiger et reliées entre elles en parallèle par des moyens conducteurs (7) de manière à définir un signal de sortie analogique, ledit substrat semi-conducteur (2) comprenant une pluralité de points d'extraction (8) électriquement isolés entre eux, chacun desquels est apte à être relié à un secteur correspondant (6) par des moyens conducteurs (7) de façon à ce que lesdits signaux analogiques puissent être pris et traités d'une manière indépendante par une unité logique (9), **caractérisée en ce qu'**un ou plusieurs desdits secteurs (6) sont désactivés au cas où au moins une desdites photodiodes (5) appartenant auxdits un ou plusieurs secteurs (6) ne fonctionnerait pas ou résulterait bruyante.

2. Méthode selon la revendication 1), où ladite opération d'inhibition desdits un ou plusieurs secteurs (6) prévoit que lesdits un ou plusieurs secteurs (6) sont désactivés à niveau logique, au cas où ladite unité logique (9) serait du type numérique.

3. Méthode selon la revendication 1), où ladite opération d'inhibition desdits un ou plusieurs secteurs (6) prévoit la désactivation des connexions physiques entre lesdits un ou plusieurs points d'extraction (8) correspondant auxdits un ou plusieurs secteurs (6) et ladite unité logique (9), au cas où ladite unité logique (9) serait du type analogique.

4. Méthode selon l'une quelconque des revendications précédentes, où ladite unité logique (9) produit en sortie au moins un signal résultant proportionnel au numéro desdits photons.

5. Méthode selon la revendication 4), où ladite unité logique (9) produit en sortie aussi des données relatives au temps de détection desdits photons.

6. Méthode selon l'une quelconque des revendications précédentes, où lesdits points d'extraction (8) sont positionnés sur le deuxième côté (10) dudit substrat semi-conducteur (2) opposé audit premier côté (4) où ladite surface sensible (3) est disposée par la connexion avec des colonnes conductrices (11) réalisées de façon à ce qu'elles passent à travers ledit substrat semi-conducteur (2).

7. Méthode selon l'une quelconque des revendications précédentes, où lesdits moyens conducteurs (7) comprennent une bande métallique (71) réalisée sur ladite surface sensible (3) pour la connexion en parallèle desdits photodiodes (5) appartenant à chacun desdits secteurs (6).

8. Méthode selon l'une quelconque des revendications précédentes, où ledit dispositif photomultiplicateur (1) comprend un élément de support diélectrique (13) essentiellement laminaire accouplé, à hauteur d'une première parmi ses faces (14), audit deuxième côté (10) dudit substrat semi-conducteur (2) et, à hauteur de sa deuxième face (16) opposée à ladite première face (14), à ladite unité logique (9) ayant une pluralité d'entrées (17), chacune desquelles est apte à être reliée à un desdits points d'extraction (8).

9. Méthode selon la revendication 8), où ledit dispositif photomultiplicateur (1) comprend une pluralité de câblages filaires (18) disposés de façon à passer à travers une ou plusieurs ouvertures (15) définies dans ledit élément de support diélectrique (13) et aptes à relier chacune desdites entrées (17) à un point d'extraction correspondant (8).

10. Méthode selon l'une quelconque des revendications précédentes 8) ou 9), où ledit élément de support diélectrique (13) est une carte à circuits imprimés (PCB) (131).

11. Méthode selon l'une quelconque des revendications précédentes, où chacun desdits secteurs (6) comprend lesdits photodiodes (5) dans un numéro inférieur à vingt.

12. Méthode selon l'une quelconque des revendications précédentes, où ledit dispositif photomultiplicateur

(1) comprend une résistance (12) de polysilicium reliée en série entre chacun desdits photodiodes (5) et les moyens conducteurs correspondants (7).

13. Méthode selon l'une quelconque des revendications précédentes, où ladite unité logique (9) est un circuit intégré (91).

14. Méthode selon l'une quelconque des revendications précédentes, où ladite unité logique (9) est du type numérique.

15. Méthode selon l'une quelconque des revendications de 1) à 13), où ladite unité logique (9) comprend un ou plusieurs amplificateurs/ additionneurs du type analogique.

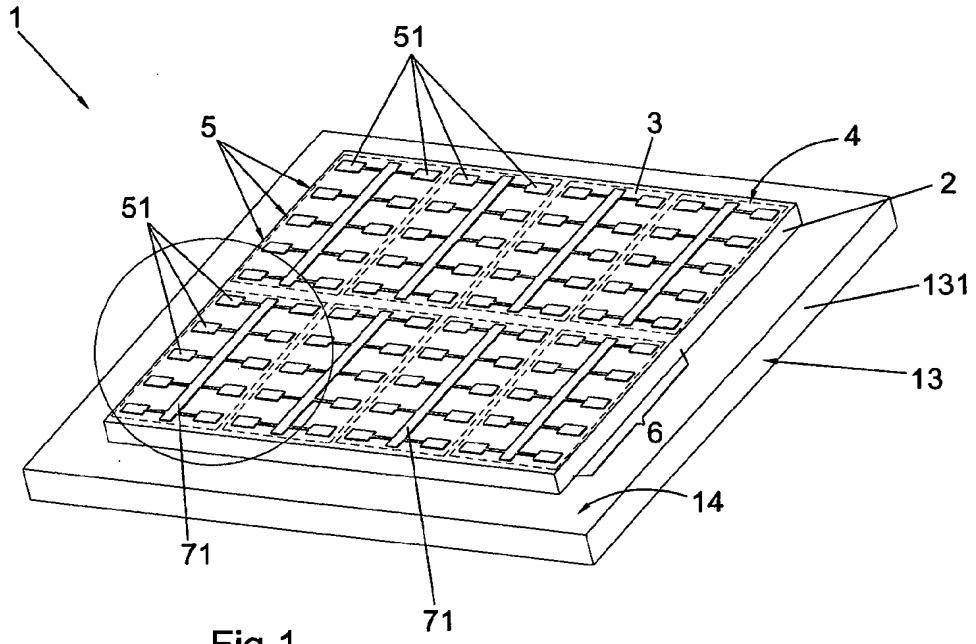


Fig.1

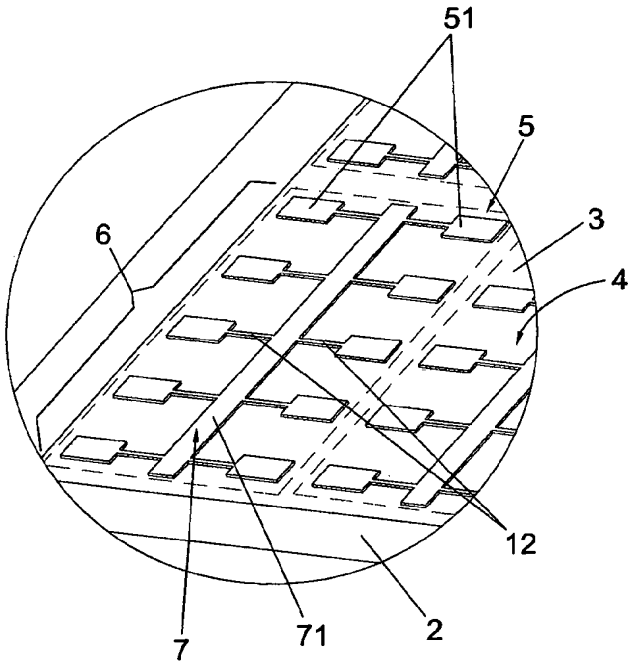


Fig.2

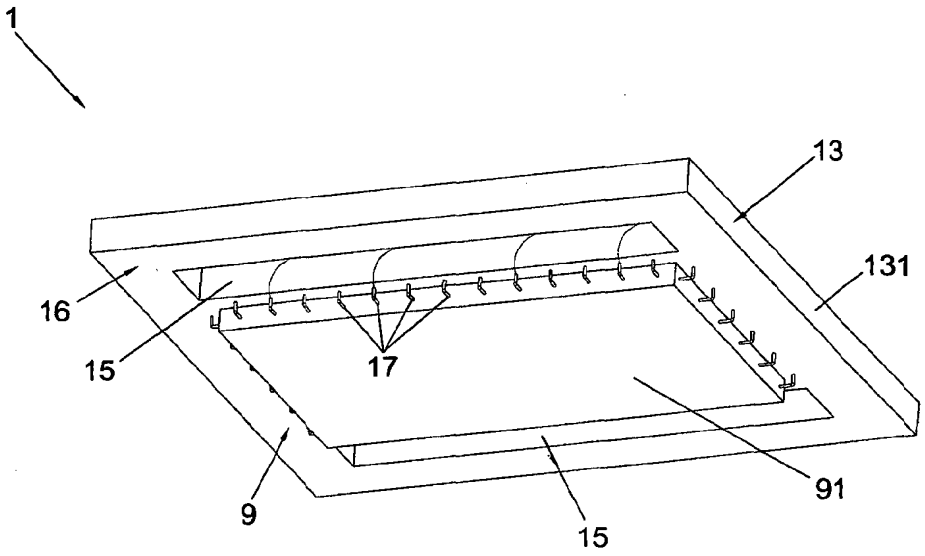


Fig.3

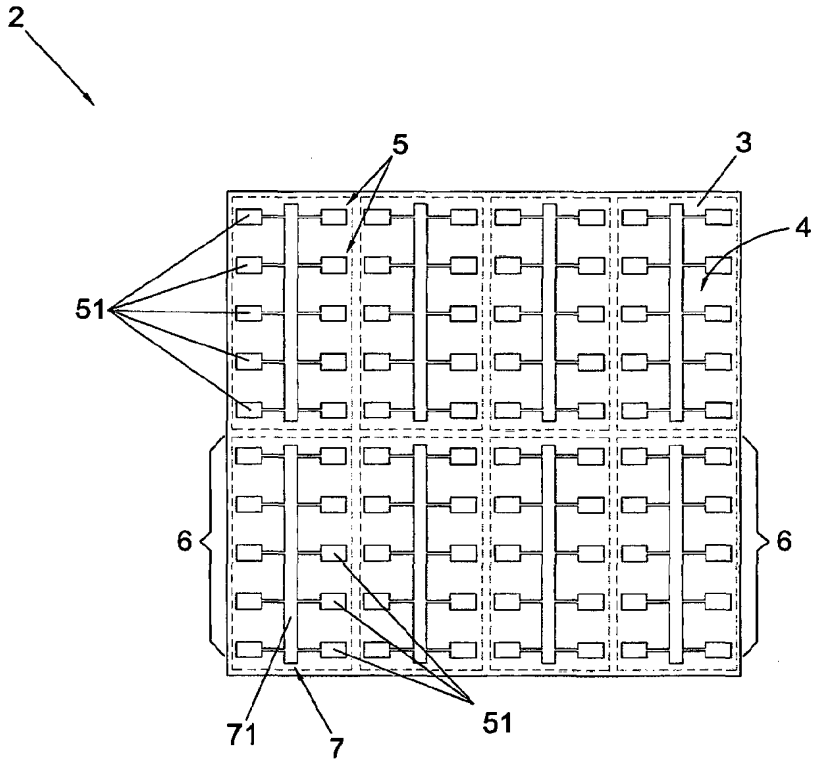


Fig.4

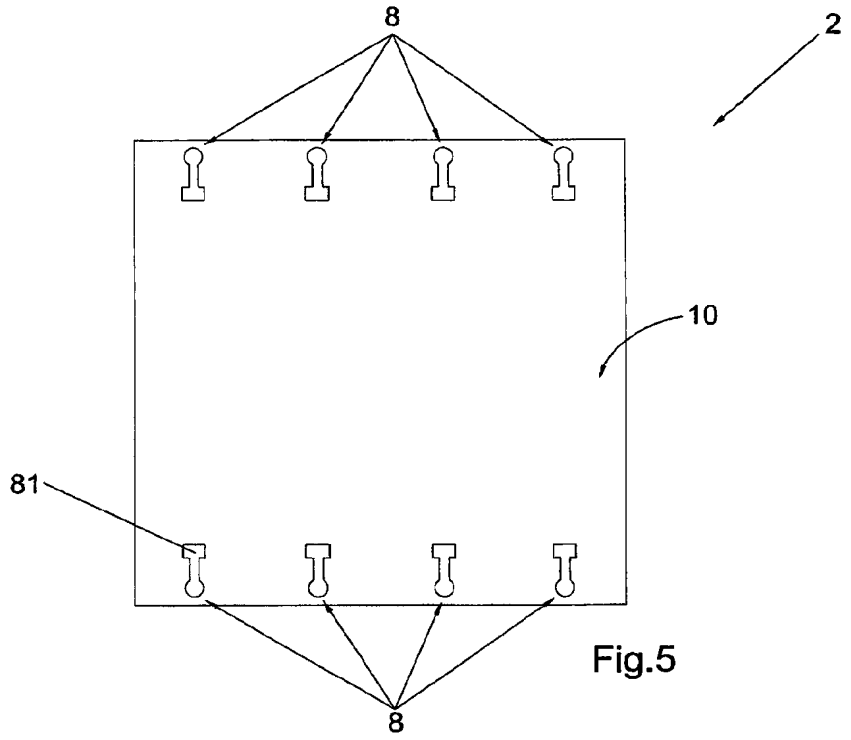


Fig.5

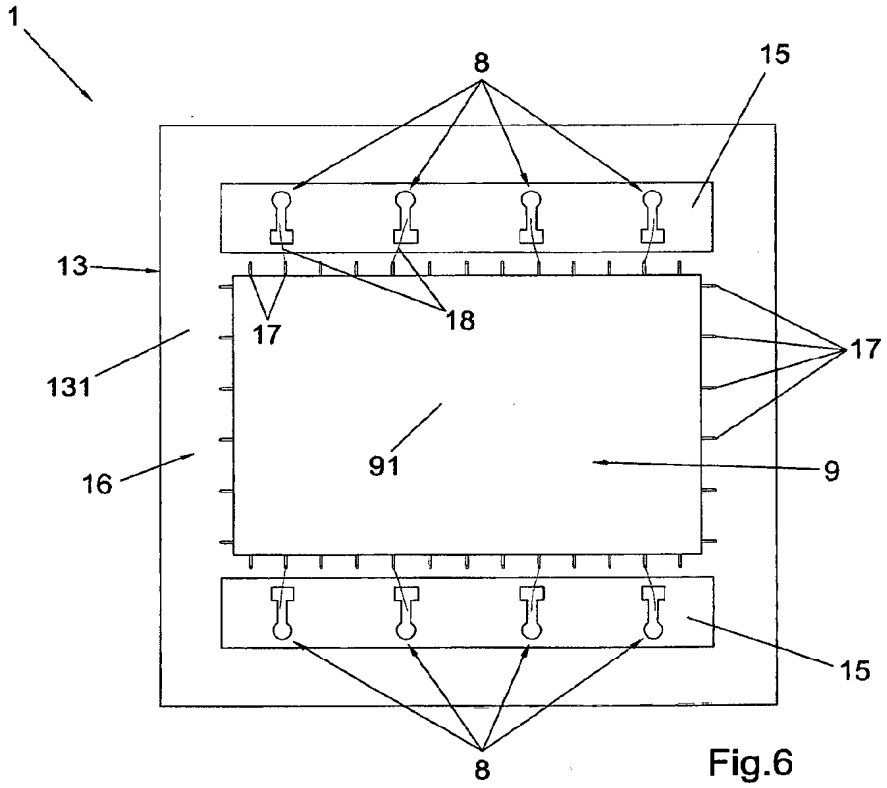


Fig.6

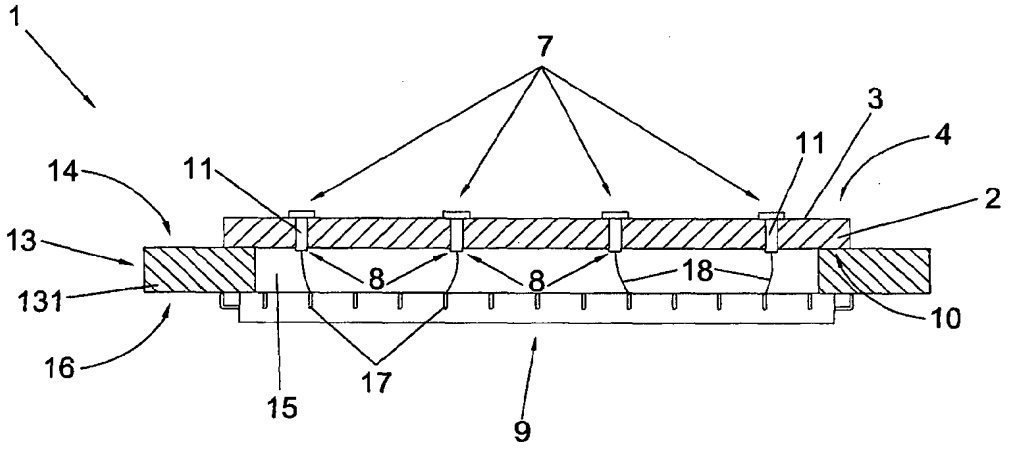


Fig.7

REFERENCES CITED IN THE DESCRIPTION

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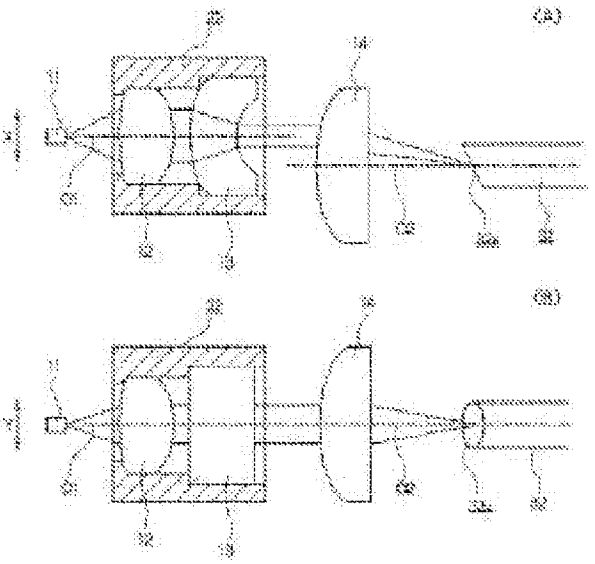
Bibliographic data: JPH05333245 (A) — 1993-12-17

No title available

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Classification:	**- international:** ***G02B6/42;*** (IPC1-7): G02B6/42 **- cooperative:** **G02B6/4206**; G02B6/4207
Application number:	JP19920170191 19920603
Priority number(s):	JP19920170191 19920603

Abstract of JPH05333245 (A)

PURPOSE:To correct laser light which have elliptic or long circular divergence characteristics into almost circular luminous flux and to facilitate the assembling of optical components for the correction and the mutual positioning of the respective optical components. CONSTITUTION:A collimator lens 12 and a cylinder lens 13 are held by the same holding member 22 and mutually positioned, and also positioned about a semiconductor 11. The condenser lens 14 and the core 32 of an optical fiber are previously set coaxially. Then the position of the holding member 22 is adjusted on an X-Y plane to align optical axes O1 and O2 with each other in the Y direction and the quantity of deviation between the optical axes O1 and O2 is set in the X direction to put laser light, which is made incident on the slanting end surface 32a of the core 32, in phase.



(19)日本国特許庁（ＪＰ）　(12)**公開特許公報（Ａ）**　(11)特許出願公開番号

特開平5－333245

(43)公開日　平成５年(1993)12月17日

(51)Int.Cl.[5]	識別記号	庁内整理番号	ＦＩ	技術表示箇所
Ｇ０２Ｂ　6/42		7132－2K		

審査請求　未請求　請求項の数３(全　６　頁)

(21)出願番号　特願平4－170191

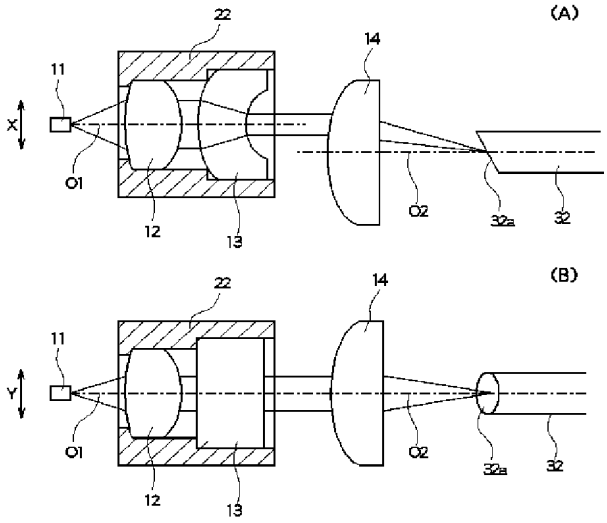
(22)出願日　平成４年(1992)６月３日

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(54)【発明の名称】　光通信用光学装置およびその調整方法

(57)【要約】
【目的】　楕円または長円の発散特性を有するレーザ光をほぼ真円の光束に矯正できるようにし、且つこの矯正のための光学部品の組付けおよび各光学部品の相互位置合わせを容易にできるようにする。
【構成】　コリメートレンズ１２とシリンダーレンズ１３とが同じ保持部材２２により保持されて互いに位置合わせされ、さらに半導体レーザ１１とも位置合わせされている。また集光レンズ１４と光ファイバーのコア３２は予め同軸に設定されている。そして保持部材２２をＸ－Ｙ平面にて位置調整することにより、Ｙ方向では光軸Ｏ１とＯ２を一致させ、Ｘ方向では光軸Ｏ１とＯ２のずれ量を設定して、コア３２の傾斜端面３２ａに入射するレーザ光の位相を整合できるようにする。

図３



【特許請求の範囲】
【請求項１】　半導体レーザと、この半導体レーザからの発散光を平行光にするコリメートレンズと、コリメートレンズの次に配置され前記半導体レーザの発散特性に基づく光束の断面形状を矯正するシリンダーレンズと、このシリンダーレンズにより矯正された光を光ファイバーの端面に集光させる集光レンズとが設けられており、前記シリンダーレンズと、コリメートレンズまたは集光レンズのいずれか一方とが、同じ保持部材により保持されていることを特徴とする光通信用光学装置。
【請求項２】　光ファイバーの端面はファイバー軸の直交面に対して傾斜して形成されており、この光ファイバーの軸と集光レンズの光軸とが同軸に配置され、コリメートレンズと集光レンズのそれぞれの光軸が前記光ファイバーの端面の傾斜方向にずれて配置され、光ファイバーの端面に対し集光光束が位相整合される角度により入射される請求項１記載の光通信用光学装置。
【請求項３】　請求項１または２記載の光通信用光学装置を使用し、前記保持部材と他方のレンズとの相対位置を合わせることを特徴とする光通信用光学装置の調整方法。
【発明の詳細な説明】
【０００１】
【産業上の利用分野】本発明は、光信号の増幅部などに使用される光通信用光学装置に係り、特に、半導体レーザから楕円または長円状に発散される光束を矯正する機能を有し、且つ組立てならびに調整作業を効率よくできるようにした光通信用光学装置およびその調整方法に関する。
【０００２】
【従来の技術】図１０は、光通信装置における光信号の増幅部を示している。この増幅部では、励起用光学装置１にて励起用光源から発せられたレーザ光が光ファイバーＦ２内に送られ、光ファイバーＦ１にて伝達される光信号Ｓと前記励起用のレーザ光とがカップラ２にて混合され、アイソレータ３を介してＥｒ（エルビウム）光ファイバーＦｅに送られる。Ｅｒ光ファイバーＦｅ内にて、光信号Ｓが励起用レーザ光により励起されることにより、光信号Ｓが増幅される。前記励起用光学装置１では、図９に示すように、励起用光源である半導体レーザ１ａからのレーザ光が、コリメートレンズ１ｂにより平行光束とされ、集光レンズ１ｃにより、前記光ファイバーＦ２のコア端面に集光されて入射される。
【０００３】
【発明が解決しようとする課題】前記励起用光源の半導体レーザ１ａとして、例えば波長が０．９８μｍの歪超格子ＩｎＧａＡｓの半導体レーザなどを使用した場合、図７に示すように、半導体チップからの発散光の角度が直交方向のそれぞれにおいて相違し、その光束断面が真円ではなく楕円または長円形状になる。図９に示すような光学装置において、図７に示すような楕円または長円形状の発散光をそのまま集光させて光ファイバーＦ２のコア端面に入射させると、結合効率が低下し光源から光ファイバーＦ２のコアに入射する光量が低下する。図８は半導体レーザ１ａからの発散光のビーム直径の比（ωｖ／ωｈ）と、光ファイバーＦ２への光結合効率のレベル低下（単位：ｄＢ）との関係を線図により示している。この線図に示すように、ビーム直径の比（ωｖ／ωｈ）が大きくなるにしたがって、結合効率のレベル低下が顕著になる。
【０００４】そこで、前記励起用光学装置１には、図７に示すような楕円または長円の発散角度の相違を矯正するためのプリズムなどの光学部品を設けることが必要になる。ところがこの種の光学部品を光学装置内に設けた場合、この光学部品とレンズや半導体レーザなどの他の光学部品および光ファイバーＦ２の端面などとの位置合わせ作業が非常に煩雑なものとなる。
【０００５】本発明は上記従来の課題を解決するものであり、ビーム直径比の矯正が必要となる半導体レーザを使用した場合に、この矯正のための光学部品を位置合わせ容易に組み込むことができる光通信用光学装置およびその調整方法を提供することを目的としている。
【０００６】
【課題を解決するための手段】本発明による光通信用光学装置は、半導体レーザと、この半導体レーザからの発散光を平行光にするコリメートレンズと、コリメートレンズの次に配置され、前記半導体レーザの発散特性に基づく光束の断面形状を矯正するシリンダーレンズと、このシリンダーレンズにより矯正された光を光ファイバーの端面に集光させる集光レンズとが設けられており、前記シリンダーレンズと、コリメートレンズまたは集光レンズのいずれか一方とが、同じ保持部材により保持されていることを特徴とするものである。
【０００７】さらに、前記光ファイバーの端面はファイバー軸の直交面に対して傾斜して形成されており、この光ファイバーの軸と集光レンズの光軸とが同軸に配置され、コリメートレンズと集光レンズのそれぞれの光軸が前記光ファイバーの端面の傾斜方向にずれて配置され、光ファイバーの端面に対し集光光束が位相整合される角度により入射されるものである。
【０００８】また、本発明による調整方法は、前記いずれかの光通信用光学装置を使用し、前記保持部材と他方のレンズとの相対位置を合わせることを特徴とするものである。
【０００９】
【作用】上記手段では、半導体レーザからの発散ビームの形状を矯正する光学部品としてシリンダーレンズが使用されている。このシリンダーレンズがコリメートレンズまたは集光レンズと同じ保持部材により保持されていることにより、シリンダーレンズといずれかのレンズと

の光軸合わせを保持部材に組込む段階で行うことができる。よって装置組立ての際の調整としては、前記保持部材を他方のレンズや半導体レーザに対して位置合わせするだけで、各光学部品相互の位置調整を簡単で且つ高精度にできるようになる。

【００１０】

【実施例】以下、本発明について図面を参照して説明する。図１以下は本発明の実施例として、光通信装置の増幅部に使用される励起用光学装置を示している。図１は、励起用光学装置１０の構造を示す平断面図、図６はこの励起用光学装置１０に組込まれた光学部品の配置を示す斜視図である。図６に示すように、この光学装置１０には、励起用光源として例えば波長が０．９８μｍの歪超格子ＩｎＧａＡｓの半導体レーザ１１と、半導体レーザ１１からの発散光を平行光束にするコリメートレンズ１２と、半導体レーザ１１から発せられるビームの直径比を矯正するシリンダーレンズ１３と、シリンダーレンズ１３により矯正されたレーザ光を光ファイバーＦ２の端面１５に対して集光させて入射させる集光レンズ１４とが設けられている。

【００１１】半導体レーザ１１と、シリンダーレンズ１３との配置関係は、半導体レーザ１１から発せられたレーザ光束の長径方向（光束径ωｖ方向）が、シリンダーレンズ１３の円周面に沿う方向に向けられ、レーザ光の短径方向（光束径ωｈ方向）がシリンダーレンズ１３の円周面と直交する方向（円周面の軸方向）へ向けられている。よって、レーザ光の長径方向がシリンダーレンズ１３により集束されて、断面がほぼ真円となるように矯正される。この矯正された光束が集光レンズ１４により集光されて、光ファイバーＦ２の端面１５に入射される。

【００１２】図１に示す励起用光学装置１０は、光ファイバーＦ２を着脱できるコネクタ方式のものである。図１の実施例では、前記半導体レーザ１１が金属ケース２１内に収納されている。コリメートレンズ１２とシリンダーレンズ１３は互いに光軸が一致するように調整され、且つ光軸方向の距離が設定された状態で保持部材２２内に保持されている。この保持部材２２は金属ケース２１の前端部に溶接または接着により固定されているが、この固定作業の際に、半導体レーザ１１の発光点とコリメートレンズ１２の光軸とが一致するように位置合わせ調整され、さらに半導体レーザ１１とコリメートレンズ１２の光軸方向の距離も調整され設定される。そして前記金属ケース２１と保持部材２２とが、支持筒２３の内部に固定される。この支持筒２３は、小径ブロック２４の外周に装着され、支持筒２３が小径ブロック２４に対しＺ方向へ進退できるように取り付けられる。

【００１３】前記小径ブロック２４は大径ブロック２５の突き当て面Ａに突き当てられ、相対位置の調整後に接着または溶接により互いに固定される。大径ブロック２５内には集光レンズ１４が保持されている。またその右側にはスペーサ２６が設けられ、このスペーサ２６に嵌合されたスリーブ２７に、前記光ファイバーＦ２の先端部が嵌着される。この光ファイバーＦ２のクラッド３１およびコア３２の先端面はほぼ球面に形成されている。図１では省略しているが、前記光ファイバーＦ２の先部はコネクタに保持されており、このコネクタがコネクタブロック２８に装着される際に、光ファイバーＦ２の先端がスリーブ２７に嵌着されてコア３２と集光レンズ１４の光軸との位置合わせが行われ、且つ光ファイバーＦ２の先端（コア３２の先端面３２ａ）がスペーサ２６に突き当てられることにより、集光レンズ１４とコア先端面３２ａとの距離Ｌ１が決められる。

【００１４】このように、大径ブロック２５側においては、前記集光レンズ１４の取り付け位置、スペーサ２６およびスリーブ２７の寸法により、光ファイバーＦ２がスリーブ２７に嵌着された状態で、集光レンズ１４の光軸とコア３２の光軸とが一致し、また集光レンズ１４とコア３２の先端面３２ａとの距離Ｌ１が設定できるようになっている。一方、前記小径ブロック２４側においては、金属ケース２１と保持部材２２の相互位置設定により、半導体レーザ１１、コリメートレンズ１２およびシリンダーレンズ１３の光軸が一致し、またそれぞれの光軸方向の位置が設定されている。

【００１５】よってこの光学装置１０を組立てる際には、突き当て面Ａに突き当てた小径ブロック２４を大径ブロック２５に対しＸ－Ｙ平面内にて微動さることにより全ての光学部品の光軸合わせができる。また小径ブロック２４に嵌装された支持筒２３をＺ方向へ微動させることにより、シリンダーレンズ１３と集光レンズ１４との距離Ｌ２の調整もできるようになっている。

【００１６】図２および図３は、図１に示す光学装置１０における各光学部品の位置関係を実施例別に示している。図２と図３のそれぞれにおいて、（Ａ）は図６のａ矢視方向の平面図、（Ｂ）は同図のｂ矢視方向の側面図を示している。図２（Ａ）（Ｂ）は、コリメートレンズ１２、シリンダーレンズ１３、集光レンズ１４、コア３２が全て同軸に位置する場合を示している。前記のように小径ブロック２４側にてコリメートレンズ１２とシリンダーレンズ１３とが予め軸合わせされ、大径ブロック２５側にて集光レンズ１４の光軸とコア３２の軸とが予め軸合わせされているため、図１に示す光学装置１０の組立て作業において、小径ブロック２４を突き当て面Ａに沿ってＸ－Ｙ平面内にて微動させるだけで、前記各光学部品が同軸となるように調整できる。なお図２（Ａ）（Ｂ）では、コア３２の先端面３２ａは軸に対して垂直面である。よってこの先端面３２ａに反射防止膜などの反射防止処理を施して、コア３２の先端面３２ａに入射した光の反射光が半導体レーザ１１に戻るのを防止する必要がある。

【００１７】図３（Ａ）（Ｂ）は、コア３２への入射光が先端面３２ａにて反射されて半導体レーザ１１側に戻るのを防止するために、コア３２の先端面３２ａが軸の直交面に対して傾斜して形成されている。図３では、コア３２の先端面３２ａの傾斜方向がＸ方向へ向けられている。そしてコリメートレンズ１２およびシリンダーレンズ１３の光軸Ｏ１に対し、集光レンズ１４およびコア３２の光軸Ｏ２がＸ方向へずれている。このずれ量は、コア３２の傾斜先端面３２ａからコア３２内にレーザ光の位相が整合する状態で入射できるように設定される。すなわち図４に示すように、先端面３２ａへ入射される集束光の光軸をＢ、その入射角をαとし、コア３２の軸Ｏ２に沿う出射角をβとし、コア３２の屈折率をｎｃとすると、ｓｉｎ（α）＝ｎｃ・ｓｉｎ（β）である。なお図３（Ｂ）に示すように、前記光軸Ｏ１と光軸Ｏ２は、Ｙ方向においては一致している。図３に示すような光軸Ｏ１とＯ２の設定は、図１において小径ブロック２４を突き当て面Ａに沿って微動させて調整することにより可能である。

【００１８】次に図５（Ａ）（Ｂ）は、本発明の第２実施例を示している。この実施例では、突き当て面Ａの左側において、コリメートレンズ１２が半導体レーザ１１と軸合わせされて設置されている。すなわちコリメートレンズ１２を保持する保持部材４１が半導体レーザ１１の金属ケース２１の先端面に固定されるなどして両者の軸が一致させられ、この金属ケース２１と保持部材４１とが、例えば図１に示す小径ブロック２４に支持されている。また突き当て面Ａの右側には、シリンダーレンズ１３、集光レンズ１４ならびにコア３２が互いに位置決めされて設けられている。すなわちシリンダーレンズ１３と集光レンズ１４とが共通の保持部材４２に保持されており、この保持部材４２内にて、両レンズ１３と１４の光軸が一致するように設定されている。

【００１９】上記保持部材４２は例えば図１に示す大径ブロック２５に保持され、この保持部材４２とスリーブ２７に保持される光ファイバーＦ２との位置関係が互いに同軸となるように予め設定される。またコア３２の先端面３２ａは軸の直交面に対して傾斜しており、この傾斜方向はＹ方向に沿うように設定されている。組立て調整では、突き当て面Ａに沿って小径ブロック２４をＸ－Ｙ面にて移動させることにより、突き当て面Ａを挟む左右の光学部品の光軸Ｏ１とＯ２との関係を調整する。この調整では、図５（Ｂ）に示すように、光軸Ｏ１とＯ２とがＹ方向にずれるように設定する。このずれ量は、コア３２の先端面３２ａに対する入射光の光軸Ｂが図４に示したのと同じ関係となるように調整される。また図５（Ａ）に示すように前記光軸Ｏ１とＯ２はＸ方向には一致するように調整される。

【００２０】図５の実施例では、図３と同様にコリメートレンズ１２の光軸と集光レンズ１４の光軸とをずらすことにより、コア３２の傾斜した先端面３２ａに対し、レーザ光の位相を整合させて入射させることができるようにしている。ここで図３ではコリメートレンズ１２とシリンダーレンズ１３の光軸が予め一致しているが、図５では、後の調整により、コリメートレンズ１２の光軸とシリンダーレンズ１３の光軸とがずれる状態になる。ここでこの光軸のずれ方向がシリンダーレンズ１３の円周面に沿う方向（Ｘ方向）であると、シリンダーレンズ１３の円周面によりレーザ光に収差が発生する。そこで図５に示す実施例では同図（Ｂ）に示すように、光軸Ｏ１とＯ２とをシリンダーレンズ１３の円周面と直交する方向へずらし、同図（Ａ）に示すようにＸ方向では両光軸Ｏ１とＯ２を一致させている。このように光軸をシリンダーレンズ１３の集光に無関係なシリンダー軸方向へずらすことにより、収差が生じないようにしている。

【００２１】なお、図３の実施例では、コア３２の先端面３２ａの傾斜方向と光軸Ｏ１とＯ２のずれ方向が互いに一致していれば、こ傾斜方向および光軸のずれ方向はＸ－Ｙ平面上のどの方向であってもよい。また、上記実施例は、光通信の増幅部における励起用光学装置について説明したが、本発明は楕円または長円の発散光を発する半導体レーザを使用した光モジュールであればどのような用途のものであっても実施可能である。

【００２２】

【発明の効果】以上のように本発明によれば、半導体レーザの発散特性を矯正する光学部材としてシリンダーレンズを使用し、このシリンダーレンズをコリメートレンズまたは集光レンズと共通の保持部材に保持させたので、この両レンズの光軸合わせを予め行うことができる。よって装置全体の調整としてはこの保持部材により保持されているシリンダーレンズおよび一方のレンズを、他方のレンズ、半導体レーザなどに合わせるだけでよい。よって調整作業が簡単で且つ高精度な光結合ができる光学装置を構成できる。

【図面の簡単な説明】

【図１】本発明による光学装置の第１実施例を示す平断面図、

【図２】（Ａ）（Ｂ）は光学部品の光軸の設定状態を示す平面図および側面図、

【図３】（Ａ）（Ｂ）は他の実施例による光学部品の光軸の設定状態を示す平面図および側面図、

【図４】コアの傾斜端面への入射角の説明図、

【図５】（Ａ）（Ｂ）は本発明の第２実施例の光学装置における光学部品の光軸の設定状態を示す平面図および側面図、

【図６】光学装置の部品構成を示す斜視図、

【図７】半導体レーザの発散特性を示す斜視図、

【図８】半導体レーザからの発散光のビーム径比と光ファイバーへの結合効率との関係を示す線図、

【図９】従来の光学装置の部品配置説明図、

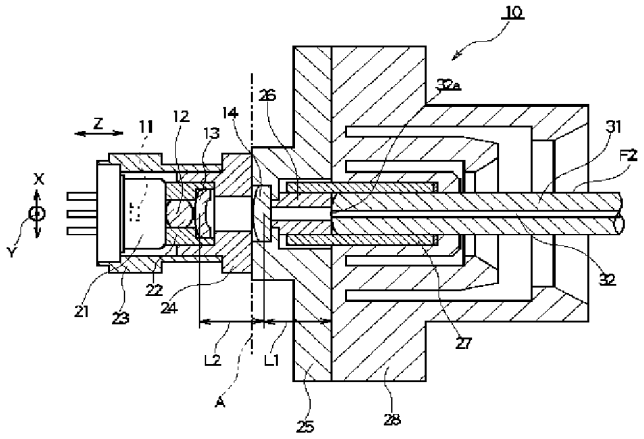
【図１０】光通信装置における信号増幅部を示す説明図、

【符号の説明】

１０　励起用光学装置
１１　半導体レーザ
１２　コリメートレンズ
１３　シリンダーレンズ
１４　集光レンズ
２１　金属ケース
２２　保持部材
２３　支持筒
２４　小径ブロック
２５　大径ブロック
２６　スペーサ
２７　スリーブ
２８　コネクタブロック
Ｆ２　光ファイバー
３１　クラッド
３２　コア
３２ａ　先端面

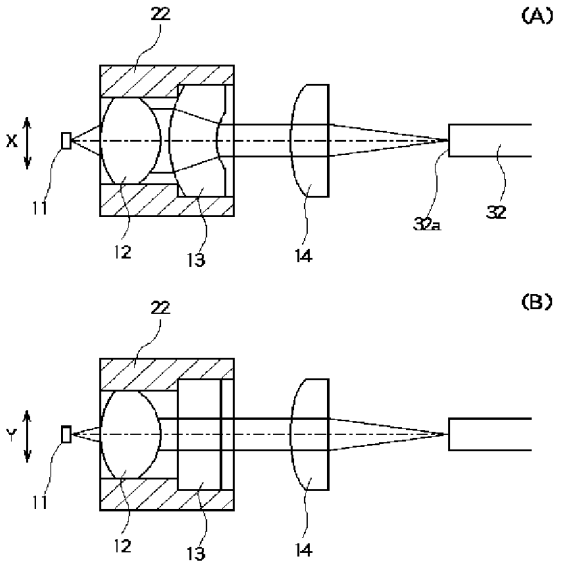
【図１】

図１



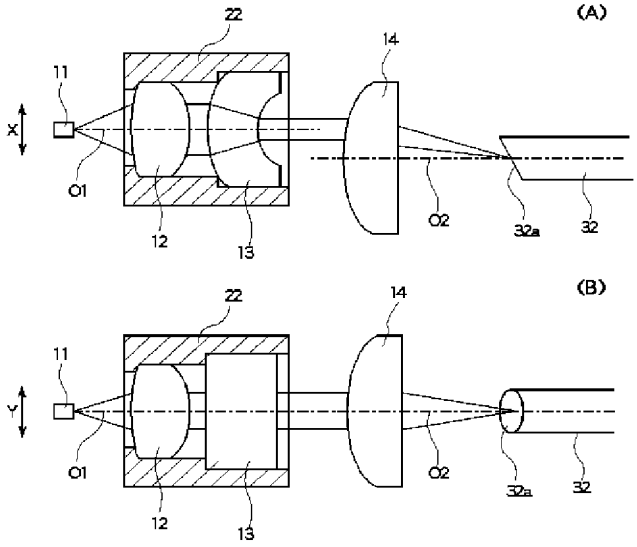
【図２】

図２



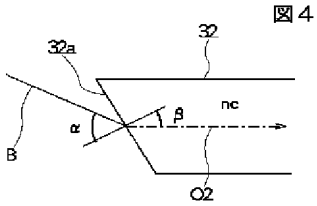
【図３】

図３



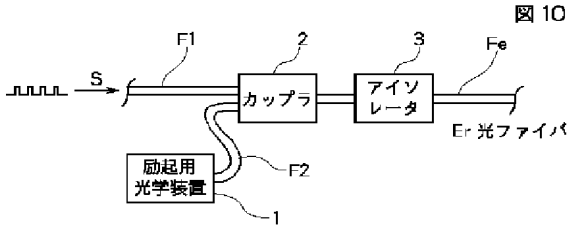
【図４】

図４



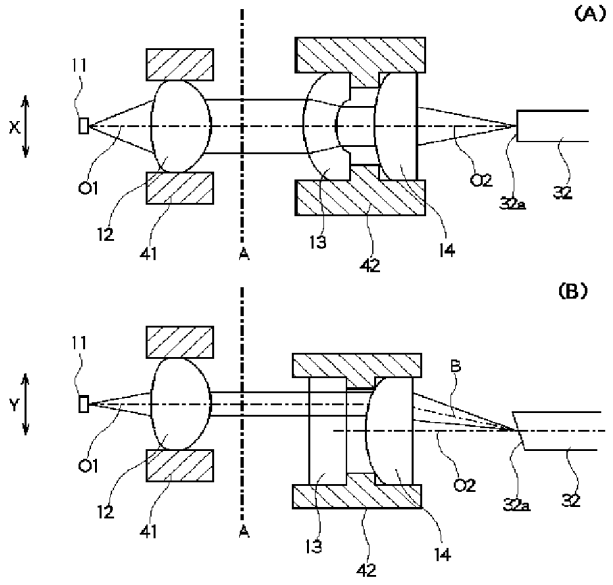
【図１０】

図 10



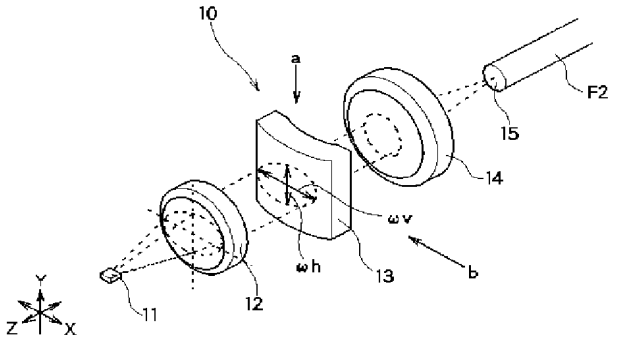
【図５】

図５



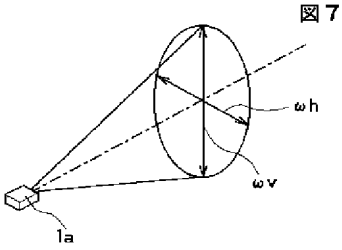
【図６】

図６



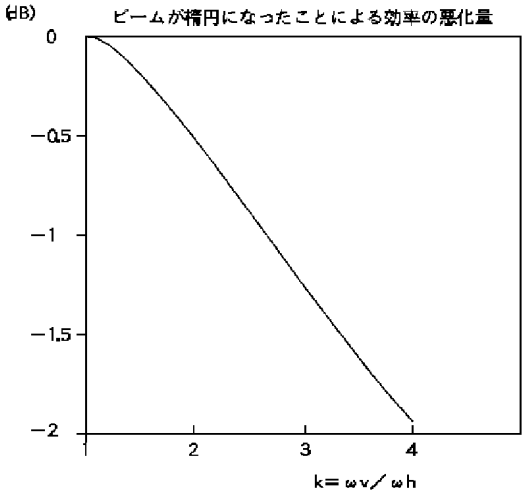
【図７】

図７



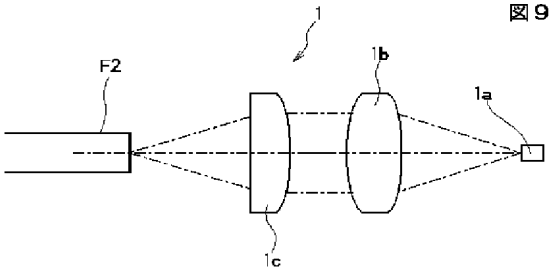
【図８】

図８



【図９】

図９



PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DONALD E. SCHREIBER
POST OFFICE BOX 2926
KINGS BEACH, CA 96143-2926

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	20 DEC 2013
Applicant's or agent's file reference 2294PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2013/043453	30 May 2013	30 May 2012

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G01N 15/14 (2013.01)
USPC - 356/337

Applicant CYTOJENE CORP.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	04 December 2013	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/043453

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/043453

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:
   - ☐ paid additional fees
   - ☐ paid additional fees under protest and, where applicable, the protest fee
   - ☐ paid additional fees under protest but the applicable protest fee was not paid
   - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - ☐ complied with
   - ☒ not complied with for the following reasons:

See first supplemental box.

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - ☐ all parts
   - ☒ the parts relating to claims Nos. 1-73

Form PCT/ISA/237 (Box No. IV) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/043453

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-73	YES
	Claims	None	NO
Inventive step (IS)	Claims	1-55	YES
	Claims	56-73	NO
Industrial applicability (IA)	Claims	1-73	YES
	Claims	None	NO

2. Citations and explanations:

Claims 56-58, 60-62, 65-67, 69-71 lack an inventive step under PCT Article 33(3) as being obvious over Fukuda et al., hereinafter referred to as Fukuda, in view of Dubelaar.

Regarding claim 56, Fukuda discloses a LD based optical subsystem for impinging a beam of light into a viewing zone in which a particle is present (cell analyzer 10 for flow cytometry, abstract, comprising semiconductor laser 53, pars. 37, 44; fig. 8), the optical subsystem comprising: a) a LD for emitting a diverging beam of light from an edge thereof (semiconductor laser 53, pars. 37, 44; fig. 8), the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis (radial laser light emitted from semiconductor laser 53, par. 38, has an elongated elliptical shape, par. 40; fig. 5); b) a collimating lens for converting the diverging beam of light emitted from said LD into a collimated elliptical beam of light (radial laser light emitted from the semiconductor laser 53 is converted to parallel rays by the collimator lens 52a, par. 38), wherein the minor axis of said collimated elliptical beam of light is oriented parallel to a direction in which particles pass through the viewing zone (lens system 52 produces a beam spot with a smaller diameter in the direction of the flow cell 51, par. 40; fig. 5); c) a beam compressing optical element for reducing the size of said elliptical beam of light at the viewing zone (condenser lenses 52d, 52e collect the light, pars. 38, 39, and reduce the size of the beam within the flow cell 51, figs. 8-9) whereby a width of said major axis of said elliptical beam of light oriented perpendicular to the direction in which particles pass through the viewing zone (lens system 52 produces a beam spot with a larger diameter in the direction of the flow cell 51, par. 40; fig. 5); and d) a cylindrical focusing element positioned with an axis of said cylindrical focusing element being oriented perpendicular to the direction in which particles pass through the viewing zone (a cylinder lens system comprising cylinder lenses 52b, 52c, par. 47, with lens axes perpendicular to the flow direction within flow cell 51, figs. 8, 9) whereby: said minor axis of said beam of light becomes focused at the viewing zone (light passes through lens system 52 such that it is focused on collecting point A within the measurement sample flowing in the flow cell 51, par. 38, where the shorter diameter of the beam spot is vertical along the direction of flow, par. 40; fig. 8); and the size of said major axis of said elliptical beam of light at the viewing zone remains essentially unchanged (light passes through lens system 52 such that it is collected on a second collecting point B behind the flow cell 51, par. 39, where the larger diameter of the beam spot is perpendicular to the flow direction, par. 39; fig. 9), but does not disclose a cylindrical focusing element positioned adjacent to the viewing zone. However, Fukuda discloses modifications may be made to the disclosed optical systems (lens system 52 is not limited to the structure described and may be variously modified, par. 41); furthermore, the particular arrangement of optical components is a design choice for one of ordinary skill in the art based on considerations such as available space and the chosen refractive power of optical components. Thus, it would have been obvious to one of ordinary skill in the art at the time of the invention to provide a cylindrical focusing element positioned adjacent to the viewing zone in the invention of Fukuda in order to reduce the overall space requirements for the optical subsystem and provide the necessary space for chosen optical components, since rearranging parts of an invention involves only routine skill in the art. Fukuda further does not disclose the width of said major axis of said elliptical beam is less than a width of a sheath flow. Dubelaar is in the field of particle detection systems with optical subsystems (detection of particles flowing through a flow cell including means to irradiate the particles and detect the light emitted, abstract), and teaches the width of an optical beam is less than a width of a sheath flow (laser beam 20 is focused by means 50 such that laser beam 20 is contained within sheath 33 surrounding suspension stream 30 within the flow cell defined by walls 11, p. 8, lines 18-30; figs. 2A-2B). It would have been obvious to one of ordinary skill in the art at the time of the invention to provide a beam width less than a width of a sheath flow as taught by Dubelaar in the invention of Fukuda in order to reduce the reception of spurious optical signals which may result if the beam spot is irradiated on elements outside the width of the sheath flow.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/043453

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Claim 56 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claim 56 is indefinite for the following reason: Claim 56 is lacking antecedent basis for "said liquid sheath flow" recited. For the purpose of the international opinion, claim 56 is interpreted as reciting "a liquid sheath flow".

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/043453

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Continuation of Box IV. Lack of Unity of Invention.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Species I) a laser diode based optical subsystem, figures 2, 2A, 3A, 3B, 4A, 4B

Species II) a composite microscope objective, figures 5A, 5B, 5C, 8, 9A

Species III) a fluidic subsystem, figures 14-15

Species IV) a peristaltic pump, figures 17-24

Species V) a wavelength division multiplexer, figures 25-30B

The claims are deemed to correspond to the species listed above in the following manner:

Species I) figures 2, 2A, 3A, 3B, 4A, 4B; claims 56-73

Species II) figures 5A, 5B, 5C, 8, 9A; claims 74-129

Species III) figures 14-15; claims 130-145

Species IV) figures 17-24; claims 146-161

Species V) figures 25-30B; claims 162-173

The following claims are generic: claims 1-55.

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

Species I) figures 2, 2A, 3A, 3B, 4A, 4B; have the special technical feature of a laser diode based optical subsystem. This special technical feature is not present in species II, III, IV or V.

Species II) figures 5A, 5B, 5C, 8, 9A; have the special technical feature of a composite microscope objective. This special technical feature is not present in species I, III, IV or V.

Species III) figures 14-15; have the special technical feature of a fluidic subsystem. This special technical feature is not present in species I, II, IV or V.

Species IV) figures 17-24; have the special technical feature of a peristaltic pump. This special technical feature is not present in species I, II, III or V.

Species V) figures 25-30B; have the special technical feature of a wavelength division multiplexer. This special technical feature is not present in species I, II, III or IV.

Species I, II, III, IV and V lack unity of invention because even though the inventions of these species require the technical feature of an optical system having liquid flow, directing light, and having a microscope, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of WO 2005/033654 A2 (TUNG et al) 14 April 2005 (14.04.2005) page 18, lines 21-29; page 19, line 1; 15-16.

Since none of the special technical features of the Species I, II, III, IV and V inventions is found in more than one of the inventions, unity of invention is lacking.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/043453

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V. Written Opinion

Regarding claim 65, Fukuda discloses a method for delivering an elliptically shaped beam of light using a LD based optical subsystem (cell analyzer 10 for flow cytometry, abstract, comprising semiconductor laser 53, pars. 37, 44; fig. 8; and associated methods, title, where the beam has an elongated elliptical shape, par. 40; fig. 5), the beam of light having a smooth profile at a focus of a minor axis thereof that is located at a viewing zone through which a sample liquid flows (the beam is collected (focused) at point A within the flow cell 51, par. 38, and has a smooth profile as indicated by the optical configuration of figs. 8, 9), the method comprising the steps of: a) providing a LD that emits a diverging beam of light from an edge thereof (semiconductor laser 53, pars. 37, 44; fig. 8), the diverging beam of light having an elliptically shaped cross-sectional profile with both a major axis and a minor axis (radial laser light emitted from semiconductor laser 53, par. 38, has an elongated elliptical shape, par. 40; fig. 5); b) impinging the diverging beam of light emitted by the LD upon a collimating lens for converting the diverging beam of light emitted therefrom into a collimated elliptical beam of light (radial laser light emitted from the semiconductor laser 53 is converted to parallel rays by the collimator lens 52a, par. 38) wherein the minor axis of said collimated elliptical beam of light is oriented parallel to a direction in which sample liquid passes through the viewing zone (lens system 52 produces a beam spot with a smaller diameter in the direction of the flow cell 51, par. 40; fig. 5); c) after passing through said collimating lens, impinging the collimated elliptical beam of light upon an beam compressing optical element for reducing the size of said elliptical beam of light at the viewing zone (condenser lenses 52d, 52e collect the light, pars. 38, 39, and reduce the size of the beam within the flow cell 51, figs. 8-9) whereby a width of said major axis of said elliptical beam of light oriented perpendicular to the direction in which sample liquid passes through the viewing zone (lens system 52 produces a beam spot with a larger diameter in the direction of the flow cell 51, par. 40; fig. 5); and d) after passing through said beam compressing optical element, impinging the beam of light upon a cylindrical focusing element with an axis of said cylindrical focusing element being oriented perpendicular to the direction in which sample liquid passes through the viewing zone (a cylinder lens system comprising cylinder lenses 52b, 52c, par. 47, with lens axes perpendicular to the flow direction within flow cell 51, figs. 8, 9) whereby: said minor axis of said beam of light becomes focused at the viewing zone (light passes through lens system 52 such that it is focused on collecting point A within the measurement sample flowing in the flow cell 51, par. 38, where the shorter diameter of the beam spot is vertical along the direction of flow, par. 40; fig. 8); and the size of said major axis of said elliptical beam of light at the viewing zone remains essentially unchanged (light passes through lens system 52 such that it is collected on a second collecting point B behind the flow cell 51, par. 39, where the larger diameter of the beam spot is perpendicular to the flow direction, par. 39; fig. 9), but does not disclose a cylindrical focusing element positioned adjacent to the viewing zone. However, Fukuda discloses modifications may be made to the disclosed optical systems (lens system 52 is not limited to the structure described and may be variously modified, par. 41); furthermore, the particular arrangement of optical components is a design choice for one of ordinary skill in the art based on considerations such as available space and the chosen refractive power of optical components. Thus, it would have been obvious to one of ordinary skill in the art at the time of the invention to provide a cylindrical focusing element positioned adjacent to the viewing zone in the invention of Fukuda in order to reduce the overall space requirements for the optical subsystem and provide the necessary space for chosen optical components, since rearranging parts of an invention involves only routine skill in the art. Fukuda further does not disclose the sample liquid being hydrodynamically focused within the viewing zone by a liquid sheath flow that also flows through the viewing zone, where a width of said elliptical beam becomes less than a width of said liquid sheath flow. Dubelaar is in the field of particle detection systems with optical subsystems (detection of particles flowing through a flow cell including means to irradiate the particles and detect the light emitted, abstract), and teaches a sample liquid being hydrodynamically focused within the viewing zone by a liquid sheath flow that also flows through the viewing zone (the particle suspension is injected under laminar conditions into a particle free sheath fluid which narrows down and accelerates the particle suspension to a very thin stream of suspension that is carried through the center of the flow cell, p. 1, lines 18-21; figs. 1-2), where a width of an optical beam becomes less than a width of said liquid sheath flow (laser beam 20 is focused by means 50 such that laser beam 20 is contained within sheath 33 surrounding suspension stream 30 within the flow cell defined by walls 11, p. 8, lines 18-30; figs. 2A-2B). It would have been obvious to one of ordinary skill in the art at the time of the invention to provide a sheath flow and a beam width less than a width of a sheath flow as taught by Dubelaar in the invention of Fukuda in order provide a laminar flow of particles to be detected (Dubelaar, p. 1, lines 10-27) and to reduce the reception of spurious optical signals which may result if the beam spot is irradiated on elements outside the width of the sheath flow.

Regarding claims 57, 66, Fukuda discloses a cuvette having rectangularly-shaped cross-section (flow cell 51, par. 37, shown as a cuvette, fig. 8, with a rectangular cross-section, figs. 4, 9), and wherein the viewing zone is located within a channel having a rectangularly-shaped cross-section that is located within said cuvette (the viewing zone within the channel within flow cell 51 with which the light beam interacts is shown to have a rectangularly-shaped cross-section, figs. 4, 9).

Regarding claims 58, 67, Fukuda does not disclose a cuvette having a tubularly-shaped cross-section, and wherein the viewing zone is located within a channel having a circularly-shaped cross-section that is located within said cuvette. Dubelaar teaches a cuvette having a tubularly-shaped cross-section (elongated cuvette 10, p. 7, lines 23-26, with a tubularly-shaped cross-section, fig. 1), and wherein the viewing zone is located within a channel having a circularly-shaped cross-section that is located within said cuvette (cuvette 10 has transparent wall 11 allowing the passage of laser beam 20, p. 7, lines 23-29, and channel with a circularly-shaped cross section through which particles flow and interact with beam 20, fig. 1). It would have been obvious to one of ordinary skill in the art at the time of the invention to provide a cuvette having a tubularly-shaped cross-section with a channel having a circularly-shaped cross-section as taught by Dubelaar in the invention of Fukuda in order to provide an optical intersecting area which is a cross section of the fluid stream, allowing accurate determination of the particle flux (Dubelaar, p. 4, lines 1-12).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US2013/043453

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claims 60, 69, Fukuda discloses said cylindrical focusing element is in optical contact with an entrance face of said rectangularly-shaped cuvette (cylinder lens system comprising lenses 52b and 52c, par. 37, is in optical contact with the entrance face of flow cell 51, figs. 8, 9).

Regarding claims 61, 70, Fukuda discloses said cylindrical focusing element is separated from said rectangularly-shaped cuvette [claim 61], by establishing a spacing between said cylindrical focusing element and said cuvette [claim 70] (cylinder lens system comprising lenses 52b and 52c, par. 37, is physically separated by an established space from flow cell 51, figs. 8, 9).

Regarding claims 62, 71, Fukuda as modified provides a tubularly-shaped cuvette as shown above, and Fukuda further discloses said cylindrical focusing element is separated from said tubularly-shaped cuvette [claim 62], by establishing a spacing between said cylindrical focusing element and said cuvette [claim 71] (cylinder lens system comprising lenses 52b and 52c, par. 37, is physically separated by an established space from flow cell 51, figs. 8, 9).

Claims 59, 63, 68, 72 lack an inventive step under PCT Article 33(3) as being obvious over Fukuda in view of Dubelaar, and further in view of Hollinger et al., hereinafter referred to as Hollinger.

Regarding claims 59, 68, Fukuda does not disclose the sample liquid and the liquid sheath flow form a jet stream in which the viewing zone is located. Hollinger is in the field of optically analyzing particles in a stream (flow-through particle analyzer using optical measurements, abstract), and teaches a sample liquid and a liquid sheath flow form a jet stream in which a viewing zone is located (a sheath liquid hydrodynamically focuses the particle stream and jets the same in a liquid jet from the nozzle, abstract; col. 2, lines 24-48). It would have been obvious to one of ordinary skill in the art at the time of the invention to provide a sample liquid and a sheath flow forming a jet stream as taught by Hollinger in the invention of Fukuda in order to enable particle sorting and introduction of the sheath without interfering with the optical assembly (Hollinger, col. 2, lines 48-57).

Regarding claims 63, 72, Fukuda as modified provides a jet stream as shown above, and Fukuda further teaches said cylindrical focusing element is separated from said jet stream [claim 63] by establishing spacing between said cylindrical focusing element and said jet stream [claim 72] (cylinder lens system comprising lenses 52b and 52c, par. 37, is physically separated by an established space from flow cell 51 and particle and sheath streams contained therein, figs. 8, 9).

Claims 64, 73 lack an inventive step under PCT Article 33(3) as being obvious over Fukuda in view of Dubelaar, and further in view of Wells.

Regarding claims 64, 73, Fukuda does not disclose a step of inserting a polarization conditioning element between the collimating lens and the beam compressing optical element whereby the collimated elliptical beam of light passes through the polarization conditioning element. Wells is in the field of flow cytometry (title; abstract), and teaches inserting a polarization conditioning element between a collimating lens and a beam compressing optical element (element 50 rotates the plane of polarization of the beam and is placed between collimating lens 3 and focusing lens 5, par. 22; fig. 5) whereby the collimated elliptical beam of light passes through the polarization conditioning element (the elliptical beam emitted from the laser diode passes through collimating lens 3 and then has its plane of polarization rotated by element 50, par. 22; fig. 5). It would have been obvious to one of ordinary skill in the art at the time of the invention to provide a polarization conditioning element as taught by Wells in the invention of Fukuda in order to enable detection of side-scattered light (Wells, par. 22).

Claims 1-55 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest:

Regarding claim 1, a flow cytometer comprising: a peristaltic pump for supplying the sample liquid carrying the particles, the sample liquid being hydrodynamically focused with the viewing zone by the liquid sheath flow, the peristaltic pump comprising: a compressible tube sandwiched between said rollers and the arcuate curved track of said pump housing; and a wavelength division multiplexer for separating into multiple colored bands a beam of light emitted initially from the viewing zone and imaged by the composite microscope objective into an optical fiber for transmission to the WDM, the WDM comprising: a collimating optical element, a focusing optical element, and an imaging relay optical element, in combination with the remaining aspects of the claim.

Claims 2-55 meet the criteria due to their dependence from novel claim 1.

The prior art, as shown above, details some aspects of the invention, however none of the prior art teaches all of the missing limitations either alone or in combination as specified.

Claims 1-73 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/043453

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 15/14 (2013.01)
USPC - 356/337
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - G01N 15/08, 10, 14, 21/01, 03, 05, 47, 53, 62, 64 (2013.01)
USPC -356/311, 317, 318, 337; 435/288.7; 436/164, 171, 172

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - G01N 15/08, 10, 14, 1434, 21/01, 03, 05, 47, 53, 62, 64 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0091746 A1 (FUKUDA et al) 09 April 2009 (09.04.2009) entire document	56-73
Y	WO 2001/40764 A2 (DUBELAAR) 07 June 2001 (07.06.2001) entire document	56-73
Y	US 4,515,274 A (HOLLINGER et al) 07 May 1985 (07.05.1985) entire document	59, 63, 68, 72
Y	US 2006/0221325 A1 (WELLS) 05 October 2006 (05.10.2006) entire document	64, 73
A	US 2002/0141902 A1 (OZASA et al) 03 October 2002 (03.10.2002) entire document	1-73
A	US 2008/0024758 A1 (TABATA) 31 January 2008 (31.01.2008) entire document	1-73
A	WO 2005/033654 A2 (TUNG et al) 14 April 2005 (14.04.2005) page 18, lines 21-29; page 19, line 1, 15-16	1-73

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 December 2013	20 DEC 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/043453

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-73

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

Continuation of Box III.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Species I) a laser diode based optical subsystem, figures 2, 2A, 3A, 3B, 4A, 4B

Species II) a composite microscope objective, figures 5A, 5B, 5C, 8, 9A

Species III) a fluidic subsystem, figures 14-15

Species IV) a peristaltic pump, figures 17-24

Species V) a wavelength division multiplexer, figures 25-30B

The claims are deemed to correspond to the species listed above in the following manner:

Species I) figures 2, 2A, 3A, 3B, 4A, 4B; claims 56-73

Species II) figures 5A, 5B, 5C, 8, 9A; claims 74-129

Species III) figures 14-15; claims 130-145

Species IV) figures 17-24; claims 146-161

Species V) figures 25-30B; claims 162-173

The following claims are generic: claims 1-55.

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

Species I) figures 2, 2A, 3A, 3B, 4A, 4B; have the special technical feature of a laser diode based optical subsystem. This special technical feature is not present in species II, III, IV or V.

Species II) figures 5A, 5B, 5C, 8, 9A; have the special technical feature of a composite microscope objective. This special technical feature is not present in species I, III, IV or V.

Species III) figures 14-15; have the special technical feature of a fluidic subsystem. This special technical feature is not present in species I, II, IV or V.

Species IV) figures 17-24; have the special technical feature of a peristaltic pump. This special technical feature is not present in species I, II, III or V.

Species V) figures 25-30B; have the special technical feature of a wavelength division multiplexer. This special technical feature is not present in species I, II, III or IV.

Species I, II, III, IV and V lack unity of invention because even though the inventions of these species require the technical feature of an optical system having liquid flow, directing light, and having a microscope, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of WO 2005/033654 A2 (TUNG et al) 14 April 2005 (14.04.2005) page 18, lines 21-29; page 19, line 1, 15-16.

Since none of the special technical features of the Species I, II, III, IV and V inventions is found in more than one of the inventions, unity of invention is lacking.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
Shishima, Gina N.
FULBRIGHT & JAWORSKI L.L.P.
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701
ETATS-UNIS D'AMERIQUE

NORTON ROSE FULBRIGHT
IPT DOCKETING
Docketed ☐ Not Req'd ☐ Confirmation ☐
Initials 1st _____ Initials 2nd _____
APR 14 2015
Attorney _____
Docket No. _____
Action Req'd Date Due

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference BECC.P0003WO	Date of mailing *(day/month/year)* 9 April 2015 (09-04-2015) **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2014/067682	International filing date *(day/month/year)* 26 November 2014 (26-11-2014)
Applicant IRIS INTERNATIONAL, INC.	RECEIVED F & J AUSTIN INTL APR 14 2015

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   How? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 338.82.70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 - 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	Authorized officer PORRACHIA, Isabelle Tel: +31 (0)70 340-4447

Form PCT/ISA/220 (July 2014)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
BECC.P0003WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2014/067682	26 November 2014 (26-11-2014)	4 December 2013 (04-12-2013)
Applicant		
IRIS INTERNATIONAL, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed
      ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (See Box No. II)

3. ☒ **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   ☒ the text is approved as submitted by the applicant
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   ☒ the text is approved as submitted by the applicant
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. 37
      ☐ as suggested by the applicant
      ☒ as selected by this Authority, because the applicant failed to suggest a figure
      ☐ as selected by this Authority, because this figure better characterizes the invention
   b. ☐ none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/067682

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see annex

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/067682

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N15/14
ADD. G01N21/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 889 319 A1 (ABB RESEARCH LTD [CH]) 7 January 1999 (1999-01-07) figure 1 page 6, line 42	1-15, 130-143
X	BROGIOLI DORIANO ET AL: "Heterodyne near-field scattering", APPLIED PHYSICS LETTERS, AMERICAN INSTITUTE OF PHYSICS, US, vol. 81, no. 22, 25 November 2002 (2002-11-25), pages 4109-4111, XP012032611, ISSN: 0003-6951, DOI: 10.1063/1.1524702 the whole document	1-15, 130-143
X	WO 93/16368 A1 (CAMBRIDGE CONSULTANTS [GB]) 19 August 1993 (1993-08-19) page 14 - page 15; figure 6	1-5

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 February 2015	09/04/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Mauritz, Jakob

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	WO 2013/181453 A2 (CYTOJENE CORP [US]; CHEN YONG QIN [US]) 5 December 2013 (2013-12-05) the whole document	1-15
A	US 2012/274925 A1 (CHEN YONG [US] ET AL) 1 November 2012 (2012-11-01) figure 3	1-15

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/067682

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0889319 A1	07-01-1999	NONE	
WO 9316368 A1	19-08-1993	AT 166458 T	15-06-1998
		DE 69318632 D1	25-06-1998
		DE 69318632 T2	25-02-1999
		DK 0579829 T3	08-03-1999
		EP 0579829 A1	26-01-1994
		JP 3144687 B2	12-03-2001
		JP H06507025 A	04-08-1994
		WO 9316368 A1	19-08-1993
WO 2013181453 A2	05-12-2013	EP 2861956 A2	22-04-2015
		TW 201405116 A	01-02-2014
		WO 2013181453 A2	05-12-2013
US 2012274925 A1	01-11-2012	CN 103430009 A	04-12-2013
		EP 2702389 A1	05-03-2014
		US 2012274925 A1	01-11-2012
		WO 2012149041 A1	01-11-2012

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-8, 130-136(completely); 9-15, 137-143(partially)

   heterodyne mode detector

   ---

2. claims: 9-15, 137-143(all partially)

   microscope objective

   ---

3. claims: 14, 15, 142, 143(all partially)

   ellipsoidal mirror

   ---

4. claims: 15, 143(all partially)

   flat mirror

   ---

5. claims: 16-129, 144-190

   system

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2014/067682	26.11.2014	04.12.2013

International Patent Classification (IPC) or both national classification and IPC
INV. G01N15/14 ADD. G01N21/53

Applicant
IRIS INTERNATIONAL, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Telephone No. +31 70 340-0

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 16-129, 144-190(completely); 9-15, 137-143(partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for the whole application or for said claims Nos. 16-129, 144-190(completely); 9-15, 137-143(partially)

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-8, 130-136(completely); 9-15, 137-143(partially)

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6-8, 131-136(completely); 9-15, 137-143(partially)
	No:	Claims	1-5, 130
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-8, 130-136(completely); 9-15, 137-143(partially)
Industrial applicability (IA)	Yes:	Claims	1-8, 130-136(completely); 9-15, 137-143(partially)
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

   and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

   see form 210

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1 EP 0 889 319 A1 (ABB RESEARCH LTD [CH]) 7 January 1999 (1999-01-07)

D2 BROGIOLI DORIANO ET AL: "Heterodyne near-field scattering", APPLIED PHYSICS LETTERS, AMERICAN INSTITUTE OF PHYSICS, US,
vol. 81, no. 22, 25 November 2002 (2002-11-25), pages 4109-4111, XP012032611,
ISSN: 0003-6951, DOI: 10.1063/1.1524702

D3 WO 93/16368 A1 (CAMBRIDGE CONSULTANTS [GB]) 19 August 1993 (1993-08-19)

D4 WO 2013/181453 A2 (CYTOJENE CORP [US]; CHEN YONG QIN [US]) 5 December 2013 (2013-12-05)

Re Item IV

Lack of unity of invention

1 This Authority considers that the application does not meet the requirements of unity of invention and that there are at least five inventions covered by the claims indicated as follows:

- Group I: claims: 1-8, 130-136 (completely); 9-15, 137-143 (partially when referring back to claims 2-8, 131-136) **heterodyne mode detector**
- Group II: claims: 9-15, 137-143 (all partially when referring back to the independent claim 1 or 130, resp.) **microscope objective**
- Group III: claims: 14, 15, 142, 143 (all partially when referring back to the independent claim 1 or 130, resp.) **ellipsoidal mirror**
- Group IV: claims: 15, 143 (all partially when referring back to the independent claim 1 or 130, resp.) **flat mirror**
- Group V: claims: 29-129,144-190: **optical system**

2 The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:

3 The prior art has been identified as EP0889319 (=D1, Fig. 7) and discloses a light detection system comprising:

- a concave mirror (Fig. 1, *Hohlspiegel 30*) for reflecting light that propagates from a viewing zone; and
- a detector for measuring axial light loss produced by an object in the viewing zone by detecting light reflected by the concave mirror (column 6, line 42: *Auswerteeinheit 12*).

4 The only common technical feature of claim 1 with all of the other independent claims (except for the corresponding method claim 130) is a "concave mirror".

5 This feature is known from D1 and cannot therefore unite the independent claims by providing a single general *inventive* concept.

6 Therefore the claims are split into two groups,

- group (a) with claims 1-15,130-143
- group (b) with the remaining claims (29-129,144-190)

7 Group (a) is subdivided further, as the claims contained therein are also not unitary:

8 The following technical features of dependent claims 2-15 and 130-143 make a contribution over the prior art and can be considered as a special technical feature within the meaning of Rule 13.2 PCT:

- claims 2-8 (and partially claim 9-15 when referring back to claim 2-8): **detector [arranged to] measure in a heterodyne mode coherent interference of the forward scattered light and the remaining light**
- claims 9 (when referring back to claim 1, including 10-13 partially when referring back to claim 1 via claim 9): **microscope objective**
- claim 14 (when referring back to claim 1): **ellipsoidal mirror**
- claim 15 (when referring back to claim 1): **flat mirror and lens**

9 The technical effect and problem solved by these special technical features can therefore be construed as:

1) unmixing FSC and direct light

2) minimising the viewing zone

3) improving the concave mirror

4) replacing the concave mirror

10 This appears to show lack of corresponding technical effect as well. Consequently, neither the objective problem underlying the subjects of the claimed inventions, nor their solutions defined by the special technical features allow for a relationship to be established between the said inventions, which involves a single general inventive concept.

11 In conclusion, the groups of claims are not linked by common or corresponding special technical features and define five different inventions not linked by a single general inventive concept.

The application, hence does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

12 The application relates to a plurality of inventions, or groups of inventions, in the sense of Rule 13.1 PCT. They have been divided as defined above. If the applicant pays additional fees for one (or more) not yet searched group(s) of invention(s), then the further search(es) may reveal further prior art that gives evidence of a further lack of unity 'a posteriori' within one (or more) of the not yet searched group(s). In such a case only the first invention in this (each of these) group(s) of inventions, which is considered to lack unity of invention, will be the subject of a search. No further invitation to pay further additional fees will be issued. This is because Article 17(3)(a) PCT stipulates that the ISA shall establish the International Search Report on those parts of the international application which relate to the invention first mentioned in the claims ('main invention') and for those parts which relate to inventions in respect of which the additional fees were paid. Neither the PCT nor the PCT guidelines provide a legal basis for further invitations to pay further additional search fees (W17/00, point 11 and W1/97, points 11-16). In such a case the non-searched claims may be the subject of one or more divisional applications after the application has entered the regional phase before the EPO (see W18/07, point 26).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

13 The present application does not meet the criteria of Article 33(2) PCT, because the subject-matter of claim 1 is not new over D1=EP0889319, please refer also to paragraph 3 above.

14 The dependent claims 2-8 (and partially claims 9-15) do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, see

- Claim 6: D2 (=*Brogioli et al.*) discloses a heterodyne detector that uses the interference of forward scatter and the remaining light to avoid using a beam mask.
- Claim 2-5,7,8 and (and partially claims 9-15): These features are merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.

Re Item VI

Certain documents cited

Certain published documents

Application No Publication No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date claimed *(day/month/year)*
PCT/US2013/043453 WO2013/181453	5.12.2013	30.05.2013	30.12.2012 18.10.2012 19.10.2012 29.04.2013

This document is cited because it could become relevant when the priority of the current application lapses; or as a prior right in the national phases. Pages 42-43 and Fig. 25 disclose a detector at point 906 and a concave mirror 907.

Re Item VII

Certain defects in the international application

15 Partial claim 10: It is not sufficiently disclosed how the claimed arrangement would work (Article 5 PCT). Note that the optical axis of a cylindrical lens is perpendicular to the plane square surface and the light beams emanating from the lens towards the mirror would strike the mirror on its thin edge (= optical axes perpendicular).

Re Item VIII

Certain observations on the international application

16 The application does not meet the requirements of Article 6 PCT, because the following is unclear:

- Claim 1/15(partially): The inclusion of a flat mirror in the definition of a concave mirror is not clear from the wording of claim 1 alone and against the common understanding of "concavity", meaning a surface of a body where two points on it can be connected with a straight line such that the line exits the body.
- Claim 3: The direction of the axis refers to an entity that is not part of the claimed subject-matter.
- Claim 4,5: The "light reflected" does not refer to any tangible apparatus features, but rather a way of using the apparatus.
- Claim 6: It is unclear what limitations "...is in a heterodyne mode..." involves for the detector.
- Claim 7: the word "source" seems to be missing.

(19)日本国特許庁（ＪＰ）　(12) **公開特許公報（A）**　(11)特許出願公開番号

特開平8－29726

(43)公開日　平成８年(1996)２月２日

(51)Int.Cl.[6]	識別記号	庁内整理番号	ＦＩ	技術表示箇所
Ｇ０２Ｂ　27/09				
3/02				
			Ｇ０２Ｂ　27/ 00	Ｅ

審査請求　未請求　請求項の数４　ＦＤ　（全　８　頁）

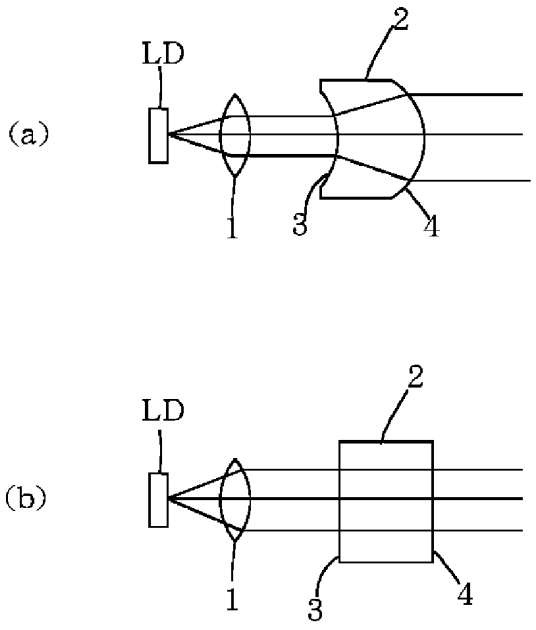
(21)出願番号　特願平6－183940

(22)出願日　平成６年(1994)７月13日

(71)出願人　000004112
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東京都千代田区丸の内３丁目２番３号
(72)発明者　大滝　桂
東京都品川区西大井１丁目６番３号　株式会社ニコン大井製作所内
(74)代理人　弁理士　山口　孝雄

(54)【発明の名称】　ビーム整形光学系

(57)【要約】
【目的】　光学素子の配置が容易で、全長が比較的短く、且つ収差補正の良好なビーム整形光学系を提供すること。
【構成】　本発明においては、楕円形断面を有する光束をほぼ円形断面を有する平行光束に変換するビーム整形光学系において、少なくとも１つの面が全体的に円筒面状の整形レンズを備え、該整形レンズの前記円筒面状の面の横断形状は実質的に非円形である。好ましい態様によれば、前記整形レンズは、互いに相似な２つの楕円筒面からなり、該２つの楕円筒面の横断形状である２つの楕円は、焦点を共有する。さらに好ましくは、前記整形レンズの２つの楕円筒面は、光束の入射側に凹面を向けている。



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【特許請求の範囲】

【請求項１】　楕円形断面を有する光束をほぼ円形断面を有する平行光束に変換するビーム整形光学系において、

少なくとも１つの面が全体的に円筒面状の整形レンズを備え、該整形レンズの前記円筒面状の面の横断形状は実質的に非円形であることを特徴とするビーム整形光学系。

【請求項２】　前記整形レンズは、互いに相似な２つの楕円筒面からなり、該２つの楕円筒面の横断形状である２つの楕円は、焦点を共有することを特徴とする請求項１に記載のビーム整形光学系。

【請求項３】　前記整形レンズの２つの楕円筒面は、光束の入射側に凹面を向けていることを特徴とする請求項２に記載のビーム整形光学系。

【請求項４】　前記ビーム整形光学系は、半導体レーザからの射出光束をほぼ円形断面を有する平行光束に変換する光学系であって、

前記半導体レーザ側から順に、前記半導体レーザからの楕円形断面を有する光束を平行光束に変換するためのコリメータレンズと、該コリメータレンズを通過した楕円形断面を有する平行光束を所定方向に拡大してほぼ円形断面を有する平行光束に変換するための前記整形レンズと、該整形レンズを通過した光を分割するためのビームスプリッタとを備えていることを特徴とする請求項１乃至３のいずれか１項に記載のビーム整形光学系。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明はビーム整形光学系に関し、特に半導体レーザからの楕円形断面を有する光束をほぼ円形断面を有する平行光束に整形する光学系に関する。

【０００２】

【従来の技術】光ディスクヘッドの光源には、半導体レーザが用いられている。半導体レーザの発光面は楕円形状をしているので、半導体レーザから放射された光束断面の強度分布も楕円である。このような楕円形断面を有する半導体レーザからの光束を効率よく利用するために、光束断面を楕円からほぼ円形に整形する、いわゆるビーム整形が行われている。

【０００３】図７は、プリズムを使用した従来のビーム整形光学系の構成を示す図である。図７のビーム整形光学系では、半導体レーザＬＤからの光束をコリメータレンズ５１で平行光束に変換し、ビーム整形プリズム５２に入射させる。ビーム整形プリズム５２に入射した光は、図中上下方向に拡大され、ほぼ円形断面を有する平行光束となって射出する。ほぼ円形断面を有する平行光束に整形された光は、ビームスプリッター５３を透過し、対物レンズ５４を介してディスク５５上に集光される。ディスク５５からの反射光は、対物レンズ５４を介してビームスプリッター５３で図中上方に偏向され、信号検出される。

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【０００４】プリズムは簡単な構成の整形光学素子であるが、半導体レーザＬＤおよびコリメートレンズ５１の光路とビームスプリッター５３および対物レンズ５４の光路とがビーム整形プリズム５２を挟んで傾いてしまう。このため、各光学素子の配置が困難となる。また、レーザ光が整形プリズム５２に斜入射（入射角θ＝７０°～８０°）する。このため、整形プリズム５２の入射面におけるレーザ光の反射を抑えるために、整形プリズム５２の入射面に多層膜からなる反射防止膜を設ける必要があり、コストアップになり易い。

【０００５】図８は、整形プリズムの代わりに円筒レンズを用いた従来のビーム整形光学系の構成を示す図である。（ａ）はガリレオタイプの整形光学系を、（ｂ）はケプラータイプの整形光学系をそれぞれ示している。

【０００６】図８（ａ）に示すように、ガリレオタイプの整形光学系では、半導体レーザＬＤからの光束をコリメータレンズ６１で平行光束に変換する。平行光束に変換された光は、図面の紙面内において負屈折力を有し且つ光軸を含み紙面と直交する面内において屈折力を有しない円筒レンズ６２に入射する。円筒レンズ６２に入射した光は図中上下方向に適宜拡大された後、図面の紙面内において正屈折力を有し且つ光軸を含み紙面と直交する面内において屈折力を有しない円筒レンズ６３に入射する。円筒レンズ６３を通過してほぼ円形断面を有する平行光束に整形された光は、ビームスプリッター６４を透過し、対物レンズ６５を介してディスク６６上に集光される。

【０００７】一方、図８（ｂ）に示すように、ケプラータイプの整形光学系では、半導体レーザＬＤからの光束をコリメータレンズ６１で平行光束に変換する。平行光束に変換された光は、図面の紙面内において正屈折力を有し且つ光軸を含み紙面と直交する面内において屈折力を有しない円筒レンズ６７に入射する。円筒レンズ６７に入射した光は図中上下方向に適宜拡大された後、図面の紙面内において正屈折力を有し且つ光軸を含み紙面と直交する面内において屈折力を有しない正レンズ６３に入射する。こうして、円筒レンズ６３を通過した光は、ほぼ円形断面を有する平行光束に整形される。

【０００８】図８（ａ）に示すガリレオタイプおよび図８（ｂ）に示すケプラータイプのいずれの場合も、収差を抑えるにはＮＡ（開口数）を小さくする必要がある。すなわち、２つの円筒レンズの間隔を大きくする必要があり、その結果光学系の全長が大きくなってしまう。

【０００９】

【発明が解決しようとする課題】上述のように、整形プリズムを使用した従来のビーム整形光学系では、プリズムを介して光軸が折れ曲がるため、各光学素子の配置が困難となるという不都合があった。また、レーザ光が整

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形プリズムに大きな入射角で斜入射するため、プリズムの入射面に反射防止膜を設ける必要があり、コストアップになり易いという不都合があった。一方、円筒レンズを使用した従来のビーム整形光学系では、収差を抑えるために、光学系の全長が大きくなってしまうという不都合があった。

【００１０】本発明は、前述の課題に鑑みてなされたものであり、光学素子の配置が容易で、全長が比較的短く、且つ収差補正の良好なビーム整形光学系を提供することを目的とする。

【００１１】

【課題を解決するための手段】前記課題を解決するために、本発明においては、楕円形断面を有する光束をほぼ円形断面を有する平行光束に変換するビーム整形光学系において、少なくとも１つの面が全体的に円筒面状の整形レンズを備え、該整形レンズの前記円筒面状の面の横断形状は実質的に非円形であることを特徴とするビーム整形光学系を提供する。

【００１２】好ましい態様によれば、前記整形レンズは、互いに相似な２つの楕円筒面からなり、該２つの楕円筒面の横断形状である２つの楕円は、焦点を共有する。さらに好ましくは、前記整形レンズの２つの楕円筒面は、光束の入射側に凹面を向けている。

【００１３】

【作用】図１は、本発明のビーム整形光学系の構成および作用を説明するための図であり、光軸をＺ軸としたＸＹＺ直交座標系において（ａ）はＸＺ平面に沿った断面図を、（ｂ）はＹＺ平面に沿った断面図をそれぞれ示している。

【００１４】図１のビーム整形光学系では、たとえば半導体レーザＬＤからの光束がコリメータレンズ１に入射して平行光束に変換される。コリメータレンズ１を通過した楕円断面を有する平行光束は、整形レンズ２に入射する。整形レンズ２は、（ａ）に示すように、ＸＺ平面において入射ビームを所定の倍率で拡大整形する。すなわち、ＸＺ平面において整形レンズ２の入射面３は入射する平行光束を発散する屈折面であり、射出面４は発散する光束を平行光束に収れんする屈折面である。

【００１５】一方、（ｂ）に示すように、整形レンズ２の双方の面３および４はＹＺ平面においては屈折力を有しないので、入射ビームは拡大されることなくそのまま透過する。こうして、整形レンズ２を通過した光は、ほ＊

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＊ぼ円形断面を有する平行光束に整形される。

【００１６】このように、本発明のビーム整形光学系では、少なくとも１つの面が全体的に円筒面状の整形レンズを備えている。なお、図１では、整形レンズ２の双方の面３および４が全体的に円筒面状に形成されている。そして、整形レンズ２の面３および面４の少なくともいずれか一方の面のＸＺ平面における横断形状が実質的に非円形状に、たとえば楕円形に形成されている。なお、本明細書において、少なくとも１つの面が全体的に円筒面状であり、その円筒面状の面の横断形状が実質的に非円形であるようなレンズを「非球面円筒レンズ」という。

【００１７】このように、本発明のビーム整形光学系では、整形レンズが１群１枚のレンズ成分で構成されているが、収差補正上有利ないわゆる非球面を導入した非球面円筒レンズである。このため、全長が短い光学系であるにもかかわらず、収差補正が良好になされる。

【００１８】また、本発明のビーム整形光学系を光ディスクヘッドに適用すると、半導体レーザＬＤ、コリメートレンズ、ビームスプリッタおよび対物レンズを、一直線になった光軸上に位置決めすることができるので、各光学素子の配置が容易になる。なお、近年の加工技術の進歩により、横断形状が実質的に非円形の円筒面状の面もガラスモールドによって容易に量産加工することができるので、実質的なコストアップにはならない。

【００１９】次に、横断形状が実質的に非円形の円筒面状の面の形状について考えてみる。一般に、２次曲面の組み合わせで様々な無収差光学系を構成することができることは、よく知られている。本発明の整形レンズでは、互いに相似な２つの楕円筒面からなり、２つの楕円筒面の横断形状である２つの楕円が焦点を共有するように形成することにより、アプラナチックな（球面収差がなく且つ正弦条件を満足する）アフオーカル系を構成することができることを以下に示す。

【００２０】図２は、アプラナチックなアフオーカル系を構成する整形レンズの作用を説明する図である。まず、入射面Ｓ１によって、平面波ＡＡ’を球面波ＢＢ’（中心Ｏ、半径ｆ１）に変換するには、光路長ＡＢとＡ’Ｂ’とが等しくなればよい。すなわち、ｒ１＝ＯＡとすると、光路長ＡＢおよび光路長Ａ’Ｂ’はそれぞれ式（１）および（２）で表される。

$$光路長AB = n \cdot (f1 - r1) \quad (1)$$

$$光路長A'B' = f1 - r1 \cdot \cos\theta \quad (2)$$

【００２１】式（１）および（２）から、面Ｓ１の形状※　※は極座標を用いて次の式（３）のように表わされる。

$$r1 = (n-1) \cdot f1 / (n - \cos\theta) \quad (3)$$

式（３）は、楕円を表す式である。こうして、式（３）で表される楕円筒面Ｓ１によって平面波ＡＡ’は、焦点Ｏを中心とする球面波ＢＢ’に変換される。

【００２２】同様に、射出面Ｓ２によって球面波ＣＣ’（中心Ｏ）を平面波ＤＤ’（Ｄ’は中心Ｏから距離ｆ２だけ離れている）に変換するためには、光路長ＣＤと光路長Ｃ’Ｄ’とが等しくなればよい。すなわち、ｒ２＝ＯＣとすると、光路長ＣＤおよび光路長Ｃ’Ｄ’はそれ

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ぞれ式（4）および（5）で表される。

光路長ＣＤ＝ｆ２－ｒ２・ｃｏｓθ　　（4）

光路長Ｃ’Ｄ’＝ｎ・（ｆ２－ｒ２）　　（5）

【００２３】式（4）および（5）から、面Ｓ２の形状＊　＊は極座標を用いて次の式（6）のように表わされる。

ｒ２＝（ｎ－１）／ｆ２／（ｎ－ｃｏｓθ）　　（6）

式（6）も、楕円を表す式である。こうして、式（6）で表される楕円筒面Ｓ２によって球面波ＣＣ’は、平面波ＤＤ’に変換される。

※楕円筒面Ｓ２の横断形状の楕円の長焦点距離（長半径）である。

【００２５】こうして、楕円筒面Ｓ１によって平面波ＡＡ’が球面波ＢＢ’に変換され、次いで楕円筒面Ｓ２によって球面波ＣＣ’が平面波ＤＤ’に変換される。さらに、球面波ＢＢ’とＣＣ’とがその中心（すなわちＯ）を共有する。すなわち、式（3）および（6）でそれぞれ表される２つの楕円筒面を有する整形レンズによって、球面収差のないアフォーカル系を構成することができることがわかる。

【００２４】式（3）および（6）を参照してわかるように、図２において、楕円筒面Ｓ１と楕円筒面Ｓ２は互いに相似で、且つ焦点Ｏを共有するように配置されている。すなわち、楕円筒面Ｓ１の横断形状である楕円の２つの焦点のうち図中左側の焦点Ｏは、楕円筒面Ｓ２の横断形状である楕円の２つの焦点のうち図中左側の焦点位置と一致している。なお、図２において、点Ａは光が楕円筒面Ｓ１に入射する点であり、点Ｃは光が楕円筒面Ｓ２から射出する点である。また、ｆ１は楕円筒面Ｓ１の横断形状の楕円の長焦点距離（長半径）であり、ｆ２は※

【００２６】また、入射前の光線の高さＨ１および射出後の光線の高さＨ２は、それぞれ式（7）および（8）で表される。

入射前の光線の高さ　Ｈ１＝ｒ１・ｓｉｎθ　　（7）

射出後の光線の高さ　Ｈ２＝ｒ２・ｓｉｎθ　　（8）

【００２７】さらに、楕円筒面Ｓ１と楕円筒面Ｓ２とは相似であることから、次の式（9）で表される関係が成★立する。

Ｈ２／Ｈ１＝ｒ２／ｒ１＝ｆ２／ｆ１＝一定（ビーム整形比）　（9）

このように、式（3）および（6）でそれぞれ表される２つの楕円筒面を有する整形レンズでは、アフォーカル系での正弦条件、すなわち入射光線の高さＨ１と射出光線の高さＨ２との比Ｈ２／Ｈ１が一定であるという条件を満足している。換言すれば、図２の整形レンズは、正☆弦条件を満足したアプラナチックなアフオーカル系である。

【００２８】楕円筒面Ｓ１および楕円筒面Ｓ２の実際の面形状は、次の式（１０）乃至（１３）に示す関係にしたがって決められる。

ｍ＝ｆ２／ｆ１　　（１０）

ｄ＝ｆ２－ｆ１＝（ｍ－１）・ｆ１　　（１１）

ａ１＝（ｎ－１）ｆ１／ｎ　　（１２）

ａ２＝（ｎ－１）ｆ２／ｎ＝ｍ・ａ１　　（１３）

【００２９】ここで、

ｆ１：楕円筒面Ｓ１の長焦点距離

ｆ２：楕円筒面Ｓ２の長焦点距離

ｍ　：ビーム整形倍率

ｄ　：楕円筒面Ｓ１と楕円筒面Ｓ２との間の光軸に沿った距離（中心厚）

ｎ　：レンズの屈折率

ａ１：楕円筒面Ｓ１の近軸曲率半径

ａ２：楕円筒面Ｓ２の近軸曲率半径

【００３０】このように、所要のビーム整形比ｍから、◆

長半径＝ｎ・ｆ／（ｎ＋１）　　（１４）

短半径＝ｆ・〔（ｎ－１）／（ｎ＋１）〕$^{1/2}$　　（１５）

∴楕円率＝（１－１／ｎ^{2}）$^{1/2}$　　（１６）

◆楕円筒面Ｓ１および楕円筒面Ｓ２の屈折力（すなわち焦点距離）が決まり、屈折力からレンズ厚が決まる。楕円筒面Ｓ１および楕円筒面Ｓ２の形状は、式（3）および（6）で与えられる。なお、θ＝０およびθ＝πのときの動径ｒの平均値が長半径であり、θ＝ｃｏｓ$^{-1}$（１／ｎ）の時のｒ×ｓｉｎθが短半径であるから、楕円筒面の長半径、短半径および楕円率はそれぞれ次の式（１４）乃至（１６）で表される。

このように、整形レンズの楕円筒面の形状は屈折率ｎのみで決まり、楕円筒面の軸上間隔ｄはビーム整形倍率できまる。

【００３１】

【００３２】図２では、入射側に凹面を向けた楕円筒面Ｓ１および同じく入射側に凹面を向けた楕円筒面Ｓ２を有するガリレオ型に相当するタイプの整形レンズを示したが、図３には、入射側に凸面を向けた楕円筒面Ｓ１および同じく入射側に凹面を向けた楕円筒面Ｓ２を有するケプラー型に相当するタイプの整形レンズを示している。なお、図３においても、楕円筒面Ｓ１と楕円筒面Ｓ

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２は互いに相似で、且つ焦点Ｏを共有するように配置されている。

【００３３】図２のガリレオ型の整形レンズのレンズ厚＊

$$D1 = f2 - f1 = (m-1) \cdot f1 \qquad (17)$$

$$D2 = f2 + f1 = (m+1) \cdot f1 \qquad (18)$$

このように、同じビーム整形比で考えると、図２のガリレオ型の整形レンズの方が、図３のケプラー型の整形レンズよりもレンズ厚を小さくすることができるし、形状にも無理がないことがわかる。

【００３４】なお、全体的に円筒面状の２次曲面に高次の非球面係数を加えれば、設計の自由度が更に増えるので設計はより容易になる。この場合、整形レンズを構成する２面のうち１面だけを高次の非球面係数を用いた、※

$$S(y) = (y^2/R)/\left[1+\left(1-k\cdot y^2/R^2\right)^{1/2}\right] + C_2\cdot y^2 + C_4\cdot y^4 + C_6\cdot y^6 + C_8\cdot y^8 + C_{10}\cdot y^{10} + \cdots \qquad (19)$$

また、非球面の近軸曲率半径ｒは、次の数式（２０）で★

$$r = 1/(2\cdot C_2 + 1/R) \qquad (20)$$

【００３６】上述のような非球面円筒レンズをたとえば光ディスクヘッドに適用する場合、図４（ａ）に示すように、整形レンズ４２は通常、コリメートレンズ４１とビームスプリッタ４３との間に配置される。この場合、ビームスプリッタ４３には整形レンズ４２を介してほぼ円形断面に整形された光束が入射するので、ビームスプリッタ４３は正方形状で比較的大きくなる。

【００３７】一方、図４（ｂ）に示すように、整形レンズ４２をビームスプリッタ４３と対物レンズ４４との間に配置すると、ビームスプリッタ４３には未だ拡大されていない楕円断面を有する光束が入射する。その結果、ビームスプリッタ４３は長方形状となり、１／ビーム整形比（＝１／２～１／３）程度に比較的小さく構成することができる。また、本発明の非球面円筒レンズは、ビーム整形以外にも、画角の大きくないアナモルフィック光学系（縦横の倍率の異なる光学系）にも適用することができる。

【００３８】

【実施例】本発明の実施例を、添付図面に基づいて説明する。図５は、比較例として従来の２群２枚の円筒面レンズ、すなわち入射側から順に平凹型の円筒レンズおよび平凸型の円筒レンズを配置したビーム整形レンズを示している。また、図６は、本発明の実施例１乃至３にかかる１群１枚の楕円筒レンズからなるビーム整形レンズ

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＊Ｄ１および図３のケプラー型の整形レンズのレンズ厚Ｄ２は、それぞれ次の式（１７）および（１８）で表される。

※いわゆる球面形状にするだけでも収差は良好になる。非球面形状は、光軸に垂直な方向の高さをｙ、高さｙにおける光軸方向の変位量をＳ（ｙ）、基準の曲率半径をＲ、円錐係数をｋ、ｎ次の非球面係数をＣn としたとき、以下の数式（１９）で表される。

【００３５】

【数１】

★定義される。

を示している。図６のビーム整形レンズは、図１の整形レンズに対応しており、入射側の楕円筒面Ｓ１と射出側の楕円筒面Ｓ２とは互いに相似であって焦点を共有している。

【００３９】各比較例および各実施例において共通の諸元の値は、次のとおりである。

入射ビーム径　＝２mm×５mm

出射ビーム径　＝５mmφ

ビーム整形倍率＝２．５×

波長λ　　＝６８０mm

なお、ΔＷ（λrms ）はＲＭＳ球面収差を、ΔWd （λrms ）はレンズ全体を３０’偏心させた時のＲＭＳ波面収差を、ＴＬ（mm）は全長（第一面から最終面までの距離）をそれぞれ示している。このように、長さの単位は全てmmである。

【００４０】次の表（１）に、比較例１および２の諸元の値を掲げる。表（１）において、ｒ１は平凹円筒レンズの凹面の曲率半径を、ｒ２は平凸円筒レンズの凸面の曲率半径を、ｄ１は平凹円筒レンズの中心厚を、ｄ２は平凸円筒レンズの中心厚を、ｔは２つのレンズの間の軸上空気間隔を表す。なお、屈折率はｄ線（λ＝５８７．６ｎm）に対する値を示している。

【００４１】

【表１】

	比較例１	比較例２
ガラス	ＢＫ７	ＢＫ７
屈折率	１．５１０２１	１．５１０２１
ｒ１	７．６５３２	６．１２２５
ｄ１	１．０	１．０
ｒ２	１９．１３２９	１５．３０６３
ｄ２	２．０	２．０
ｔ	２０．５１	１９．０

9　　10

ＴＬ（mm）	２３．５	１６．０
ΔＷ（λrms）	０．０１１	０．０２１

【００４２】次の表（２）に、実施例１乃至３の諸元の値を掲げる。表（２）において、ａ１は楕円筒面Ｓ１の近軸曲率半径を、ａ２は楕円筒面Ｓ２の近軸曲率半径を、ｆ１は楕円筒面Ｓ１の長焦点距離を、ｆ２は楕円筒面Ｓ２の長焦点距離を、ｄは整形レンズの中心厚を表＊す。なお、屈折率はｄ線（λ＝５８７．６ｎｍ）に対する値を示している。

【００４３】

【表２】

	実施例１	実施例２	実施例３
ガラス	ＣａＦＫ９５	同左	同左
屈折率	１．４３２４５１	同左	同左
ｄ	４．０	６．０	８．０
ｆ１	２．６６６６	４．０００	５．３３３３
ｆ２	６．６６６６	１０．０００	１３．３３３３
ａ１	０．８０５１	１．２０７６	１．６１０１
ａ２	２．０１２６	３．０１９０	４．０２５３
ＴＬ（mm）	４．０	６．０	８．０
ΔＷ（λrms）	０．０	０．０	０．０
ΔＷｄ（λrms）	０．００８	０．００６	０．００５

【００４４】比較例１および２から明らかなように、従来の整形レンズでは球面収差を０．０２λｒｍｓに抑えるためにＮＡを小さくする必要から、全長ＴＬが２０ｍｍ程度必要となる。一方、実施例１乃至３から明らかなように、各実施例の整形レンズでは球面収差が０で且つ全長が４乃至８ｍｍと比較例よりはるかに短くて済むことのがわかる。更に、作用で前述したように各実施例の整形レンズでは正弦条件も満足されているので、レンズ全体が光軸に対して３０’偏心しても発生する収差は０．０１λｒｍｓ以下と極めて小さいことがわかる。換言すれば、上述の実施例の整形レンズを光学系に組み込む際に偏心に対する位置決め精度を緩くすることができる。

【００４５】

【効果】以上説明したように、本発明のビーム整形光学系では、光学素子の配置が容易で、全長が比較的短く、且つ収差補正の良好なビーム整形光学系を実現することができる。

【図面の簡単な説明】

【図１】本発明のビーム整形光学系の構成および作用を説明するための図であり、光軸をＺ軸としたＸＹＺ直交座標系において（ａ）はＸＺ平面に沿った断面図を、（ｂ）はＹＺ平面に沿った断面図をそれぞれ示している。

【図２】アプラナチックなアフオーカル系を構成する整形レンズの作用を説明する図である。

【図３】入射側に凸面を向けた楕円筒面Ｓ１および同じく入射側に凹面を向けた楕円筒面Ｓ２を有するケプラー型に相当するタイプの整形レンズを示している。

【図４】本発明の整形レンズたとえば光ディスクヘッドに適用する場合の光学素子配置について説明する図である。

【図５】比較例として従来の２群２枚の円筒面レンズ、すなわち入射側から順に平凹円筒レンズおよび平凸円筒レンズを配置したビーム整形レンズを示している。

【図６】本発明の実施例１乃至３にかかる１群１枚の楕円筒レンズからなるビーム整形レンズを示している。

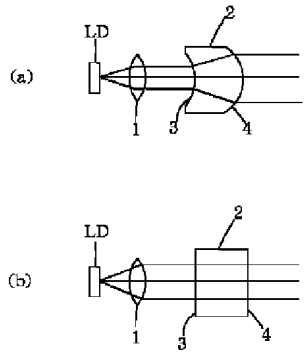
【図７】プリズムを使用した従来のビーム整形光学系の構成を示す図である。

【図８】整形プリズムの代わりに円筒レンズを用いた従来のビーム整形光学系の構成を示す図である。

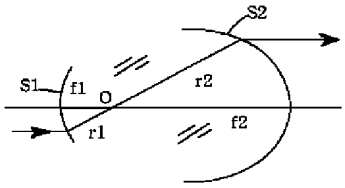
【符号の説明】

１	コリメータレンズ
２	整形レンズ
３	入射側楕円筒面
４	射出側楕円筒面
Ｓ１	入射側楕円筒面
Ｓ２	射出側楕円筒面

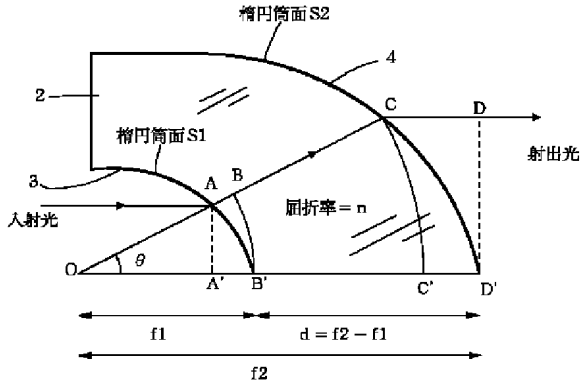
【図１】



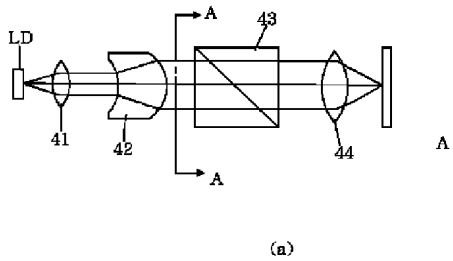
【図３】



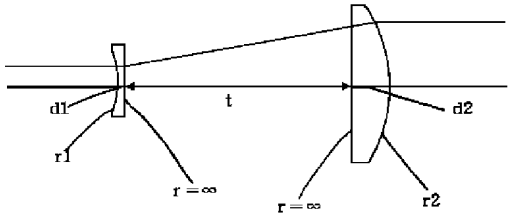
【図２】



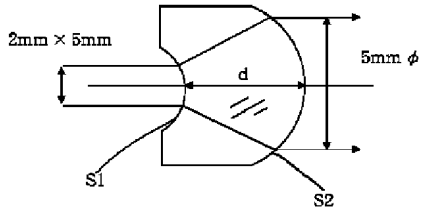
【図４】



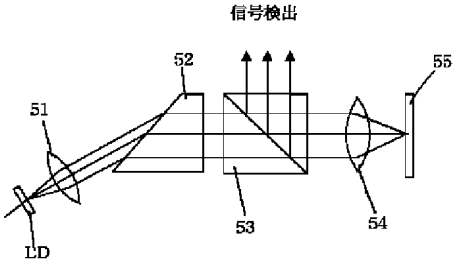
【図５】



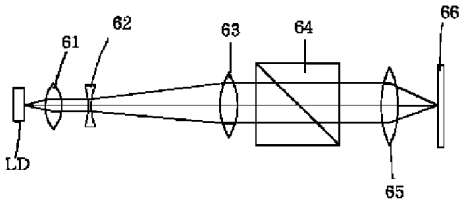
【図６】



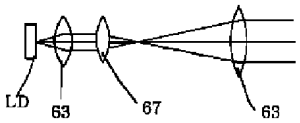
【図７】



【図８】



(a)



(b)

1 of 1 DOCUMENT

JP08029726A 1996-02-02 BEAM SHAPING OPTICAL SYSTEM (en)

English Abstract:

PURPOSE: To easily arrange optical elements, to relatively shorten an overall length and to improve aberration correction by specifying the constitution of a beam shaping optical system which converts a luminous flux having an elliptic section to parallel luminous fluxes having nearly a circular section.

CONSTITUTION: This beam shaping optical system has a shaping lens at least one faces of which are cylindrical overall and the cross sectional shape of the cylindrical surface of the shaping lens is substantially non-circular. The luminous flux from a semiconductor laser LD is made incident on a collimator lens 1 and is converted to the parallel luminous fluxes. The parallel luminous fluxes which are past the collimator lens 1 and have an elliptic section are made incident on the shaping lens 2. The shaping lens 2 enlarges and shapes the incident beam at a prescribed magnification on an X-Z plane (a). On the other hand, both faces 3 and 4 of the shaping lens 2 have no refracting power at a Y-Z plane (b) and, therefore, the incident beam transmits these faces as it is without enlargement. The light past the shaping lens 2 is thus shaped to the parallel luminous fluxes having nearly the circular section.

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Inventors: OTAKI KATSURA

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ECLA: G02B3/04; G02B3/06
IPC[6]: G02B 27/09 A
IPC[6]: G02B 3/02 B
IPC[8]: G02B27/09 (2006-01-01; C, F, I, 2006-03-10; R, M, JP)
IPC[8]: G02B27/09 (2006-01-01; A, F, I, 2006-03-10; R, M, JP)
IPC[8]: G02B3/02 (2006-01-01; C, , I, 2005-10-08; R, M, EP)
IPC[8]: G02B3/02 (2006-01-01; A, L, I, 2006-03-10; R, M, JP)
IPC[8]: G02B3/06 (2006-01-01; A, , I, 2005-10-08; R, M, EP)
JP-FI-CLASS: G02B27/00.E

Patent References Cited-Forward:

Publication Number
JP3974761B2

English Description:

0001 :

technical field: the present invention with respect to the beam-shaping optical system, especially from a

semiconductor laser having an elliptic cross-section having a substantially circular cross section of the luminous flux shaping optical system of the parallel light flux.

0002 :

prior art: source of the disk head, semiconductor laser is used. The light emitting surface of the semiconductor laser is an elliptical shape, the light flux radiated from the semiconductor laser intensity distribution of elliptical cross section. With such an elliptic cross-section from the semiconductor laser in order to efficiently utilize the luminous flux, luminous flux from the ellipse cross section is substantially circular, is called a beam shaping is performed. The

0003: fig. 7, which is a drawing showing the constitution of the conventional beam-shaping optical system using a prism. Fig. 7 of the beam-shaping optical system, a semiconductor laser light from the LD bundle is converted into a parallel light flux by the lens 51, and is made incident on the prism beam 52. The light incident on the prism beam 52, is enlarged in the vertical direction in the figure, and a parallel luminous flux having a nearly circular cross section that is emitted. The parallel luminous flux having a nearly circular cross section, the light shaping, transmitted through beam splitter 53, through the objective lens 54 is collected on the disk 55. The reflected light from the disk 55, through the objective lens 54 is deflected upward in the figure by splitter 53, signal detection.

0004: shaping of the prism optical element is a simple constitution, and an optical path of a semiconductor laser and a beam splitter 53 [...] 51 LD and objective lens 54 and the optical path of the beam shaping prism 52 being inclined sides. Therefore, it is difficult to arrange and each of the optical elements. Furthermore, the laser beam is obliquely incident on the prism 52 (incident angle θ=70°-80 °). Therefore, in the incident surface of the prism 52 in order to suppress the reflection of the laser beam, the multilayer film on the incident surface of the prism 52 from the necessity of providing an anti-reflection coating, an increase in cost. The

0005: fig. 8, which is a drawing showing the constitution of the conventional beam-shaping optical system in place of the cylindrical lens using a prism. (A), (b) an optical system for shaping the galileian type [...] respectively represent a shaping of the optical system. As shown in

0006: fig. 8 (a), in a type of galileian shaping optical system, a semiconductor laser light from the LD collimator 61 are converted into parallel light fluxes in a bundle. The light converted into parallel light fluxes, within the plane of the drawing and includes a negative refractive power in a plane perpendicular to the optical axis and refracting power in the paper is not incident on the cylindrical lens 62. The light incident on the cylindrical lens 62 in the vertical direction in the figure after appropriately enlarged, the plane of the drawing in which contains the optical axis and having positive refractive power in a plane orthogonal to the paper and having no refractive power incident on the cylindrical lens 63. Passing through the cylindrical lens 63 is shaped into a parallel beam having a substantially circular cross section, the light, transmitted through beam splitter 64, through the objective lens 65 is converged on a disk 66.

0007: on the other hand, as shown in fig. 8 (b), an optical system for shaping the [...] , semiconductor laser light from the LD collimator 61 are converted into parallel light fluxes in a bundle. The light converted into parallel light fluxes, the plane of the drawing in which contains the optical axis and having positive refractive power in a plane orthogonal to the paper and having no refractive power incident on the cylindrical lens 67. The light incident on the cylindrical lens 67 in the vertical direction in the figure after appropriately enlarged, the plane of the drawing in which contains the optical axis and having positive refractive power in a plane perpendicular to the paper surface in refractive power and does not have a positive lens 63. Therefore, the light passing through the cylindrical lens 63, having a substantially circular cross section is shaped into a parallel light flux.

0008: fig. 8 (a) and fig. 8 (b) show galileian [...] shown in any case of a type, to suppress the aberration in the NA (numerical aperture) must be small. In other words, one of the cylindrical lens 2 it is necessary to increase the spacing, length of the optical system becomes large as a result thereof.

0009 :

problems to be solved by the invention: as mentioned above, the prism is used in the conventional beam-shaping optical system, the optical axis through the prism for bending, and is difficult to arrange that each of the optical elements. Furthermore, the laser beam is obliquely incident on the prism for a large incident angle, the incident surface of the prism to the necessity of providing an anti-reflection film, apt to overcome a cost increase. On the other hand, the cylindrical lens is used in the conventional beam-shaping optical system, in order to restrain the aberration, due to the length of the optical system. The

0010: present invention, in view of the problem described above in which, in the arrangement of the optical element, a relatively short overall length, and good aberration correction of the beam-shaping optical system and to provide.

0011 :

means for solving the problems: for solving the above problem, the present invention in, a light beam having a substantially elliptical cross section having a circular cross section is converted into a parallel luminous flux in the beam-shaping optical system, 1 at least one surface as a whole having a shaping lens having a cylindrical surface, the cylindrical surface of the shaping lens surface of non-circular cross-sectional shape is substantially and is characterized by a beam shaping optical system.

0012: in a preferred aspect, the lens has a shape, which mutually similar 2 consists of two elliptic cylindrical surface, an elliptic cylindrical surface the 2 2 of the two elliptical cross-sectional shape, sharing a focal point. Further preferably, the shaping of the elliptic cylindrical surface 2 of the lens, which is concave to the incident side of the light flux.

0013 :

operation: fig. 1 is, the constitution of the present invention and the beam shaping optical system diagram for explaining the action, and the optical axis Z axis XZ plane in the XYZ orthogonal coordinate system (a), (b) is a sectional diagram along the YZ plane respectively represent a cross-sectional diagram. The beam-shaping optical system

0014: fig. 1 of, for example, a semiconductor laser light from the LD is incident on the lens 1 is converted into a parallel light flux. The collimator 1 passing through the parallel beam having an elliptical cross section, shaping lens 2. The shaping lens 2, as shown in (a), in the XZ plane in the shaping of the incident beam. In other words, in the XZ plane of the incident surface 3 shaped lens 2 diverging incident parallel light flux by the refracting surface, the light emission surface 4 in a parallel luminous flux diverging refraction surface mismatches.

0015: on the other hand, as shown in (b), shaping lens 2 in the YZ plane of the both surfaces 3 and 4 since it does not have a refractive power, the incident beam is transmitted as it is without being expanded. Therefore, the light passing through the lens shaped 2, having a substantially circular cross section is shaped into a parallel light flux.

0016: in this way, the beam-shaping optical system of the present invention, at least one surface 1 of the lens is generally cylindrically shaped. Furthermore, in fig. 1, shaping lens 2 of both of the surfaces 3 and 4 is generally formed in the shape of a cylindrical surface. Furthermore, shaping lens 2 and surface 4 of the surface 3 of the XZ plane in at least either of the surface of non-circular cross-sectional shape is substantially in the form of, for example, is formed in an elliptical shape. Incidentally, in this specification, 1 at least one surface is generally in the form of a cylindrical surface, the cylindrical surface of the surface of non-circular cross-sectional shape is substantially a lens "non-spherical" and cylindrical lens.

0017: in this way, the beam-shaping optical system of the present invention, the lens component of shaping lens 1 group 1 are constituted, so-called non-spherical aberration correction advantageous to introduce the aspherical cylindrical lenses. Therefore, the overall length is short, in spite of the optical system, aberration correction is performed well.

0018: also, beam-shaping optical system of the present invention is applied to a light and [...] , semiconductor laser LD, [...] , objective lens and a beam splitter, a straight line can be positioned on the optical axis, the arrangement of each optical element is facilitated. Furthermore, by machining technology is advanced in recent years, a non-circular cross-sectional shape is substantially cylindrical surface [...] of mass can be machined easily, and does not increase in cost.

0019: next, non-circular cross-sectional shape is substantially cylindrical, but the shape of the surface. In general, the various no aberration can be constituted by combining a primary curved surface of the optical system 2, are well-known. In the present invention for shaping lens, which mutually similar 2 consists of two elliptic cylindrical surface, an elliptic cylindrical surface 2 of the two elliptical cross-sectional shape 2 is formed so as to share a focus, a [...] (spherical aberration and sine condition is not satisfied) to construct an afocal system is shown. The

0020: fig. 2, an afocal system [...] explains the action of shaping lens. First of all, by the incident surface S1, a spherical wave AA 'O, the center plane wave radius BB' (f1) converted into, AB and A 'B' and an optical path length becomes equal. In other words, and r1=OA and to, the optical path length AB and A 'B' respectively eq. (1) and (2) the light path length.

The optical path length AB=n · (f1-r1) (1)

optical path length A 'B' =f1-r1 ·cosθ (2)

0021 : eq. (1) and (2) from, surface S1 by using the shape of the polar eq. (3) is expressed.

R1= (n-1) · f1 / (n-cosθ) (3)

eq. (3) is, representing the ellipse. Therefore, eq. (3) by an elliptical cylindrical surface S1 is represented by the plane wave AA ', focal center BB' O and is converted into a spherical wave.

0022: similarly, exit surface S2 by a spherical wave CC '(center O) a plane wave DD' (D 'has a center O by a distance f2 and spaced) in order to transform the, optical path length of the optical path length CD and C' D ' and are equal. In other words, and r2=OC and to, the optical path length of the optical path length CD and C 'D' respectively eq. (4) and (5).

The optical path length CD=f2-r2 ·cosθ (4)

optical path length C 'D' =n· (f2-r2) (5)

0023 : eq. (4) and (5) from, the shape of the polar surface S2 to the next eq. (6) is expressed.

R2= (n-1)/ f2 / (n-cosθ) (6)

eq. (6) also, representing the ellipse. Therefore, the CC 'is converted into a spherical wave, plane wave is represented by an elliptical cylindrical surface S2 DD' eq. (6).

0024: eq. (3) and (6) recognized by referring, in fig. 2, elliptic cylindrical surface S1 and elliptic cylindrical surface S2 is similar to each other, and are arranged so that the common focus O. In other words, the cross sectional shape of the elliptic cylinder plane S1 2 at one focus of the ellipse as a focus of the left O, elliptical cross-sectional shape, an elliptic cylindrical surface S2 of the left in Figure 2 one focus and out of focus position. Furthermore, in fig. 2, the point A the light is incident on a point in an elliptical cylindrical surface S1, point C of the light emitted from the elliptical cylindrical surface S2. Furthermore, the long focal distance f1 (radial length) of the elliptical cross-sectional shape is an elliptical cylindrical surface S1, long focal distance f2 (radial length) of the elliptical cross-sectional shape of the elliptical cylindrical surface S2.

0025: therefore, elliptic cylindrical surface S1 BB 'by AA' is a plane wave is converted into a spherical wave, then CC 'is elliptical cylindrical surface S2 DD' by spherical wave is converted into a plane wave. Furthermore, spherical wave BB 'and CC' and the center (O) that is shared. In other words, eq. (3) and (6)

are respectively expressed by 2 by shaping lens having two elliptic cylindrical surface, free of spherical aberration affocal system can be constituted.

0026: in addition, the height H2 of the height H1 before and after injection of the incident light rays, respectively eq. (7) and (8).

Incident light ray height H1=r1 ·sinθ (7) before the

after injection of the ray height H2=r2 ·sinθ (8)

0027: further, elliptic cylindrical surface S1 and is similar to the elliptical cylindrical surface S2 and from it, the next eq. (9) is expressed by the relation is established.

This H2/H1=r2/r1=f2/f1= (beam shaping ratio) (9) so as to be constant, eq. (3) and (6) are represented respectively by two ellipse having a cylindrical surface 2 in the shaping lens, the sine condition of the afocal system, that is, the height H1 of the incident rays of light and the height H2 of the ratio H2/H1 of injection is constant, a condition that is satisfied. In other words, the shaping lens of fig. 2, an afocal system [...] sine condition is satisfied. The elliptical cylindrical surface S2

0028: elliptic cylindrical surface S1 and the actual shape, the next eq. (10) to (13) is determined in accordance with the relation shown.

A2= (n-1) f2/n=m · a1(13)

d=f2-f1= (m-1) · f1(11)

a1= (n-1) f1/n (12) m=f2/f1 (10)

0029: here, f1: long focal distance f2 of the elliptic cylinder plane S1:m long focal length of the elliptical cylindrical surface S2:d beam shaping magnification: elliptic cylindrical surface S1 and elliptic cylindrical surface S2 along the optical axis between the distance (thickness)

n: the refractive index of the lens a1: elliptic cylindrical surface S1 of the paraxial curvature radius a2: elliptic cylindrical surface S2 of the paraxial curvature radius

0030 : in this way, the ratio m from the required beam shaping, elliptic cylindrical surface S1 and the refractive power of the elliptical cylindrical surface S2 (i.e. focal distance) is determined, the thickness is determined from lens refracting power. An elliptical cylindrical surface S1 and the shape of the elliptical cylindrical surface S2, eq. (3) and (6). Incidentally, when the average value of the radius r θ=0 and θ=π of long radius and, in the case of short radius r×sinθ θ=cos-1 (1/n) from, the radial length of the elliptic cylinder plane, and each of the primary short radius ellipticity eq. (14) to (16).

0031 :

longer radius =n· f / (n+ 1) (14)

short radius =f· "(n-1)/ (n+ 1)" 1/2 (15)

∴ ellipticity = (1-1/n2) 1/2 (16)

in this way, the cylindrical shape of the surface of the elliptical shaped lens is decided only by the refractive index n, elliptic cylindrical surface d on the axis of the beam shaping rate determined by the interval. In the

0032: fig. 2, concave to the incident side and an elliptical cylindrical surface S1 which is concave to the incident side similarly elliptical cylindrical surface S2 of a type having a corresponding-galileian shows a shaping lens which, in fig. 3, a convex surface facing the incident side and similarly elliptical cylindrical

surface S1 which is concave to the incident side of the elliptical cylindrical surface S2 of a type having a corresponding-costitution shows the shaping lens. Furthermore, even in fig. 3, elliptic cylindrical surface S1 and elliptic cylindrical surface S2 is similar to each other, and are arranged so that the common focus O. A lens for shaping mold galileian

0033: fig. 2 of the lens thickness D1 and figs. 3 of the lens thickness D2 of shaping lens-costitution, respectively next eq. (17) and (18).

In this way D1=f2-f1= (m-1) · f1(17)

D2=f2+f1= (m+ 1) · f1(18), beam shaping and considered in the same ratio, fig. 2 is a galileian type shaping lens, fig. 3 of the lens thickness than costitution type shaping lens can be reduced, it is found that there is no unreasonable shape.

0034: in this case, the entire cylindrical surface 2 of the next higher order aspheric curved coefft. disconnected, since the degree of freedom of design is further increased, the design is made easier. In this case, shaping lens surface 2 of the surface 1 using a high-order aspheric coefficients, even by so-called spherical aberration in good shape. The non-spherical shape, the height in a direction perpendicular to the optical axis y, y in the height direction of the optical axis S (y), the amount of displacement of the reference R, the radius of curvature of the aspherical coefficient k, n conical coefft. Cn and the next time, represented by a number of eq. (19).

0035 :

[number 1]

+ C2 · y2 +C4 · y4 +C6 · y6 +C8 · y8 "1 + (1-k· y2/R2) 1/2"

S (y) = (y2/R)/

+ C10 · y10 +··· (19)

further, non-spherical radius of curvature r of the near-axis, the following number eq. (20) is defined.

0036 : r=1 / (2 · C2 + 1/R) (20), for example an aspherical cylindrical lenses as mentioned above when applied to a disk head, as shown in fig. 4 (a), shaping lens 42 is normal, and [...] 41 disposed between the splitter 43. In this case, splitter 43 through the shaping lens 42 is shaped to a substantially circular cross section, since the luminous flux is incident, splitter 43 becomes relatively large in a square shape.

0037: on the other hand, as shown in fig. 4 (b), shaping lens 42 and splitter 43 and disposed between the objective lens 44, the splitter 43 is not yet having an enlarged light flux is incident with an elliptical cross section. As a result, splitter 43 has a rectangular shape and therefore, about 1/beam shaping ratio (=1/2-1/3) can be relatively small. Furthermore, the present invention of aspheric cylindrical lenses, beam shaping, in addition, the angle of view of the optical system is not large metamorphic analites (different aspect of magnification of the optical system) can be applied.

0038 :

[a] present invention embodiment of the embodiment, based on the attached drawings. Fig. 5 is, as a comparative example of a conventional cylindrical surface of lens 2 group 2, in other words in order from the side of the incidence plane concave-convex cylindrical lens and a cylindrical lens is arranged in a beam shaping lens is shown. Furthermore, the fig. 6, the present invention embodiments 1 to 3 comprising two elliptic cylindrical lens group 1 1 shows a beam shaping lens. Fig. 6 of the beam shaping lens, a lens for shaping corresponds to fig. 1, the incident side and the exit side of the elliptic cylinder plane S1 and elliptic cylindrical surface S2 which are mutually similar to share a common focal point.

Values of various common 0039: each in each embodiment and comparative example, the following

relationships.

The incident beam diameter =2mm×5mm emitted beam diameter = 5mm φ beam shaping magnification =2.5×

wavelength λ =680mm possibility, the spherical aberration ΔW (λrms) RMS, the whole lens ΔWd (λrms) eccentric 30" at the time of wavefront aberration RMS, TL (mm) is the total length (the distance from the first surface to the final surface) respectively. In this way, all of the units mm length.

0040: in the next table (1), comparative example 1 and 2 involving various sources of value. In the table (1), r1 is the radius of curvature of the concave surface of the plano-concave cylindrical lens, r2 is the radius of curvature of the convex surface of a plano-convex cylindrical lens, the concave cylindrical lens of the flat central thickness d1, d2 of the plano-convex cylindrical lens central thickness, t 2 on the axis of the air gap between the two lenses. Furthermore, the refractive index values for d line (λ=587.6nm) is shown.

0041 :

[table 1]

comparison example 1 of a comparative example 2

glass BK7 BK 7

refractive index 1.510211.51021

d2 2.0 2.0

t 20.51 19.0

r1 7.6532 6.1225

r2 19.1329 15.3063

d1 1.0 1.0

TL (mm) 23.5 16.0

ΔW (λrms) 0.0110.021

0042 : in the next table (2), embodiments 1 to 3 involving various sources of value. In the table (2), a1 the paraxial curvature radius of the elliptic cylinder plane S1, a2 the paraxial curvature radius of an elliptical cylindrical surface S2, a long focal distance f1 of the elliptic cylinder plane S1, long focal distance f2 of an elliptical cylindrical surface S2, d represents the central thickness of shaping lens. Furthermore, the refractive index values for d line (λ=587.6nm) is shown.

0043 :

[Table 2]

embodiment 1 embodiment 2 embodiment 3

glass CaFK95 [...][...]

refractive index 1.432451 [...][...]

d 4.0 6.0 8.0

f1 2.6666 4.0005.3333

f2 6.6666 10.000 13.3333

a1 0.8051 1.2076 1.6101

a2 2.0126 3.0190 4.0253

TL (mm)

ΔWd (λrms) 0.0080.0060.005

ΔW (λrms) 0.0 0.0 0.0 4.0 6.0 8.0

0044: comparative example 1 and 2 as evidenced, spherical aberration in the conventional shaping lens in order to reduce the necessary 0. 02 λrms from NA, and requiring about 20mm TL is the overall length. On the other hand, as evidenced by the embodiments 1 to 3, in each embodiment of the shaping lens 4 to 8mm and spherical aberration is 0 and the overall length is shorter than that of the comparative example is known. Furthermore, in each embodiment described above acts as a lens for shaping the sine condition is satisfied, the entire lens 30 eccentrically with respect to the optical axis " below the aberration generated, and even very small 0. 01 λrms is known. In other words, the above-mentioned embodiment, when incorporated in an optical system for shaping lens linguistical positioning accuracy can be eccentric.

0045 :

effect: as described above, in the beam-shaping optical system of the present invention, the optical element can be easily arranged, is relatively short in length, and good aberration correction of the beam-shaping optical system can be realized.

Number of Claims: 4

ENGLISH CLAIMS:

[Claim 1] having an elliptic cross-section having a substantially circular cross-section light flux is converted into a parallel luminous flux in the beam-shaping optical system, 1 at least one surface as a whole having a shaping lens having a cylindrical surface, the cylindrical surface of the shaping lens surface of non-circular cross-sectional shape is substantially and is characterized by a beam shaping optical system.

[Claim 2] is the shaping lens, which mutually similar 2 consists of two elliptic cylindrical surface, an elliptic cylindrical surface the 2 2 of the two elliptical cross-sectional shape, and a focal point common to the beam-shaping optical system described in claim 1.

[Claim 3] 2 of the two elliptical cylindrical surface shaped lens, which is concave to the incident side of the light flux is described in claim 2 and is characterized in that the beam shaping optical system.

[Claim 4] the beam shaping optical system, the light flux emitted from a semiconductor laser having a substantially circular cross section and is converted into a parallel light flux in optical system, in order from the side of the semiconductor laser, from the semiconductor laser having an elliptic cross-section of the flux of light converted into parallel light fluxes and a collimator, said collimator having an elliptic cross-section passing through a parallel light flux by expanding in a predetermined direction of the parallel light flux having a substantially circular cross section of the lens and for converting the shaping, the shaping lens the light passing through the beam splitter for splitting and is provided with any one of claims 1 to 3 and a beam shaping optical system described in claim 1.

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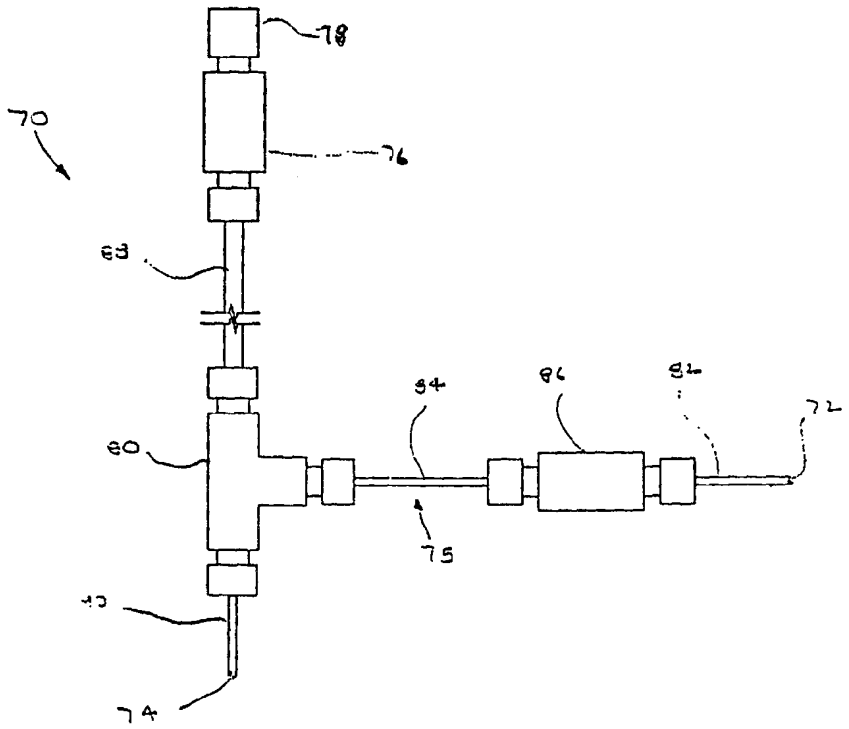
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(57) Abstract

A fluidic driver system for use with a cartridge in a microfluidic analysis instrument. The driver system includes linear pumps, differential pumps, fluidic capacitive filters, and face seal assemblies which act to provide a consistent and smooth fluid flow through the instrument, thus improving the accuracy of the analysis.



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PULSE DAMPER

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to microfluidic devices for performing analytical testing and, in particular, to a fluidic driver system for use with microfluidic cartridges.

2. Description of the Related Art

Microfluidic devices have recently become popular for performing analytical testing. Using tools developed by the semiconductor industry to miniaturize electronics, it has become possible to fabricate intricate fluid systems which can be inexpensively mass produced. Systems have been developed to perform a variety of analytical techniques for the acquisition of information for the medical field.

A process called "field-flow fractionation" (FFF) has been developed to separate and analyze molecules and particles for analysis by the use of a force applied across a flow channel carrying a variety of particle sizes. Examples of this method are taught in U.S. Patent Nos. 3,449,938; 4,147,621; 4,214,981; 4,830,756; and 5,156,039.

A related method for particle fractionation is the "Split Flow Thin Cell" (SPLITT) process. This process has been used to develop devices having mesoscale functional element capable of rapid, automated analyses of preselected molecular or cellular analytes in a range of biological and other applications. Examples of this method are taught in U.S. Patent Nos. 5,296,375; 5,304,487; 5,486,335; and 5,498,392.

Still another method used for assaying fluids involves application of electrical fields to a microfluidic system for providing capillary electrophoresis to separate materials in a flow channel. Examples of this process are taught in U.S. Patent Nos. 5,699,157; 5,779,868; and 5,800,690.

U.S. Patent No. 5,716,852 teaches yet another method for analyzing the presence and concentration of small particles in a flow cell using diffusion principles. This patent, the disclosure of which is incorporated herein by reference, discloses a channel cell system for detecting the presence of analyte particles in a sample stream using a laminar flow channel having at least two inlet means which provide an indicator stream and a sample stream, where the laminar flow channel has a depth sufficiently small to allow laminar flow of the streams and length sufficient to allow diffusion of particles of the analyte into the indicator stream to form a detection area, and having an outlet out of the channel to form a single mixed stream. This device, which is known as a T-Sensor, contains an external detecting means for detecting changes in the indicator stream. This detecting means may be provided by

any means known in the art, including optical means such as optical spectroscopy, or absorption spectroscopy or fluorescence.

A sample microfluidic analysis instrument for performing analytical testing which uses a disposable fluidic analysis cartridge is disclosed in U.S. Patent Application Serial No. 09/080,691, which was filed on May 18, 1998, the disclosure of which is incorporated herein by reference. This instrument includes a cartridge holder, a flow cytometric measuring apparatus positioned for optical coupling with a flow cytometric measuring region on the cartridge, and a second measuring apparatus positioned to be coupled with a second analysis region on the cartridge. The cartridge holder includes alignment markings to mate with cartridge alignment markings. It also includes pump mechanisms to coupled with pump interfaces on the cartridges and valve mechanisms to couple with valve interfaces on the cartridge.

In this type of system, valve and pump mechanisms are external to the cartridge, while the cartridge includes the valve and pump interfaces. Upon loading the cartridge into the apparatus, the valve and pump mechanisms engage the valve and pump interfaces. Thus, it is critical that the interfaces provide an efficient and precise coupling between the cartridge and the external mechanisms. In addition, it is imperative that these external devices provide for a smooth flow of the fluids into and out of the cartridge to ensure accurate measurements within a microfluidic analysis system.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide an efficient interface between the cartridge and the external pumps and valves of a microfluidic analysis system.

It is a further object of the present invention to provide pumping systems for using a microfluidic assembly which produce a consistently smooth and even fluid flow through the system for accurate analysis.

These and other objects of the present invention will be more readily apparent in the description that follows.

BRIEF DESCRIPTON OF THE DRAWINGS

FIG. 1 is an exploded view of a linear pump for use in the present invention;

FIG. 2 is a side elevational view of the pump of FIG. 1;

FIG. 3 is an end view of the pump of FIG. 1;

FIG. 4 is a top elevational view of the differential pump system of the present invention;

FIG. 5 is an end view of the differential pump of FIG. 4;

FIG. 6 is a plan view of the fluidic capacitor assembly of the present invention; and

FIG. 7 is a cross-sectional view of the face seal assembly of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring more particularly to the drawings, there is shown in FIGS. 1-3 a linear pump assembly, generally indicated at 10, which embodies the principles of the present invention. Pump 10 consists of a linear stepper motor unit 12, a mounting bracket 14, an optical switch 16, a precision bore glass syringe 18, a zero dead volume manifold 20, and a valve 22. Also included is a plurality of screws 24 for attaching manifold 20 to bracket 14, a pair of screws 26 and clinch nuts 28 for mounting switch 16 within bracket 14, and a pair of screws 30 and clinch nuts 32 for mounting motor unit 12 to bracket 14. As can be clearly seen in FIG. 2, motor 12, syringe 18, and manifold 20 are arranged in a linear configuration to minimize off-axis torque. Manifold 20 contains a cross channel 34 which connects to valve 22 and to fittings for connection to external fluid components (not shown). To reduce the hold up volume to near zero dead volume, the piston of syringe 18 intrudes into cross channel 34 at the end of its delivery stroke. Valve 22 is embedded within manifold 20 with short interconnections for the input and output of pump assembly 10.

In operation, linear stepper motor assembly 10 converts rotational movement to linear displacement via motor unit 12 coupled to syringe 18. Motor unit 12 offers movement per step of .001 inch and .00025 inch with a total stroke length of .520 inches. The bores of syringe 18 can range from .028 to .583 inches in diameter. By combining these components in different arrangements, a total volume displacement can range from 5 microliters to 4 milliliters.

Stepper motor unit 12 is controlled using a microstepper controller (not shown) which further portitions each full step to as much as 256 microsteps. Thus, accurate and smooth position resolution can be achieved at the microstep level, and flow rates can be as low as 1/256 of the full step rate. Flow rates using pump assembly 12 range from .0025 microliter/step to 4.314 microliter/step with a consistent and smooth flow rate to insure accuracy.

In practice, it is difficult to reliably achieve a smooth and constant fluid flow in microfluidic systems, due to stiction in the sliding piston of the syringe, and also as a result of elasticity in the drive components of the system. To avoid these obstacles for very small volume displacements, two pumps are connected to a common cross channel.

FIGS. 4 and 5 illustrate an alternative embodiment of the present invention which employs a differential pump system. Referring now to FIG. 4, a differential pump system, generally designated at 50, includes a mounting bracket 52, a pair of linear stepper motor units 54a and 54b, a pair of glass syringes 56a and 56b corresponding to motor units 54a and 54b, a pair of optical switches 58a and 58b

corresponding to motor units 54a and 54b, and a manifold 60 having a cross channel 62. Syringes 56a and 56b are coupled to motor units 54a and 54b to translate rotary motion into linear motion.

Pumps 54a and 54b are driven differentially, with one advancing while the other is receding. In this manner, the net output from cross channel 62 will be the difference in the velocities of the two pumps. Thus, the velocities and displacements of the individual pumps are located within a range above the conditions where elasticity and stiction are a major component of the volume displacement, whereby providing a constant and smooth fluid flow within the system to increase the accuracy of the analysis performed by the system.

Another device which is helpful in reducing fluctuation in the fluid flow of the microfluidic analysis system is shown in FIG. 6. Referring now to FIG. 6, there is shown a fluidic filter assembly, generally designated at 70. Assembly 70 includes an input port 72, an output port 74, a restrictor section 75, an accumulator section 76 which terminates in an end cap 78, and a T-connector 80 which couples input port 72 to accumulator 76. Assembly 70 acts as a fluidic filter which reduces any fluid pulses that are introduced into the fluid discharge by stiction and/or stepper motor cogging.

Input port 72 includes a section of tubing 82 which is preferably constructed from .04 inch internal diameter (ID) tubing in the present embodiment. Tubing 82 is coupled to restrictor section 75, which consists of a section of smaller tubing 84, by a connector 86. Tubing 84 consists of a section of .005 inch ID tubing that is at least 1 inch long, and is coupled at its opposite end to T-connector 80. Accumulator section 76 is constructed from a section of tubing 88 which has an ID of .06 inches in the present embodiment, and is 8 inches long. Tubing 88 couples T-connector 80 to accumulator section 76. Output port 74 is coupled to T-connector 80 by a section of tubing 90 having an ID of .04 inches.

In operation, fluid enters input port 72 via tubing 82 and passes through T-connector 80 which includes the accumulator section 76 which then connects to tubing 84, which acts as a flow restrictor, as it has a smaller ID. This assembly configuration acts as a filter to reduce any pulsing discharge to an acceptable smooth and continuous flow level. The fluid flow then exits port 74 via tubing 90 which is the same size as the input line. By using assembly 70, the operation of the microfluidic device can be significantly improved. During operation of this configuration, as a fluid is driven into the input port 72, passing through tubing 82 and coupling 86, it further passes through restrictor 75, developing a pressure drop across restrictor 75. This pressure will be equilibrated by a rise in fluid in accumulator 76 until the trapped volume of air is compressed to the extent required to equalize the pressure drop across restrictor 75. This configuration will exhibit a time constant dependent upon the trapped volume in accumulator 76 and the restrictivity of restrictor 75. Looking forward into the input port 70, any pulsation in the flow will be absorbed by accumulator 76. Looking backwards into exit port 74, such pressure pulses will discharge slowly according to the time constant of filter system 70.

Another device for improving the operation of a microfluidic analysis system is shown in FIG. 7. A face seal assembly, generally designated at 100, consists of a thin flexible elastomeric gasket 102 which is located within a fluidic analysis cartridge 104 as taught in Serial No. 09/808,191. Gasket 102 is located between a top plate 106 and a bottom plate 108 of cartridge 104, and is held in position by a laminate section 110. A ring-shaped surface 112 of gasket 102 is positioned between the lower surface 102a of gasket 102 and the upper surface 110a of laminate section 110 opposite a port 114 within cartridge 104. When cartridge 104 is properly positioned within the analysis device, a locating surface 116 of the device mates within an aperture 118 of cartridge 104 to ensure proper orientation. In this position, an extension 120 from a manifold 122 of the device is forced against gasket 102, causing a face seal between the two parts, and thus allowing fluid discharge from extension 120 to enter cartridge 104 in a smooth and efficient manner. Gasket 102 is constructed such that is provides a force against the bottom surface of extension 120, guaranteeing an adequate sealing force between the surfaces. Multiple face seals are employed within cartridge 104 to provide for multiple reagent and/or sample inputs. In addition, extension 120, which in the present embodiment is a tubular structure having an internal passageway 124, interacts with flexible gasket 102 to provide a spring loading effect which acts to hold the bottom surface of extension 120 tightly against gasket 102.

While the present invention has been shown and described in terms of the preferred embodiments thereof, it will be understood that this invention is not limited to this particular embodiment and that many changes of modifications may be made without departing from the true spirit and scope of the invention as defined in the appended claims.

What is claimed is:

1. A fluidic filter assembly for reducing fluctuations in fluid flow within microfluidic channels, comprising:

inlet means;

outlet means;

an accumulator section;

and means for coupling said inlet means, said outlet means, and said accumulator section,

whereby fluctuation in fluid flowing into said inlet means are reduced by said accumulator section before said fluid exits said outlet means.

2. The assembly of claim 1, wherein said inlet and outlet means comprise tubing having an internal diameter of 0.040 inches.

3. The assembly of claim 1, wherein said coupling means comprises a T-connector.

4. The assembly of claim 1, further comprising a restrictor, located between said inlet means and said coupling means, having an internal diameter less than that of said inlet means.

5. The assembly of claim 4, wherein said restrictor has an internal diameter of 0.005 inches.

6. The assembly of claim 1, wherein said accumulator includes tubing having an internal diameter of 0.06 inches.

7. The assembly of claim 6, wherein said tubing measures approximately 8 inches in length.

8. The assembly of claim 1, wherein said accumulator section includes an end cap at its end opposite said coupling means.

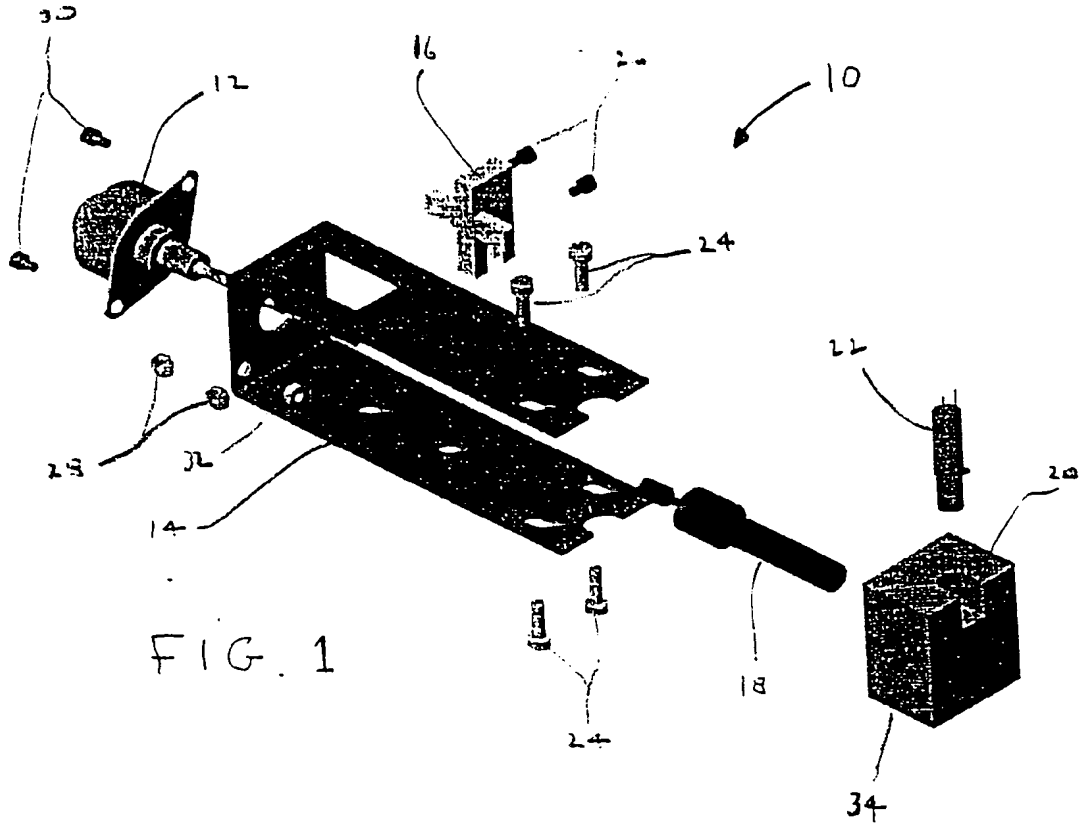


FIG. 1

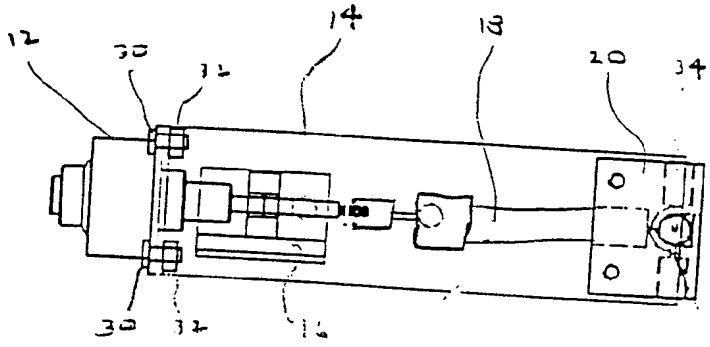


FIG. 2

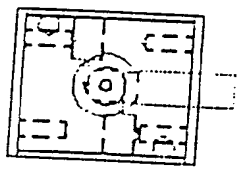


FIG. 3

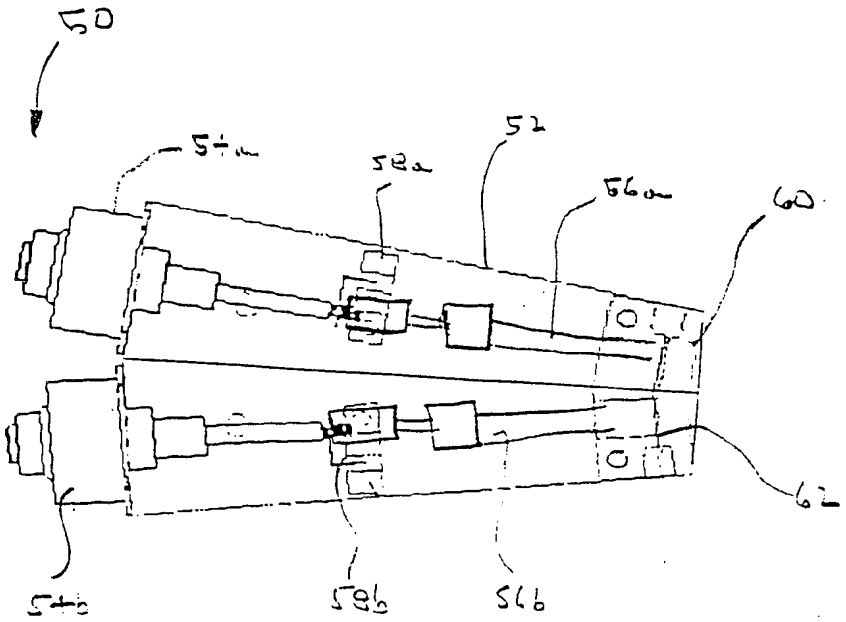


FIG. 4

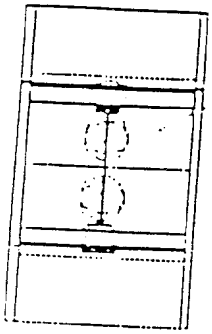


FIG. 5

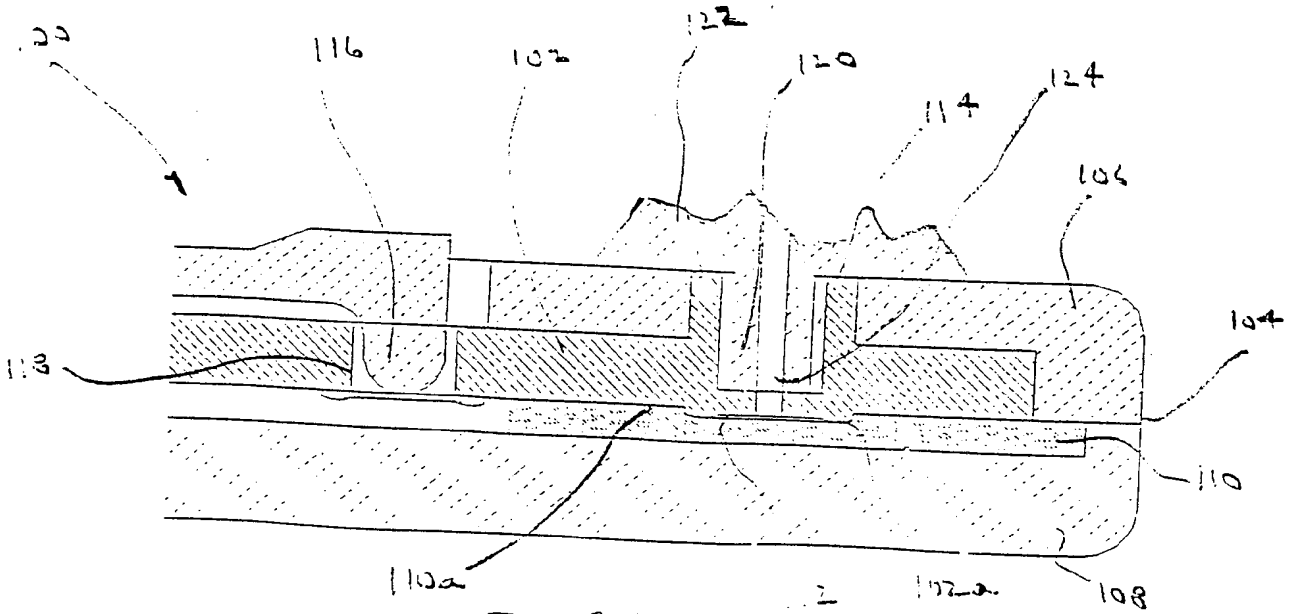


FIG. 7

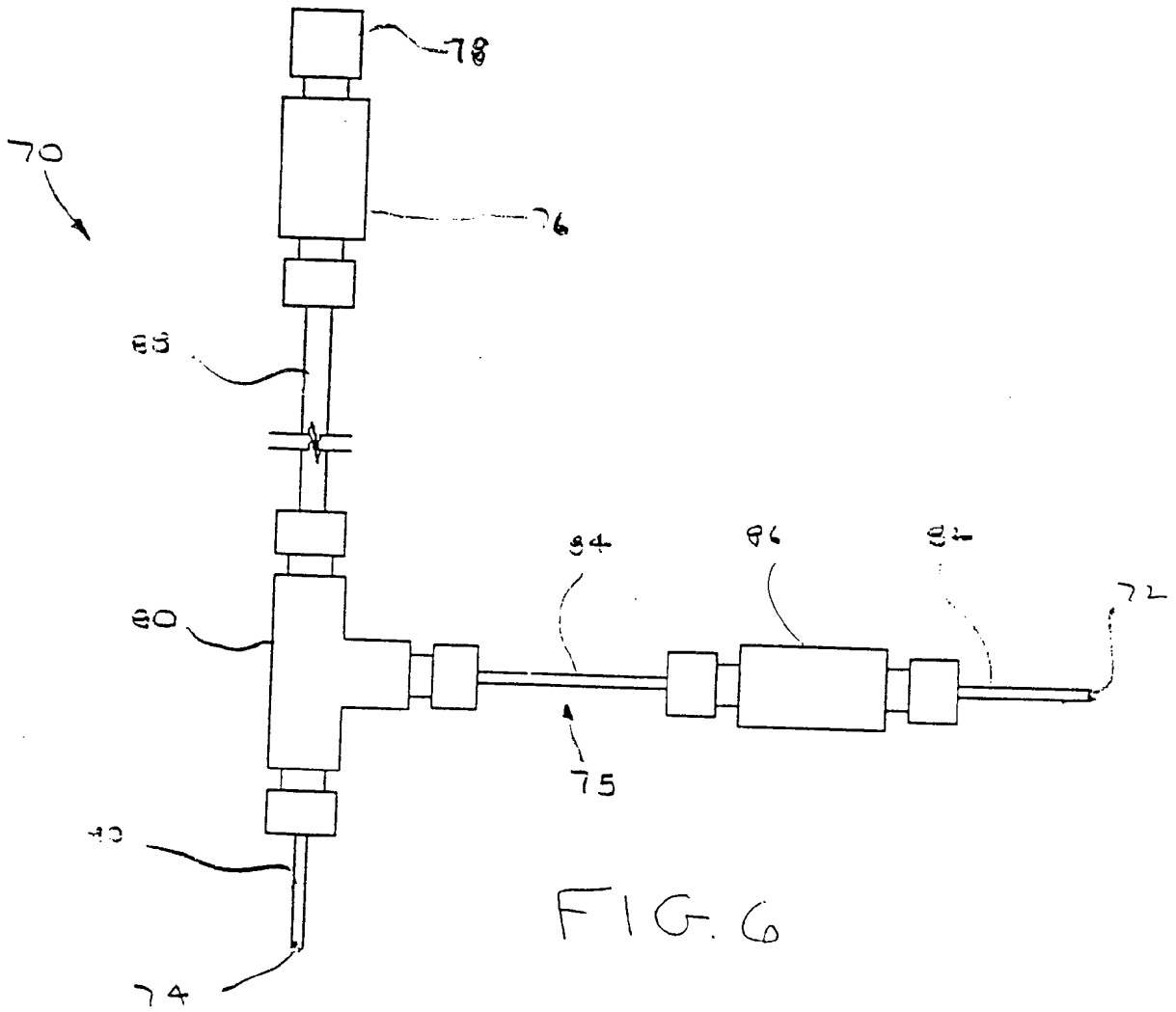


FIG. 6

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 00/06809

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N30/32 F04B11/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N F04B B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 024 061 A (GATISS JOHN WILLIAM) 17 May 1977 (1977-05-17)	1-3
A	column 4, line 47 -column 6, line 29 column 7, line 22-40 column 7, line 67 -column 8, line 28	4
X	US 4 629 562 A (KERCHER PAUL W) 16 December 1986 (1986-12-16) column 2, line 65 -column 3, line 26	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2000	28/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Zinngrebe, U

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 00/06809

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4024061 A	17-05-1977	GB 1464024 A	09-02-1977
		DE 2540060 A	25-03-1976
US 4629562 A	16-12-1986	NONE	

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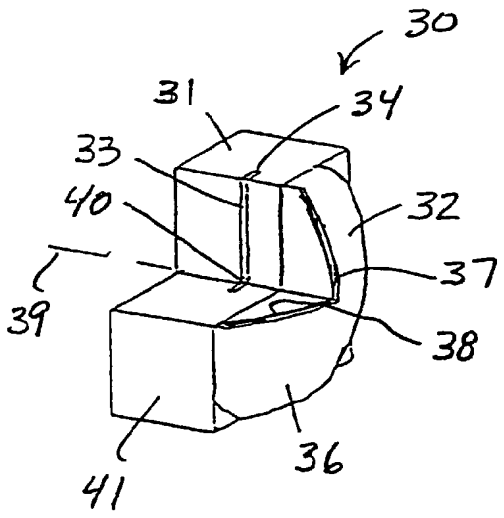
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: OPTICAL ELEMENT FOR FLOW CYTOMETRY



(57) Abstract: An optical element for use in a flow cytometer is provided that comprises a base member having a portion defining a flow channel, and an aspheric concave reflective surface having an optical axis intersecting the flow channel. Some light scattered or emitted by cells or other particulate material in a sample stream passing through the flow channel is collected and reflected by the aspheric concave reflective surface to form an image at an image plane external to the optical element.

WO 01/27590 A2

OPTICAL ELEMENT FOR FLOW CYTOMETRY

Field Of The Invention

The present invention relates to an optical element for collecting and projecting light scattered or emitted by the passage of particulate material through a beam of light. More specifically, the present invention relates to an optical element that combines the functions of a fluid flow delivery system, and a high-efficiency light gathering optic into a compact unit for use in a flow cytometer.

Background Of The Invention

Flow cytometers are used in biomedical research for counting, characterizing and sorting cells and other particulate material in a fluid. A typical flow cytometer works by passing individual cells or particles in a flow stream through an excitation site, where they are intersected by a high-intensity light beam or laser beam. If a particle is present, light from the beam is scattered by the particle, collected, and directed to a sensor that measures properties of the light to count and characterize the particles. Additional measurements may be made by staining the cells with fluorescent dyes or by reacting the cells

with fluorescent reagents that are biologically reactive with the cells, using the laser beam to induce fluorescence, and using the sensor to detect properties of the light emitted due to fluorescence to characterize various properties of the cells.

Flow cytometers are widely known and have been used for more than two decades. Numerous improvements and modifications have been developed that enable measurement of a variety of cellular properties, and enhance the accuracy and efficiency of the measurements. Also, many advancements in improving optical efficiency of the light gathering optics of flow cytometers have been achieved.

Some previously known systems improve the efficiency of light gathering by placing the excitation site within an ellipsoidal reflective chamber (typically referred to as a flow cell), or within a reflective chamber formed by a combination of spherical, ellipsoidal and other conic reflectors. These previously known systems typically are constructed so that the excitation site is located at a first focal point of the optical system, and light scattered or emitted by the sample is collected by a sensor positioned at a second focal point of the optical system.

For example, U.S. Patent No. 3,946,239 to Salzman et al., U.S. Patent No. 4,188,543 to Brunsting et al., U.S. Patent No. 4,189,236 to Hogg et al., and U.S. Patent No. 4,871,249 to Watson all describe systems that use enclosed reflective chambers. The chambers include windows for illuminating the sample stream and for light collection, and typically also have openings for introducing and removing the sample stream.

Although the light collection efficiency of the foregoing devices may be high, enclosed reflective chambers collect light scattered in all directions and direct the light to the focal spot by different paths using different numbers of surfaces, and thus do not form a true image of the sample. In addition, such previously known systems typically require additional optics to collect and redirect light in a manner useful for spectral processing and detection.

U.S. Patent 3,989,381 to Fulwyler, describes an optical chamber or flow cell having a spherical reflective portion for use in a flow cytometer. Instead of enclosing the excitation site within a reflective chamber to collect light scattered in all directions, however, Fulwyler uses a spherical reflective portion to reflect some of the scattered light, and direct it back through the sample towards a collection lens. Light passing through the collection lens is refracted into parallel beams, and is directed towards a photodetector. Thus, the spherical reflective surface functions as a light gathetering enhancer, rather than as an imaging optical element.

Refractive light collection systems, such as that described in the foregoing Fulwyler patent, may cause optical and chromatic aberrations. Such aberrations may be particularly severe near the edges of the lens, or wherever a significant refractive discontinuity is encountered. Such refractive discontinuities in the optical path also may reduce the light collection efficiency of the system. Systems that use refractive elements typically attempt to reduce such aberrations by filling the flow cell with a fluid having an index of refraction similar to the index of refraction of the material from which the

collection lens is formed, thereby decreasing the number of refractive discontinuities in the optical system. Optical aberrations may also be reduced by passing light through refractive boundaries or discontinuities at normal or near-normal angles.

In view of the foregoing, it would be desirable to provide an optical element for use in a flow cytometer that provides high-efficiency light gathering, and that produces high-quality images with reduced chromatic aberration.

It would further be desirable to provide an optical element for use in a flow cytometer that combines a flow channel for a sample stream, an excitation site, and a reflective light collection and imaging element into a single compact unit.

Summary Of The Invention

It is an object of the present invention to provide an optical element for use in a flow cytometer that provides high-efficiency light gathering, and that produces high-quality images with reduced chromatic aberration.

It is also an object of the present invention to provide an optical element for use in a flow cytometer that combines a flow channel for a sample stream, an excitation site, and a reflective light collection and imaging element into a single compact unit.

These and other objects of the present invention are accomplished by providing an optical element having a base portion defining a flow channel, and a mirror portion defining an aspheric concave reflective surface. The mirror portion preferably comprises a convex aspheric shape coated with a layer

of reflective material to form an aspheric concave reflective surface relative to the flow channel. The aspheric concave reflective surface preferably has a relatively large collection angle to provide high-efficiency light collection and to direct collected light to form an image an a plane external to the optical element.

The optical element is constructed from an optically clear material, and the base and mirror portions of the optical element may be integrally formed. Alternatively the base and mirror portions may be assembled from separate components, for example, a plano-convex element, reflectively coated on the aspheric surface, and a base element.

Where separate components are employed, the components are optically coupled with a coupling material having a refractive index matching the components joined, so as to reduce refractive discontinuities in the optical path. In addition, the sample stream preferably comprises a fluid selected to have an index of refraction that closely matches the index of refraction of the material from which the optical element is constructed.

Methods of using an optical element constructed in accordance with the present invention also are provided.

Brief Description Of The Drawings

The above and other objects and advantages of the present invention will be apparent upon consideration of the following detailed description, taken in conjunction with the accompanying drawings, in which like reference characters refer to like parts throughout, and in which:

FIG. 1 is a block diagram of a previously known flow cytometer;

FIGS. 2A and 2B are, respectively, a cutaway perspective view and a side view of an illustrative optical element constructed in accordance with the principles of the present invention;

FIG. 3 illustrates a ray trace resulting from use of the optical element of FIGS. 2; and

FIG. 4 is an exploded view of an alternative embodiment of an optical element of the present invention.

Detailed Description Of The Invention

Referring to FIG. 1, a block diagram of a previously known flow cytometer is described. Such a system is described, for example, in U.S. Patent No. 3,989,381 to Fulwyler, which is incorporated herein by reference. Flow cytometer 10 generally includes pressurized sheath fluid tank 11 coupled via inlet line 12 to sheath flow tube 13, pressurized sample fluid tank 14 coupled via inlet line 15 to sample tube 16, flow cell 17, light source 18, light collector 19, light detector 20, processing circuit 21, and waste tank 22. Light source 18 may comprise, for example, one or more lasers having different wavelengths, depending upon the intended application of flow cytometer 10.

A sample to be analyzed or sorted by flow cytometer 10, generally cells or other particulate material, is mixed with saline solution or distilled water in sample tank 14 to form a sample fluid. The sample may be treated with stains, fluorescent reagents or dyes to provide emissions in predetermined wavelength regions when subjected to predetermined

types of illumination. The sample fluid is injected under pressure into sample tube 16.

Sheath fluid, which generally comprises the same fluid used to form the sample fluid, is injected into sheath flow tube 13 and forms an annular flow coaxial with the sample fluid. Because the sheath fluid travels past the exit of sample tube 16 at much greater velocity than the sample fluid, the sheath fluid entrains the slower moving sample fluid. As the sample fluid attains the velocity of the surrounding sheath fluid, the diameter of the sample fluid stream generally is reduced to a diameter on the order of the thickness of a single cell or particle suspended in the sample fluid.

The resulting sample stream, comprising the reduced diameter sample fluid coaxially surrounded by the sheath fluid, passes through flow cell 17, where it is illuminated by beam 24 generated by light source 18. Beam 24 intersects the sample stream at excitation site 23. As described in the above-incorporated Fulwyler patent, flow cell 17 may be filled with quiescent sheath fluid through which the high-velocity sample stream passes with relatively little loss of sample material. The sample stream then passes into waste tank 22.

Flow cell 17 may include one or more spherical reflective elements that direct light from the excitation site to light collector 19. When a cell or other particulate material in the sample stream passes through the beam, the light is scattered. Alternatively, the cells in the sample may have been dyed using a fluorescent dye or a fluorescent reagent, causing them to fluoresce when the cells pass through the beam.

Some of the scattered or emitted light is reflected by the reflective interior of flow cell 17 toward light collector 19, typically a lens or other optical system, which focuses the collected light onto light detector 20. A spectral discriminating device, typically an optical filter or spectrally dispersing device 19a is placed in the optical path before the detector 20 to limit the light incident on the detector to the desired wavelength regions. Light detector 20 produces a signal indicative of properties of the detected light, and sends the signal to processing circuit 21, typically a microcomputer programmed to function as a data recorder. Processing circuit 21 may be used to count or characterize the cells or other particulate material in the sample stream.

Once the sample stream exits flow cell 17, it typically is collected in waste tank 22. Alternatively, with the addition of additional components that are not shown, but well-known in the art, system 10 may redirect some of the sample stream into a sorting chamber (not shown), based on the characterization of a sample obtained when the sample passed through flow chamber 17. Accordingly, flow cytometer may be employed for sorting cells or other particulate material having certain characteristics, as is known in the art.

Referring now to FIGS. 2A and 2B, a perspective view of optical element 30 constructed in accordance with the principles of the present invention is described. In FIG. 2A, an upper-right quadrant has been cut away to more clearly reveal internal structure of the optical element. Optical element 30 constitutes an aspheric reflective imaging viewing orifice and may

be substituted in place of flow cell 17 in flow cytometer system 10 of FIG. 1.

Optical element 30 preferably comprises base portion 31 and mirror portion 32. Base portion 31 comprises an optically clear material having a portion defining flow channel 33 having inlet 34 and outlet 35. Mirror portion 32, also preferably formed from an optically clear material, includes convex aspheric portion 36. Convex aspheric portion 36 is coated with thin layer 37 of a broadband reflecting material, such as aluminum, a broadband dielectric interference stack, or gold, which is deposited using techniques that are per se known, for example, by vapor deposition.

When coated with reflective material, convex aspheric portion 36 forms aspheric concave reflective surface 38 having optical axis 39. Excitation site 40 is located within flow channel 33 along optical axis 39 of aspheric concave reflective surface 38. In operation, a beam of high-intensity light is directed through lateral face 41 of base portion 31 to intersect the sample stream passing through flow channel 33 at excitation site 40.

Optical element 30 preferably may be manufactured as a single compact monolithic unit, and preferably comprises a transparent optical material, such as laser grade fused silica, quartz, or an optical grade plastic or appropriate optical glass with low base refractive index or high dispersive power ("ABBE value"). Monolithic construction of optical element 30 facilitates easy assembly and replacement of the optical system in a flow cytometer, and results in an optical system that is significantly more compact than the optical systems used in a previously known flow cytometers.

High optical collection efficiency is achieved by refractive matching of the fluid passing through flow channel 33 with the transparent material from which optical element 30 is constructed. In addition, aspheric concave reflective surface 38 preferably has a relatively large collection angle (i.e. numerical aperture greater than 1.0) and covers an area such that light is collected from a cone with an apex angle of 90° or more relative to excitation site 40.

Accordingly, assuming light scattered or emitted from the cells or other particulate material within the sample stream has an isotropic distribution, a collection angle of 90° will result in approximately 20% of the total light emitted being collected by aspheric concave reflective surface 38, and used to form an image. Because aspheric optics are used to form reflective surface 38, near diffraction-limited performance is provided for on-axis operation, despite the large collection angle.

Optical element 30 may be inserted in the system of FIG. 1 in place of flow cell 17. In operation, the sample stream, either with or without an outer annulus of sheath fluid, enters optical element 30 through inlet 34. To minimize the optical aberrations, the sample stream completely fills flow channel 33. The sample stream exits optical element 30 through outlet 35.

As illustrated in FIG. 3, laser 42 generates focused excitation beam **L**, which is directed through lateral face 41 of base portion 31 orthogonal to flow channel 33. Excitation beam **L** intersects and illuminates the sample stream at excitation site 40.

As a cell or other particulate material passes through excitation site 40, illumination by excitation beam **L** causes scattered emitted light, such as fluorescence, phosphorescence and/or other types of emission to occur, depending upon the dyes, stains or reagents used in the sample stream, and the types of illumination employed.

Light scattered or emitted from the cell or particular material forms light rays **A** that strike aspheric concave reflective surface 38 of mirror portion 32. Aspheric concave reflective surface 38 reflects some of light rays **A** to form reflected rays **A'**. Rays **A'** are focused by aspheric concave reflective surface 38 to generate image **I** at image plane **P**, external to optical element 30. Image **I** may be detected using any of a variety of known photodetection means, and may be used for spectral processing and detection, or other measurements as is known in the field of flow cytometry.

In addition, because unfocused light falling on image plane **P** may represent a source of potentially interfering background noise, an apertured screen may be placed just before the image plane in alignment with optical axis 39 to mask the detector from such unfocused light. Applicant expects that contributions to the light impinging on a photodetector may be reduced, for example, to less than 0.2%, by using such an apertured screen.

Advantageously, the reflective optics used to collect, redirect, and generate the image in the optical element of the present invention are expected to introduce little or no chromatic aberrations. Although the light travels through refractive materials

(i.e. the optical element), and encounters boundaries having significant refractive discontinuity (e.g. when the light exits the optical element), chromatic aberration is expected to be insignificant, because the light crosses such boundaries at near normal angles. Additionally, since the fluid that fills flow channel 33 preferably has its refractive index matched to that of the material of optical element 30, the number of refractive discontinuities encountered is reduced.

Referring now to FIG. 4, an exploded view of an alternative embodiment of an optical element constructed in accordance with the present invention is described. Optical element 50 comprises two separate components: aspheric plano-convex element 52 having thin layer 53 of reflective coating disposed on aspheric convex surface 54 to form aspheric concave reflective surface 55; and base cuvette 56 defining flow channel 56. Aspheric plano-convex element 52 and base cuvette 56 preferably are constructed of the same optically clear material, and preferably are coupled together using layer 57 of a suitable optical adhesive or gel that closely matches the refractive index of the material from which components 52 and 56 are constructed.

While manufacturing optical element 50 from separate components may introduce assembly tolerance issues resulting in slight losses in optical efficiency and image quality, optical element 50 may be much easier to manufacture. In addition, aspheric plano-convex elements 52 having different curvatures may be coupled to base cuvette 56, depending upon the intended application of the flow cytometer. It will of course be apparent to one skilled in the art that the optical element of the present invention may be constructed in

other ways. For example, an optical element that is split at the position of the flow channel may provide some additional benefit in ease of manufacture.

While preferred illustrative embodiments of the present invention are described above, it will be evident to one skilled in the art that various changes and modifications may be made without departing from the invention. It is intended in the appended claims to cover all such changes and modifications which fall within the true spirit and scope of the invention.

What Is Claimed Is:

1. An optical element comprising:

a base member including a portion defining a flow channel; and

an aspheric concave reflective surface having an optical axis that intersects the flow channel to define an excitation site, the aspheric concave reflective surface optically coupled to the flow channel to generate an image at an image plane.

2. The optical element of claim 1, wherein the aspheric concave reflective surface comprises:

a mirror portion having an aspheric convex surface; and

a layer of reflective material disposed on the aspheric convex surface.

3. The optical element of claim 1, wherein the flow channel further comprises an inlet disposed on a first lateral face of the base member and an outlet disposed on an opposing lateral face of the base member.

4. The optical element of claim 1, wherein the base member defining the flow channel and the aspheric concave reflective surface are integrally formed.

5. The optical element of claim 2, wherein an optical adhesive is used to optically and mechanically couple the base member to the mirror portion.

6. The optical element of claim 2, wherein an optical gel is used to optically couple the base member to the mirror portion.

7. The optical element of claim 1, wherein the aspheric concave reflective surface has a collection angle of at least 90°.

8. The optical element of claim 1, wherein the optical element has a numerical aperture greater than 1.0.

9. The optical element of claim 1, wherein the optical element is constructed of material selected from a group consisting of laser grade fused silica, quartz, an optical grade plastic and an optical grade glass.

10. An optical element comprising:

a base member having a portion defining a flow channel;

an aspheric plano-convex portion optically coupled to the base member; and

a layer of reflective material disposed on the aspheric plano-convex element to form an aspheric concave reflective surface, the aspheric concave reflective surface having an optical axis that intersects the flow channel to define an excitation site.

11. The optical element of claim 10 wherein the base member and the aspheric plano-convex element are integrally formed.

12. The optical element of claim 10 wherein the base member and the aspheric plano-convex element are optically coupled using a material selected from a group consisting of an optical adhesive and a gel.

13. The optical element of claim 10, wherein the aspheric concave reflective surface has a collection angle of at least 90°.

14. The optical element of claim 10, wherein the optical element has a numerical aperture greater than 1.0.

15. The optical element of claim 10, wherein the aspheric concave reflective surface generates an image at a location external to the optical element.

16. The optical element of claim 10, wherein the optical element is constructed from a material selected from a group consisting of laser grade fused silica, quartz, an optical grade plastic and an optical grade glass.

17. A method of characterizing particles in a sample stream comprising:

providing an optical element comprising a base member having a portion defining a flow channel and an aspheric concave reflective surface optically coupled to the flow channel;

passing the sample stream through the flow channel;

illuminating the sample stream at an excitation site to generate scattered emitted light;

collecting the scattered emitted light using the aspheric concave reflective surface;

redirecting the scattered emitted light collected by the aspheric concave reflective surface to generate an image; and

detecting the image at the image plane to characterize the particles.

18. The method of claim 17 wherein passing the sample stream through the flow channel further comprises providing a sheath fluid having an index of refraction substantially equal to an index of refraction of a material from which the optical element is formed.

19. The method of claim 17, wherein redirecting the scattered light collected by the aspheric concave reflective surface to generate an image further comprises generating the image at an image plane at a location external to the optical element.

20. The method of claim 17 wherein collecting the scattered light using the aspheric concave reflective surface further comprises collecting scattered light from a cone-shaped region having an apex angle of at least 90° relative to the excitation site.

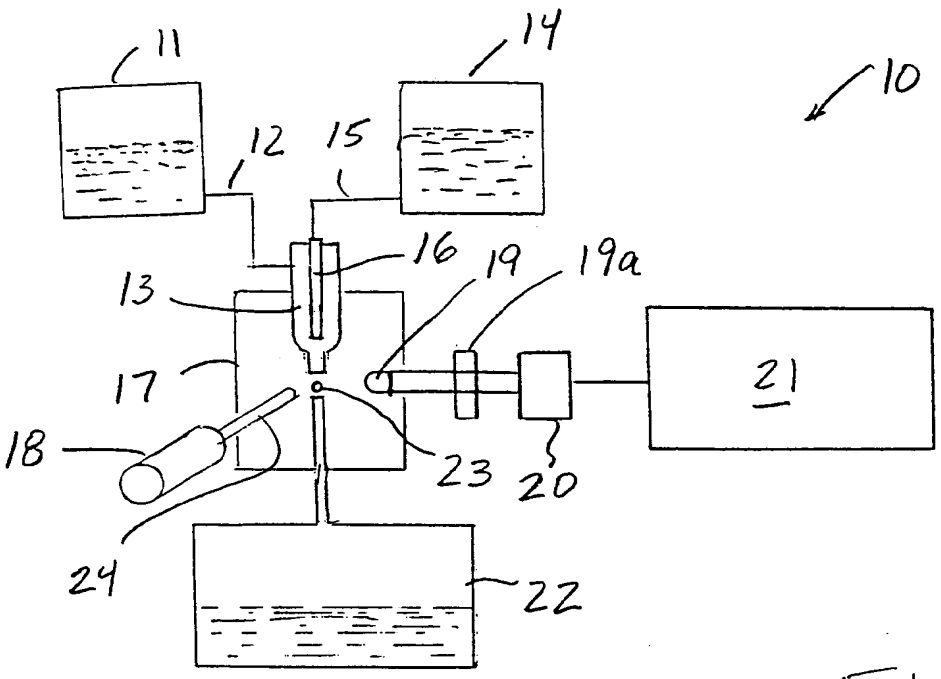


FIG. 1
(PRIOR ART)

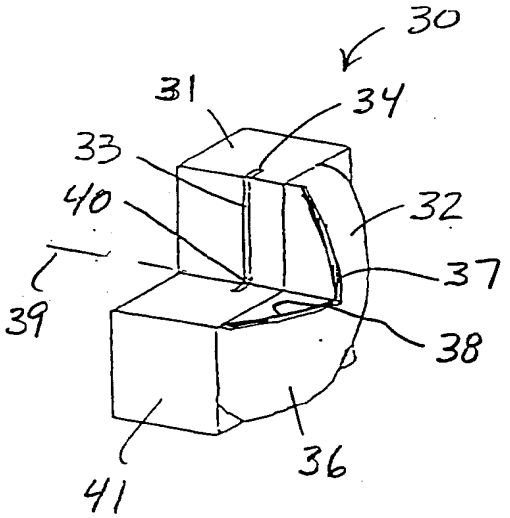


FIG. 2A

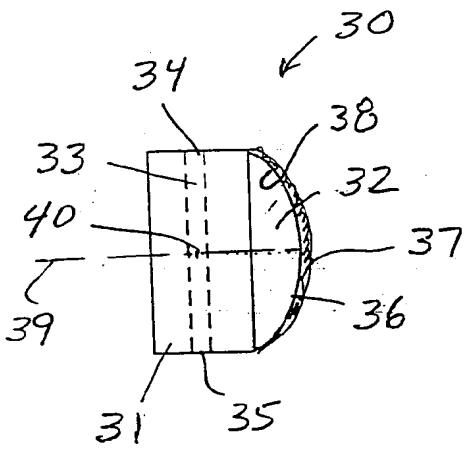


FIG. 2B

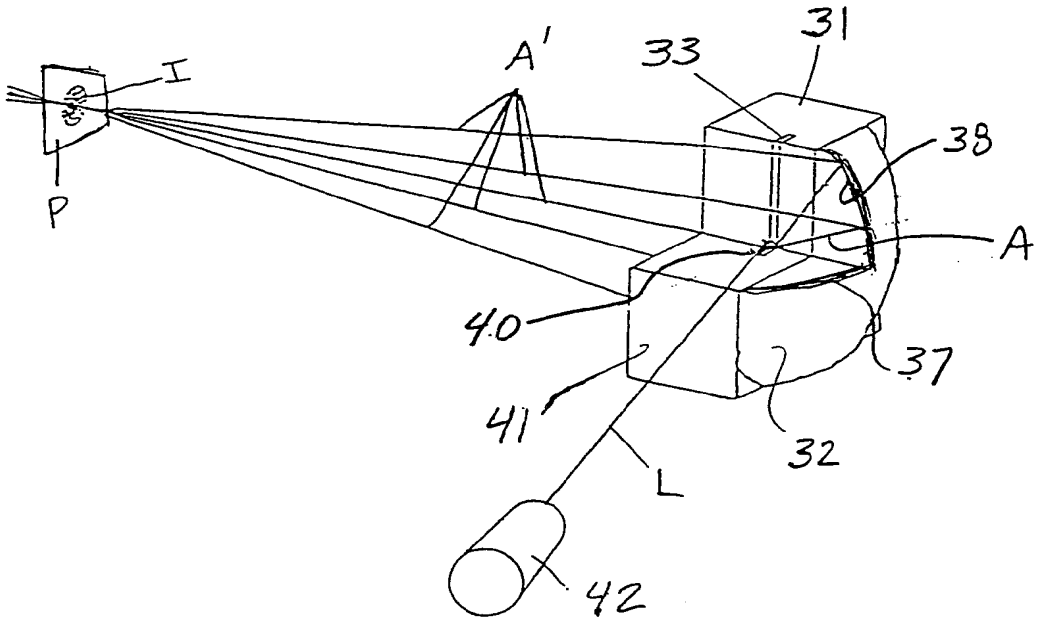


FIG. 3

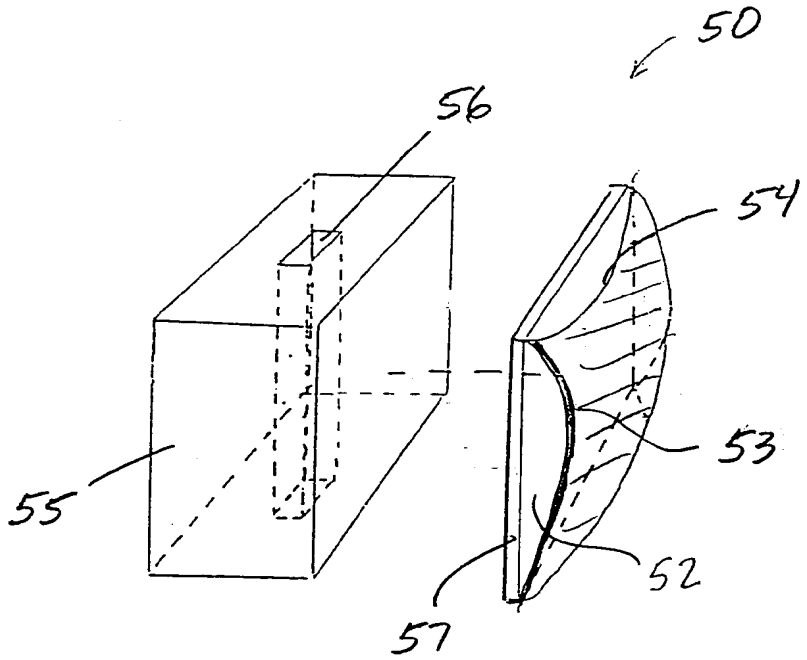


FIG. 4

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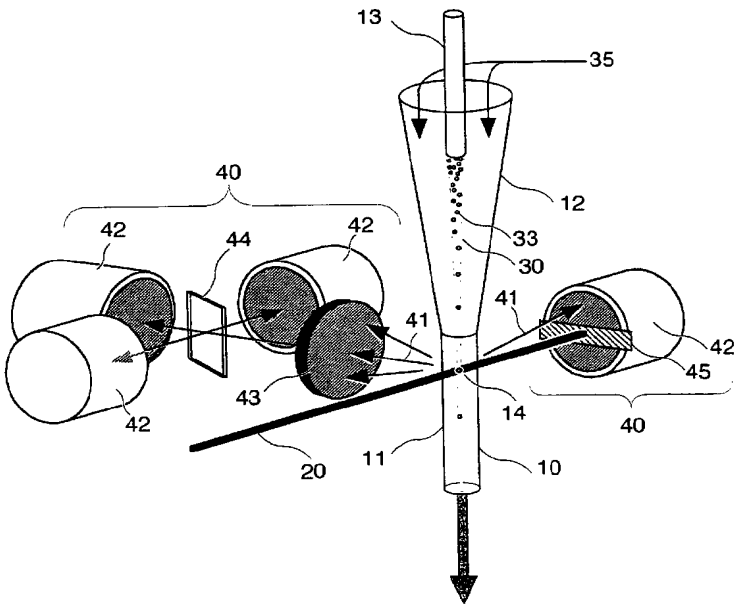
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(54) Title: APPARATUS FOR THE DETECTION OF PARTICLES



(57) Abstract: This invention provides an apparatus for the detection of individual particles in a suspension flowing through a transparent flow cell, further comprising of means to irradiate the suspension in this flow cell and means to detect the light emitted by the suspended particles. The irradiation means provides two identical coparallel and overlapping laser beams, with a lateral, relative displacement more or less equal to the beam radius, and polarized in relative orthogonal directions. This apparatus is also suited for the detection of larger particles.

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Apparatus for the detection of particles.

The present invention relates to an apparatus for the detection of particles in a fluid, comprising a fluid system with an at least partly transparent flow cell to receive the fluid, means to irradiate the fluid in this flow cell and means to detect the light emitted by the particles in the flow cell, the irradiation means comprising one or more lasers generating a beam which follows at least during operation a beam path through the fluid.

An apparatus of the kind described above is commercially available and was primarily developed for biomedical purposes. These are so-called flow cytometers, in which a suspension, usually a blood sample, is forced through the flow cell at high speed, therein intersecting a laser beam which crosses the flow cell. Microscopical particles in the stream of fluid can be detected and counted by the light they emit by scattering and possibly fluorescing, in order to determine in this way concentration thereof, and also to classify the particles based on their individual optical properties as measured. Especially different types of blood cells, but optionally also bacteria and other unicellular particles and even viruses are involved. The particle suspension is injected under laminar conditions into a particle free sheath fluid which narrows down and accelerates the particle suspension to a very thin stream of suspension that is carried through the center of the flow cell at high speed. As this forces the particles to flow through the laser beam predominantly one by one, the particles are measured on an individual basis allowing accurate counting. The detection means to detect the light fluxes emitted by the passing particles comprises of one or more photodetectors converting the light fluxes into electrical pulses. These pulses are subsequently converted into numbers allowing computer aided analysis using suitable classification software to count and classify the particles. Standard flow cytometers are most suited for particles smaller than 20-30 μm.

There is a growing need for an apparatus of the kind mentioned above that allows the measurement of larger particles and accommodates a larger flow rate of the particle suspension. This means that the fluid system and flow cell should have an accordingly larger flow through width and also that the irradiation and detection means should be

tailored to accommodate a larger diameter of the suspension stream. A problem in this respect is the requirement of a highly uniform irradiance across the full suspension stream to obtain reproducible and precise measurements. However, the irradiance distribution of a laser beam often has a Gaussian shape, limiting the useful area with an acceptable deviation from the maximum irradiance to the rather small central part of the beam. A widening of the irradiance distribution, to enlarge this area, causes a correspondingly reduced irradiance level in this area, impairing the performance of the system to detect and measure very small particles. This requires a relatively high irradiance level. If the total light output power in a Gaussian beam is limited, as with small lasers, a high irradiance would require a very narrow beam, whereas the accommodation of a uniform irradiance distribution over a rather wide suspension stream requires a wide beam, much wider compared to the suspension stream. These conflicting requirements have impaired the realization of a compact apparatus of the kind mentioned above, having the capability to measure larger particles and to accommodate larger suspension streams, such as required for the detection of algae and other planktonic particles in (surface) water.

The present invention aims to provide an apparatus of the kind mentioned in the preamble having this capability.

For this purpose an apparatus of the kind mentioned in the preamble according to the present invention is characterized in that the irradiation means comprises of two at least almost identical, Gaussian, polarized laser beams, having at least almost orthogonal relative directions of polarization, and following, at least during operation, relative parallel beam paths through the fluid while the relative distance of both beam paths is sufficiently small to allow partial overlap of both laser beams. Using according to the present invention two partly overlapping laser beams to irradiate the fluid stream results in an intensity distribution consisting of the sum of two Gaussian distributions. By choosing the overlap favorably, a significantly more uniform intensity distribution at a similar intensity level can be achieved around the center of the combined distribution, as compared to using a single Gaussian beam. Because the relative directions of the

polarization of both beams are positioned orthogonally, interference effects between both beams are circumvented, which would otherwise impair the flatness of the intensity distribution and the measurement therewith. In this manner the present invention provides a significantly wider uniform irradiation width at equal laser power compared to existing apparatus, making the apparatus according to the present invention also suited for wider fluid streams and larger particles.

In a preferred embodiment the apparatus in the present invention is characterized in that the distance between both beam axes is at least almost equal to half of the width of the laser beams, defined as the width inside which the irradiance is larger than $1/e^2$ of the maximal (axial) value, with e the mathematical base number of a natural logarithm. It appears that such an overlap yields the largest possible area in which the irradiance is at least almost uniform. Based on the available laser(s), such a fine tuning of both laser beams allows the achievement of the largest possible useful irradiation area.

In practice it appears that in an at least almost absolute laminar fluid stream a fluid speed occurs acquiring a quadratic distribution with the progression of the stream from the side of the stream to the maximum speed in the middle . This means that particles flowing through the center of the stream will reside somewhat shorter in the laser beam compared to particles flowing more closely to the side of the flow channel in the flow cell. Consequently the light emission of the central particles will be slightly less during their passage. To compensate for this a further preferred embodiment of the apparatus in the present invention is characterized in that, at least during operation, the fluid flow through the flow cell is at least almost laminar and that the distance between both beam paths is a little smaller than half of the beam width of both laser beams. By creating in this manner slightly more overlap the resulting intensity distribution around the center of the bundle will not be flat but slightly bulged towards the center, coinciding with the center of the suspension stream, which at least partly compensates for the shorter residence time of the particles in the center.

A further embodiment of an apparatus of the kind as mentioned above is characterized in that the flow cell comprises an elongated cuvette and that both parallel beam paths lie next to each other in a plane perpendicular to this cuvette intersecting the cuvette. In this manner the intersecting area is a practically perpendicular cross section of the fluid stream, allowing accurate determination of the particle flux. A further preferred embodiment of the apparatus according to the present invention is characterized here in that the polarization directions of both laser beams are positioned at least almost 45 degrees relative to the direction of breadth of the cuvette. In practice it appears that the light scattering by the particles, usually being measured in this plane, may be influenced more or less by the polarization direction of the laser beam. By positioning the directions of polarization at angles of about 45 degrees plus and minus to this plane, these kind of effects are minimized.

A special embodiment of the apparatus according to the present invention is characterized in that the fluid stream in the flow cell has a position in the overlap area of both beams. In this manner a uniform irradiance over the full width of the suspension stream can be achieved.

A further special embodiment of the apparatus according to the present invention is characterized in that the width of the fluid paths in the flow cell is sufficiently large so that both laser beams intersect across the fluid channel and not or at least almost not hit the side walls of the flow cell. By keeping in this manner both laser beams predominantly inside the fluid channel of the flow cell, adverse effects such as reflections, interference and scatter from the edge of the flow cell which could otherwise impair the intended irradiance profile and in this way the measuring results are minimized.

A further special embodiment of the apparatus according to the present invention is characterized in that the irradiation means comprise two at least almost identical lasers combined with alignment means to co-align the beam from each laser with the other beam at the desired lateral distance. Separate laser beams are used to create both laser

beams. Thanks to the alignment means these both beams are leaded in the described way besides each other. Polarization means should provide the relative orthogonal polarization directions, if needed integrated in the alignment means.

A further embodiment of the apparatus according to the present invention is characterized in that the irradiation means comprise an optical beam splitter in order to allow the use of a single laser from which the beam is split in two beams of at least almost identical diameter and intensity in such a way that the path and polarization orientation of these beams may be positioned and directed through the flow cell in the way described above. This is possible in principle with various types of components such as beam splitters and polarization prisms. The irradiation means may also comprise one or more half-wave plates to manipulate the polarization orientation at will.

A further special embodiment of the apparatus according to the present invention is characterized in that a so-called beam displacing prism or plate is used to achieve the required splitting of the single laser beam into two beams. A beam displacing prism is a specific, plan parallel crystal, for instance made from quartz or calcite and which provides two coparallel, but laterally displaced, orthogonally polarized output beams from one polarized input beam. The relative intensity of the output beams varies from zero to one (equal to the input beam) depending on the angle between the polarization state of the input beam and the optic axis of the prism material. If the orientation of the polarization state of the input laser beam is directed at least almost exactly between these two extremes (in practice at 45°), then the beam displacing prism will split this input beam into two at least almost equal, coparallel beams with relative orthogonal polarization.

One of these beams remains undisplaced from the input beam whereas the other coparallel beam is laterally displaced over a distance depending on the thickness of the prism. By choosing a sufficiently thick prism, the required overlap of both beams for the present invention can be achieved in a totally optical manner. A specific example of a beam displacer is a so-called Savart plate, comprising two equally thick beam displacing

plates glued together at an orthogonal optic axis angle. Such a component is particularly useful in the present invention.

For optimum results within the framework of the present invention it is important to fine tune the distance between both beams accurately to the beam diameter or vice versa. This overlap distance can be achieved optically and/or mechanically in the apparatus according to the present invention. Inevitably there will be some inaccuracy in the optics and the mechanics of the apparatus and particularly the beam width of the laser appears to be a less constant factor also depending on the divergence or convergence of the beam. To cope with these kinds of tolerances relatively simply, and to allow the preferred use of a beam displacer to get a constant displacement in a simple manner, a further embodiment of the apparatus according to the present invention is characterized in that the irradiation means allow fine adjustment of the beam width of the laser beams. A special embodiment of the apparatus is characterized in this respect in that the irradiation means comprises a set of anamorphic prisms. These prisms are placed behind each other in the bundle path and allow the adjustment of the beam width in one direction by rotating the prisms more or less.

As, in accordance with the present invention, the irradiation of the suspension stream is the sum of the Gaussian irradiance profiles of two partly overlapping laser beams with orthogonal polarization state, the laser light scattered by the particles will contain proportional amounts of light from both polarization states. A further embodiment of the apparatus according to the present invention is characterized in that the detection means comprise a combination of two detectors with suited polarization components, in a way that each detector measures only the light scattered by the particles in a single polarization state corresponding to one or the other of both orthogonal laser beams that make up the irradiance profile across the suspension stream. Light scattered by a particle flowing in the middle of the flow cell and intersecting the overlapping laser beams at least almost exactly through the middle of their combined irradiation profile, will contain equal amounts of both polarization states. If a particle is flowing through the combined irradiance profile of both laser beams at some distance away from the middle,

therefore more through the one than the other laser beam, then its scattered light will contain proportionally more light from this one laser with the corresponding polarization state than from the other laser with the other polarization state.
The said detectors should collect a sufficiently large portion of the light scattered by the particles to limit effects of interference at least almost sufficiently. Using a combination of two detectors as described above allows the calculation of the ratio between the measured light scatter signal from these both detectors for every individual passing particle. This ratio is directly governed by the lateral position, for instance right through the middle or some distance to the left or to the right side of the middle, of the particle trajectory through the combined irradiance profile and may be calibrated. Knowledge of this lateral position may be important for the calibration of measurements of other detectors as well for the accurate triggering or calibration of applications down stream, such as secondary irradiation and detection means or sorting or imaging-in-flow devices, for which the position of the particle trajectory may be very important in relation to certain physical properties such as flow speed, depth of focus etc.

Subsequently the present invention will be elucidated by a number of example embodiments in conjunction with accompanying drawings. The drawings are pure schematically representations and not drawn to scale. Some dimensions are strongly exaggerated particularly for clarity. Like reference numerals are used as much as possible throughout the drawings for corresponding components.

Figure 1 is a three-dimensional representation of some basic parts of an apparatus according to the present invention. Here, the flow cell comprises of an elongated cuvette (10) with an at least almost transparent wall (11) allowing the passage of a laser beam (20) with focussing means (50) (schematically depicted here). The suspension to be analyzed is carried into the relatively wide and funnel shaped upper part (12) of the cuvette in a constant flow, usually injected here through a hollow needle (13) while being surrounded by a much larger amount of co-injected particle free sheath fluid (33). Upon approaching the narrow flow channel of the cuvette the fluid cross section of both the sheath fluid and the suspension stream sharply decreases, whereas their relative

position remains. As a consequence the flow velocity increases sharply, resulting in an at least almost laminar, fast flowing suspension stream (30) in the middle, surrounded by the sheath fluid. The diameter of the sample stream is proportional to (the square root of) the suspension volume being analyzed per second. This suspensions stream intersects a laser beam (20) which is directed orthogonally through the cuvette at an intersection point (14). Particles flowing through this point scatter the laser light and may emit fluorescence light, which is being detected by a detection system (40). Detection of the light fluxes (41) emitted by the passing particles is done with photo detectors (42), combined with components such as a collection objective (43), beam splitters and/or dichroic mirrors (44) and a beam dump (45). The detectors convert the light pulses into electronic pulses. These pulses are subsequently digitized and further processed. In this manner the optical properties such as light scattering and fluorescence of about 1000 or more particles per second can be measured. The resulting data is processed and analyzed using a computer, whereas the measured data can also be used in real time in some instruments to activate a downstream device such as a sorting unit to sort out individual particles of interest from the suspension stream.

Figures 2A and 2B show the side view and top view respectively of a flow cell of an apparatus in the present invention, with flat, transparent walls (11) allowing the passage of a (schematically depicted) laser beam (20) with schematically depicted focus means (50). The flow cell contains the suspension stream 30 in the middle surrounded by the sheath fluid (33). Detection means (40), schematically depicted, are placed around the flow cell, having a 'field of view' (46) from which emitted light can be detected. Only particles inside the intersection volume (cross hatched area) of the suspension stream (30) and the laser beam (20), which should completely fall inside the detection æfield of view' (46), can be detected by the apparatus. This intersection volume is subject to certain requirements for optimum performance of the apparatus. Its volume should be as small as possible to keep the signal to noise ratio maximal. The light intensity should be uniform throughout the intersection volume and as high as possible to be able to detect small particles. To get high light intensity from a relatively small laser requires an as narrow as possible beam, at least where the particles are being measured. However, the

Gaussian distribution of the light intensity over the width requires a spot many times larger than the diameter of the suspension stream. Particles flowing through the intersection volume outside the middle (viz. figure 3) will experience a lower light intensity while covering the same optical path as particles covering the middle, will experience a higher light intensity. Figure 3 A schematically represents a Gaussian irradiance distribution by a black shading proportional to the light intensity. This is maximal in the middle and gradually decreases towards the sides. Figure 3B shows the intensity I (21) as a function of the distance r (22) from the middle according to a Gaussian distribution:

$$I(r) = I(0).\exp(-2r^2/w^2)$$

with I(0) the maximum light intensity in the center of the beam and w the distance from the middle to the side of the beam, where the intensity has fallen to $1/e^2$ (23) of the maximal intensity in the center, with e the mathematical base number for natural logarithm. The beam diameter D_b (24) is defined as 2w. To limit the variation of the measured values the variation in the light intensity should be limited. The accepted variation in light intensity (P %) yields a minimum laser beam width B_f according to:

$$B_f = \frac{D_m}{\sqrt{-\tfrac{1}{2}.\ln(1-\frac{P}{100})}}$$

with D_m the diameter of the suspension stream. If P is 5 % for example, then the beam diameter should be 6.25 times wider, yielding a beam width of 375 μm for the suspension stream diameter of 60 μm used as example here.

A large beam width yields a low variation in light intensity across the intersection volume at the cost of a relatively low light intensity level, increasing the detection limit. This detection limit can be decreased by placing a more powerful laser which increases the price of the flowcylometer. In such a case the largest part of the light is not being used because it falls outside the suspension stream while causing unnecessary background scatter particularly where intersecting the cuvette walls.

The solution applied in the apparatus according to the present invention aims to get a more efficient light distribution compared to the standard Gaussian (bell shaped) distribution because a significant broader part of the laser focus has a constant light intensity. This objective is achieved by the superposition of two Gaussian beams with coparallel - parallel beam paths and partly overlapping light distributions. These two beams may originate from a single laser. Interference of these two partly overlapping beams can yield an unwanted, strongly fluctuating intensity distribution. This however may be prevented by arranging the polarization states of these beams perpendicular to each other. This is possible in principle for normal linearly polarized laser beams as well as for circular polarized beams. The intensities are superimposed now without interference effects. With two beams of identical diameter D_b, measured with D_b between the $1/e^2$ boundary values, the ideal overlapping distance appears to be exactly 1/2 D_b or in (the beam radius) w. In this case the intensity distribution for the central cross section over the center of the combination of both beams can be written as:

$$I(r) = \tfrac{1}{2} I_0 . e^{-2(r/v)^2} . (e^{-2r/v} + e^{2r/v}) \quad with \quad v = \frac{w}{\sqrt{e}}$$

It appears that the central part of the resulting light distribution is flat, allowing a much larger suspension stream diameter, or in the case of an unchanged suspension stream diameter, a narrower total beam width with correspondingly higher light intensity.

Figures 4A and 4B show two Gaussian beams at relative distance 24 of w (= 1/2 D) with the direction of the laser beams (20) indicated. Figure 4A shows the intensities of each beam separately, whereas figure 4B shows the superimposed intensity of both beams. Owing to the Gaussian character of the original intensity distributions, all distributions including the superimposed distribution, are similarly shaped over all cross sections in the lateral direction (31), which is the flow direction of the sample stream. This is illustrated by the grid lines of figure 4B. The integration of these light distributions over the lateral direction (31) where the particle flow is therefore also similar, implying that

this type of light intensity distribution yields similar results for apparatus measuring the signal amplitudes as for apparatus measuring integrated scatter or fluorescence signals. The resulting light intensity distribution can be adjusted by slightly varying the overlapping distance at constant beam diameter or varying the beam diameters at constant overlapping distance (viz. figures 5A-5E). By slightly reducing the beam widths at constant overlapping the flat section may be widened while a small dip originates in the center. If this dip remains within the accepted variation in light intensity, this strategy would accommodate the largest possible suspension stream diameter or maximize the light intensity, viz. figure 5E. Instead of this, a slight widening of the beams reduces the flat section until a slight bulging of the center of the distribution appears (viz. figure 5B). This distribution could resemble more or less a velocity distribution of the fluid over the width of the cuvette, with a partly developed laminar Poiseuille flow profile while in the lamminar flow an even further quachatic velocity partition arises. As the integrated signal is inversely proportional to the particle velocity (lower velocities yield larger integrated signals), a light distribution as mentioned could be used to eliminate the effect of the variation in particle velocities exactly, and an optimal low spread in the integrated measured values could be obtained.

Figure 5A shows a typical Gaussian laser beam (27) as reference. The more or less 'flat' and thus acceptable section (26) of the light distribution, for the purpose of irradiation of the suspension stream, is chosen here arbitrarily as the section in which the intensity falls does not deviate more than 3% from the maximum value. The beam diameter D_b (24) is also shown as 100 percent. Next are depicted the profiles (28) resulting from the overlapping of two similar beams which allow a broader sample flow at the same intensity of light, using narrower beam widths from left to right. Figure 5E gives the largest widening (2.53 times - yielding a 6.4 times larger suspension flow) while 5B might give the best compensation for the shape of the velocity profile. Figure 5C is the exactly flat distribution, obtained using an overlap of both beams of exactly 1/2 D. Figure 6 shows the improvement at constant suspension stream diameter: a double light intensity and a strongly reduced level of light incident on the cuvette side walls at narrower distribution. Elliptical instead of round beam cross sections (at the intersection

point with the suspension stream) are sometimes applied in flow cytometers, for instance by using crossed cylindrical lenses with different focal distances instead of spherical lenses as beam focussing means. In this way the laser beam width may be reduced in the plane of the particle flow path (assumed vertical) to concentrate the light over a smaller path length (which increases the detection level) while the beam width in the perpendicular plane (assumed horizontal) may be tailored to the requirements of the light distribution across the suspension stream width. The above described principle of overlapping beams is equally valid for round beams as well as for beams with elliptical cross sections.

The most simple embodiment of an apparatus according to the present invention explained here is based on the use of a so-called Savart plate. The results given above have been based on the same. This is a combination of two ortogonal beam displacers, consisting of a relative crosswise mounted pair of identical beam displacer plates, usually made from calcite or quartz, which allows the use of a single laser to realize the two at least almost identical co-parallel beams by having its output beam normally intersect such a Savart plate. The Savart plate should be oriented in a such a way that the angle between the plane of polarization of the laser beam and the relative orthogonal planes of beam separation of the Savart plate is at least almost +45 or -45 degrees. The lateral displacement Bd of the resulting beams relative to the input beam is governed by the wavelength dependent lateral beam displacement factor, a known property of the beam displacer plate material used, the wavelength of the laser beam and the thickness of the displacer plates in the Savart plate. The overlap of the foci is $\sqrt{2}.B_d$.

Figures 7A and 7B schematically show a top view and side view respectively of an example embodiment of an apparatus according to the present invention, based on using such a Savart plate (51) for a single laser (52). The width (between $1/e^2$ points) of the laser beam incident on the Savart Plate is at least almost equal to the relative lateral beam separation of the output beams multiplied by 2: $2\sqrt{2}.B_d$.

Laser beam diameters vary widely with typical values between 1.0 and 1.8 mm between $1/e^2$ points. A relatively large beam diameter may be preferred in order to achieve the highest level of concentrating or focussing of the light in the plane along the suspension stream (assumed vertical). The latter may be achieved for example by using a cylindrical lens (54), which does not influence the distribution of light in the horizontal direction. In such a case the beam width in the perpendicular plane (assumed horizontal) may have to be reduced (for example by using a set of anamorphic prisms (53)) to achieve the preferred light distribution in conjunction with a particular beam separation. In addition, the light distribution in this horizontal plane may be further reduced by an extra cylindrical lens. Instead of tailoring the thickness of the Savart plate to the $1/e^2$ diameter of the laser beam, it is more practical to adjust the width of the laser beam by optical means to the size of the Savart plate, for instance 2.0 mm, particularly because the laser beam diameters and divergence may show quite large tolerances in practice. The width of the laser beam (assuming the horizontal diameter) can easily be adjusted in one direction by using a set of anamorphic prisms which allow later on fine tuning of the anamorphic expansion by slightly changing the prism angles with respect to the incident beam. This requires that the polarization state of the laser beam coincides with the plane of the anamorphic expansion, possibly requiring rotation of the direction of the laser beam polarization state, which can easily be achieved by for instance inserting a half-wave plate (55) in the laser beam. The directions of the polarization states are indicated in the drawings as 'hor' or 'vert' for horizontal and vertical respectively, and ±45° meaning ±45° relative to the vertical direction.

A second embodiment of the apparatus according to the present invention is depicted schematically in the figures 8A and 8B, in top and side view respectively. This embodiment is largely similar to the first embodiment, while the difference lies in the application of a single beam displacing plate in conjunction with half-wave plates (55) on its front and back side instead of a Savart plate.

The third embodiment of the apparatus according to the present invention is depicted in figures 9A and 9B, and uses two lasers for the generation of the two beams needed to

realize the coparallel overlapping pair of beams. This can be advantageous when using a type of laser with limited output power. Figure 9A shows the first laser positioned behind a polarizing cube beam splitter (57) with its polarization state oriented for maximal transmission by the beam splitter, in conjunction with the other laser placed at 90° relative angle facing the side of the polarizing cube beam splitter (57) with its polarization state oriented for maximal reflection and therefore leaving the cube beam splitter in the same direction as the beam from the first laser. Careful positioning of both lasers is required to achieve at least almost complete coparallel output beams in conjunction with the optimal lateral distance between their axes. Figure 9B shows the assembly of both lasers with the cube beam splitter placed at 45° relative to the flow cell in order to achieve the optimal orientation of the polarization states of +45° and -45° of both laser beams at the intersection point with the suspension stream (14).

Figures 10A, 10B and 10C schematically depict a possible embodiment which allows the determination of the location of a particle's trajectory relative to the irradiation light distribution according to the present invention by detection of the relative amounts of light scattered by the particle and having a state of polarization corresponding to one of both laser beams. Figure 10A schematically shows how the division of the forward light scatter flux of a particle into relative contributions of the individual laser beams making up the combined light distribution, by splitting the forwardly scattered light after collimation by a collection lens (43) in two orthogonal directions using a polarizing cube beam splitter (57) positioned such that maximum separation of both states of polarization is achieved. As a result, the light transmitted straight through the cube originates almost entirely from one of both laser beams, whereas the sidewardly reflected light originates almost entirely from the other laser beam. Adverse interference and depolarization effects can be reduced by inserting polarizers (58) in front of the detectors oriented like the corresponding laser beam. Figure 10B shows the light distribution resulting from the preferred overlapping of both laser beams, and particle trajectories (33) for two different particles. The particle flowing through the center receives equal amounts of light from both laser beams, but the particle flowing at the left side of the middle receives more light from one laser beam (27a) and less from

the other laser beam (27b). This affects the corresponding signals of the detectors (42a) and (42b). In return, the ratio between these detector signals allows the estimation of the position (22) of the flow trajectory of each particle relative to the center of the light distribution (in practice coinciding with the axis of the flow channel), especially after calibrating this relation at different ratios of the signal values (viz. figure 10C). This may be important for the calibration, timing or focussing (47) of down stream applications as a second indication and detection circuit or sorting apparatus or camera.

Although the present invention has been elucidated in the above using a limited number of examples, it should be clear that the present invention is by no means restricted to the examples given. On the contrary, many variations and embodiments are possible within the framework of the present invention for the average skilled in the art.

Claims:

1. An apparatus for the detection of particles in a fluid, comprising an at least partly transparent flow cell to receive the fluid, means to irradiate the fluid in the flow cell and means to detect the light emitted by the particles in the flow cell, with the irradiation means comprising one ore more lasers from which a beam at least during operation follows a beam path through the fluid, characterized in that the detection means are capable of creating two at least almost identical polarized laser beams with at least almost orthogonal orientation of their polarization states, which, at least during operation, have relative coparallel beam paths sufficiently close to each other to maintain partial overlap of both beams.

2. An apparatus according to conclusion 1, characterized in that the distance between both beam axes is at least almost equal to half the beam width of the laser beams, with the beam width being as the width between the points where the intensity is larger than $1/e^2$ of the maximal intensity of the bundle.

3. An apparatus according to conclusion 2, characterized in that, at least during operation, the fluid flow in the flow cell is at least predominantly laminar, and that the distance between both beam paths is slightly smaller than half the beam width of the laser beams.

4. An apparatus according to conclusion 1, 2 or 3, characterized in that the flow cell comprises an oblong cuvette normally intersected by the pair of coparallel laser beams, oriented such that both beam paths are situated in a plane perpendicular to the long axis of the cuvette.

5. An apparatus according to conclusion 4, characterized in that the polarization directions of these two laser beams are oriented at an angle of at least almost 45 degrees relative to said perpendicular plane.

6. An apparatus according to one of the previous conclusions characterized in that the fluid stream in the flow cell has a volume that is enclosed by both bundle paths.

7. An apparatus according to one of the previous conclusions characterized in that the width of the fluid channel in the flow cell allows that both laser beams intersect across the fluid channel and not or at least almost not hit the side walls of the flow cell.

8. An apparatus according to one of the previous conclusions characterized in that the irradiation means comprise two at least almost identical lasers in conjunction with alignment means in order to co-align both beams at the desired relative distance, direction and orientation of polarization state.

9. An apparatus according to one of the previous conclusions characterized in that the irradiation means comprise an optical beam splitter in order to allow the use of a single laser from which the beam is split in two beams in such a way that the path and polarization orientation of these beams may be positioned and directed through the flow cell in the preferred way.

10. An apparatus according to conclusion 9, characterized in that the irradiation means comprise a single or two crosswise positioned optical beam displacers to split the input beam in two output beams while achieving the required lateral separation of both beams.

11. An apparatus according to one of the previous conclusions characterized in that the irradiation means allows the adjustment of the beam width of the laser beams.

12. An apparatus according to conclusion 11 characterized in that the irradiation means comprise a set of anamorphic prisms.

13. An apparatus according to one of the previous conclusions characterized in that the detection means comprise a combination of two detectors which each, protected by a

polarization means, only detect the laser light polarized corresponding to one of the orthogonal beams of lasers upon intersection of the fluid fow whereby from the ratio of the light scattering signs of these detection means as measured it can be determined for each individual particle trajectory whether the lateral position of the particle was at least almost exactly in the middle of the combined irradiation volumes of both laser beams, or, and how far from the middle to the one or the other side of the flow cell.

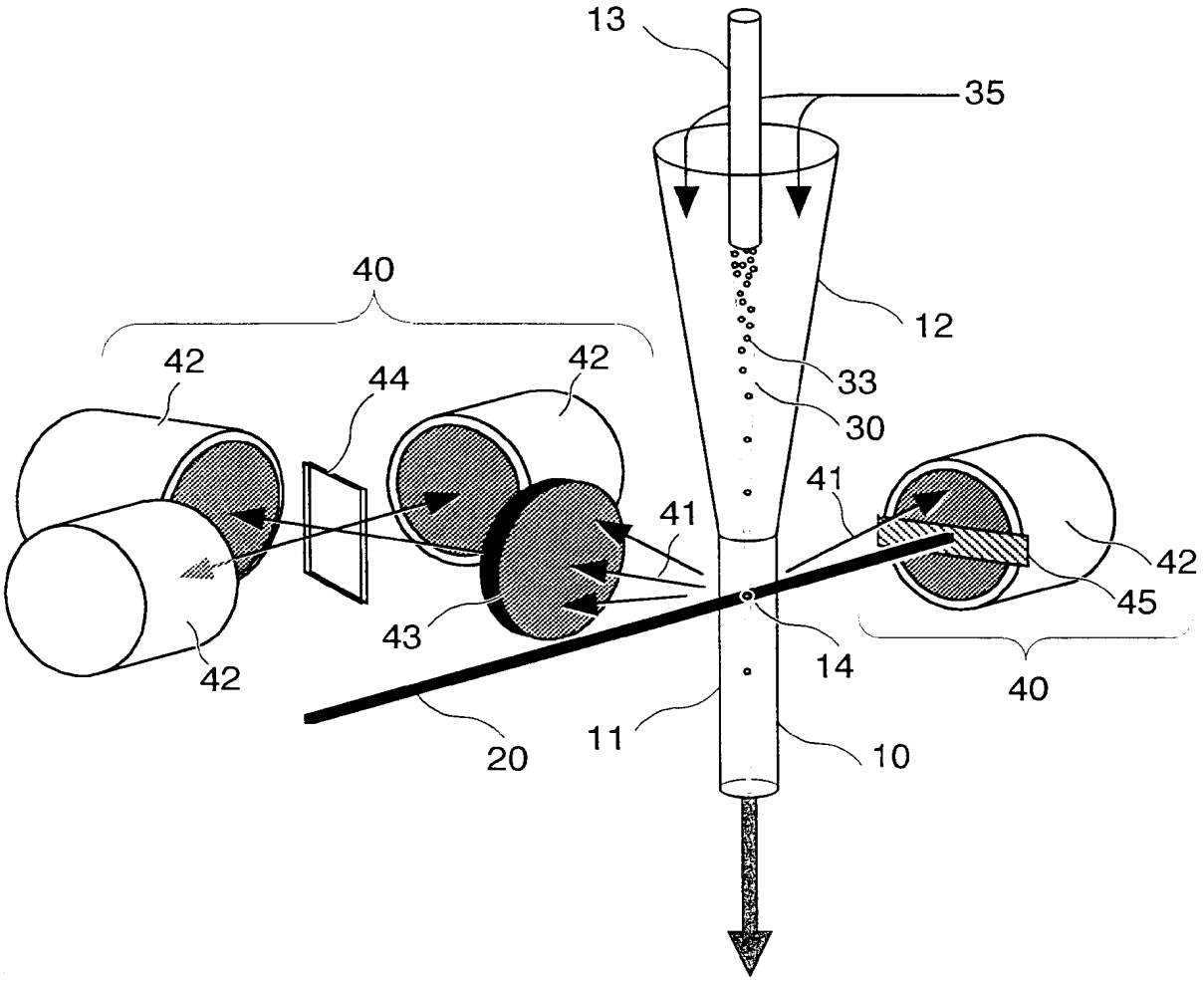


Fig. 1

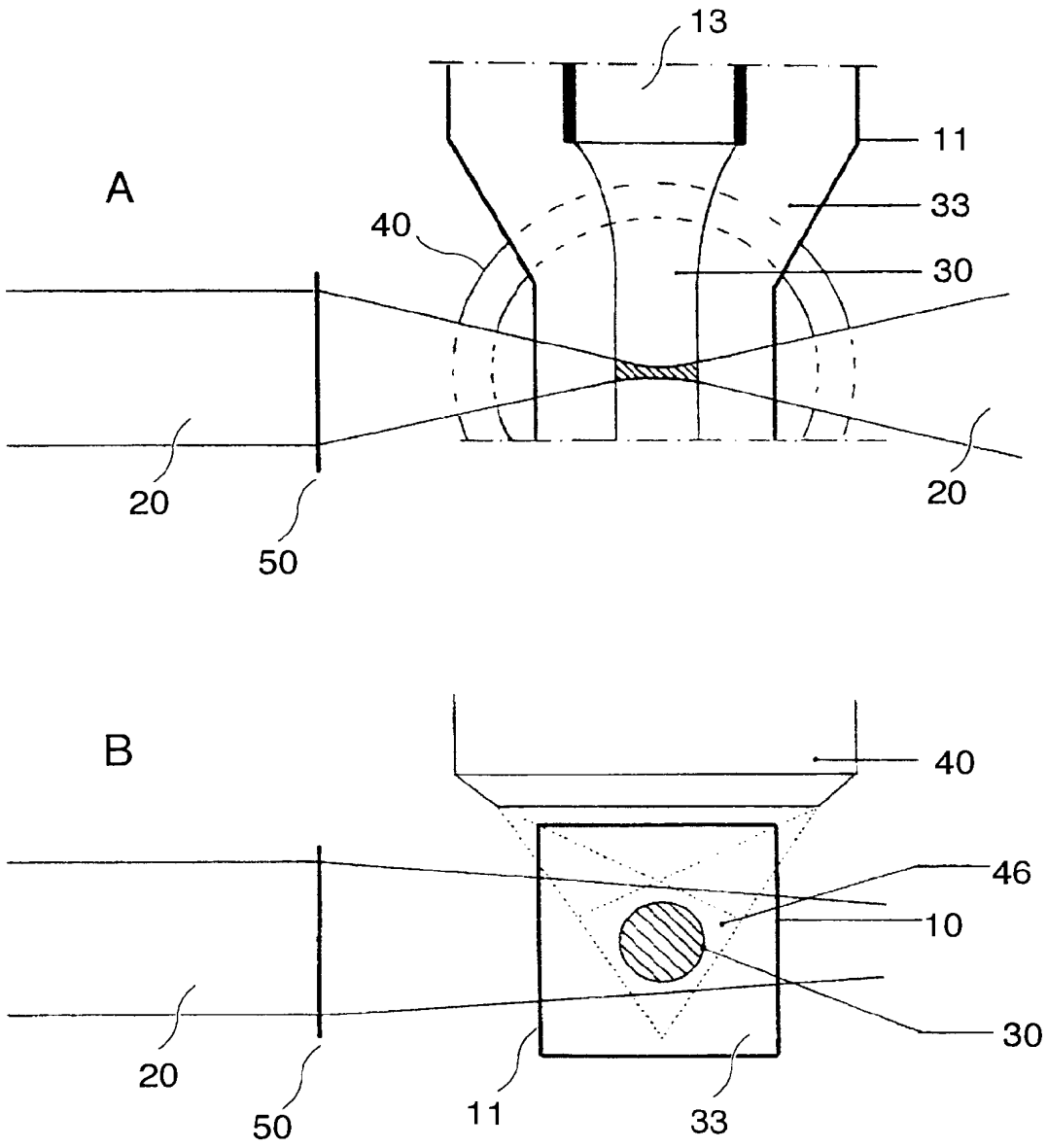


Fig. 2

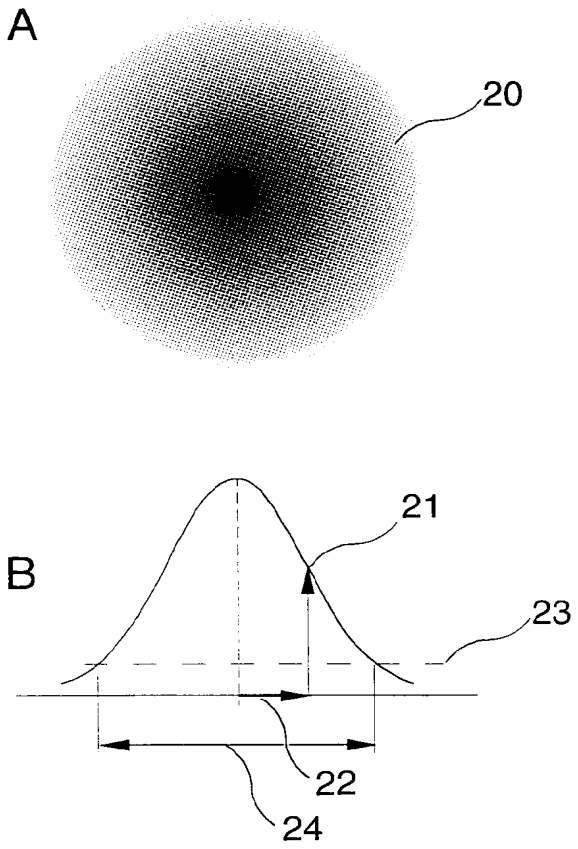


Fig. 3

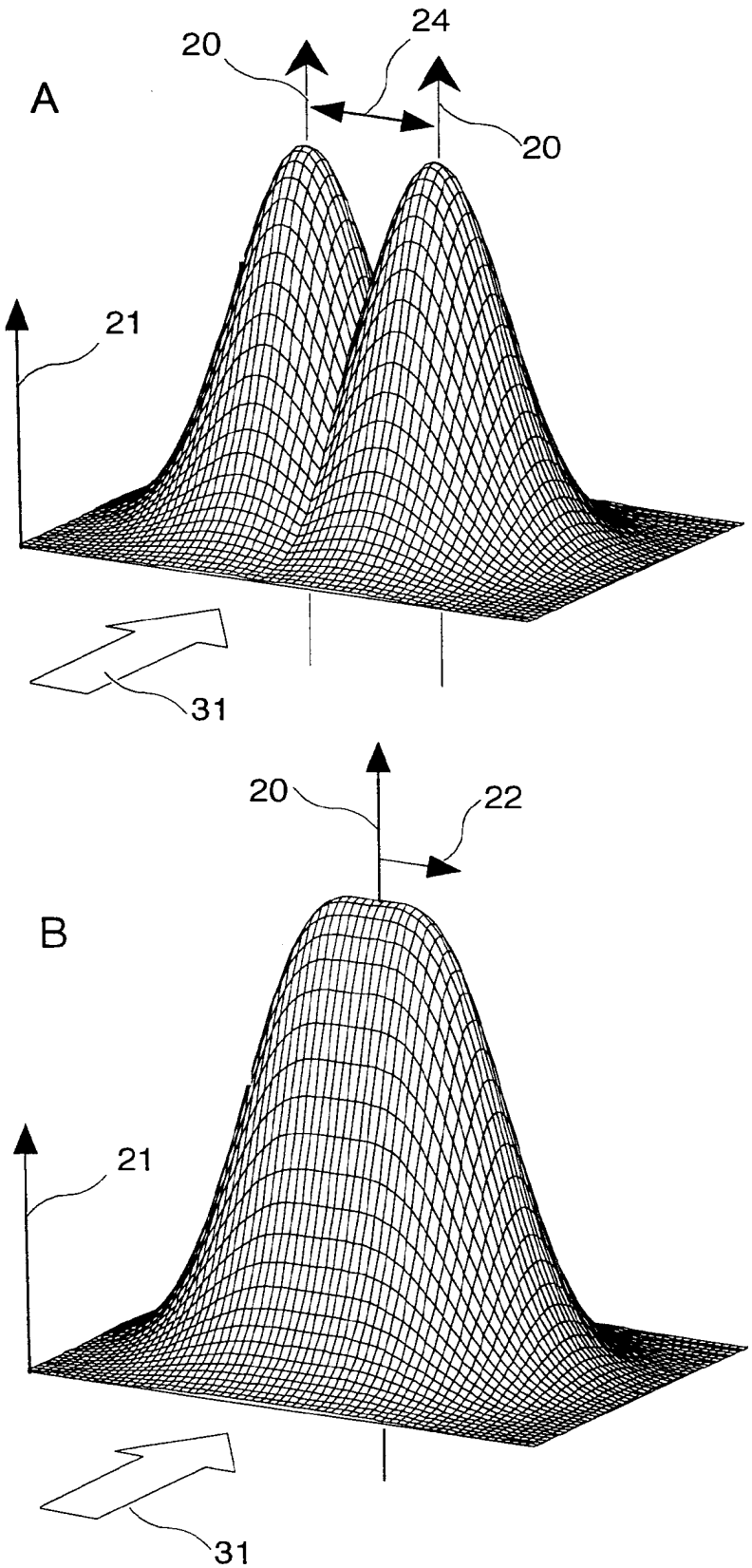


Fig. 4

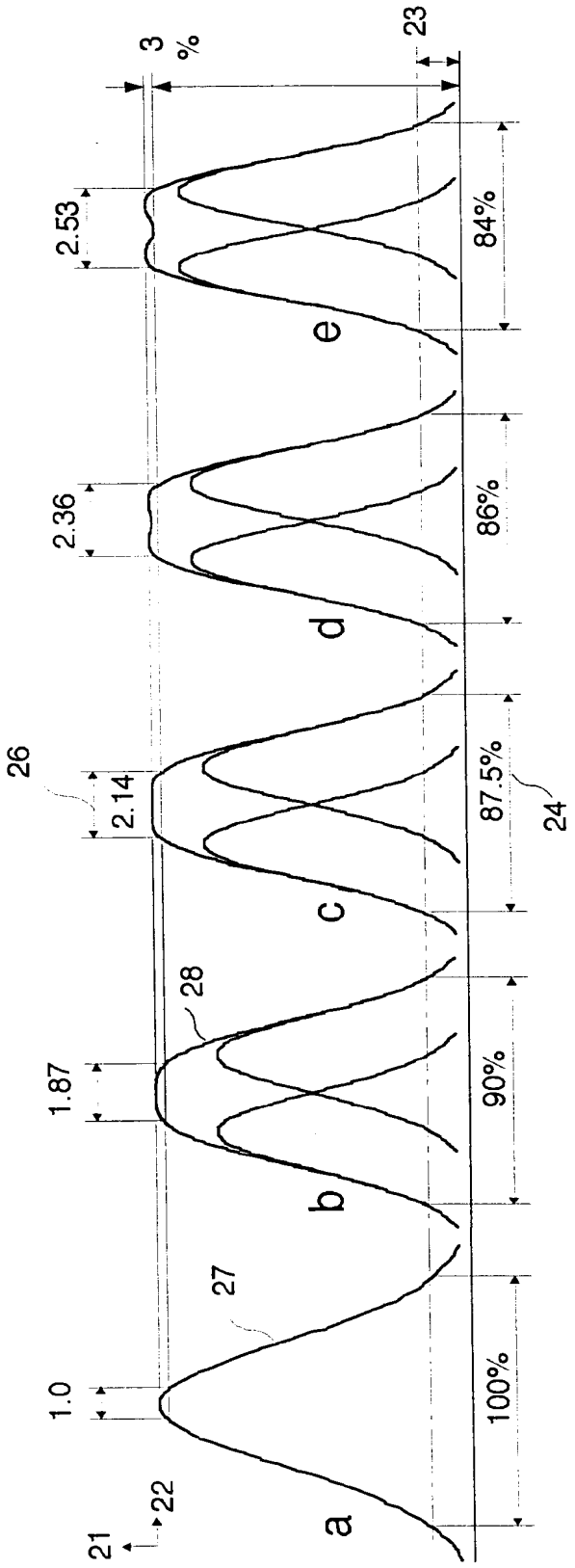


Fig. 5

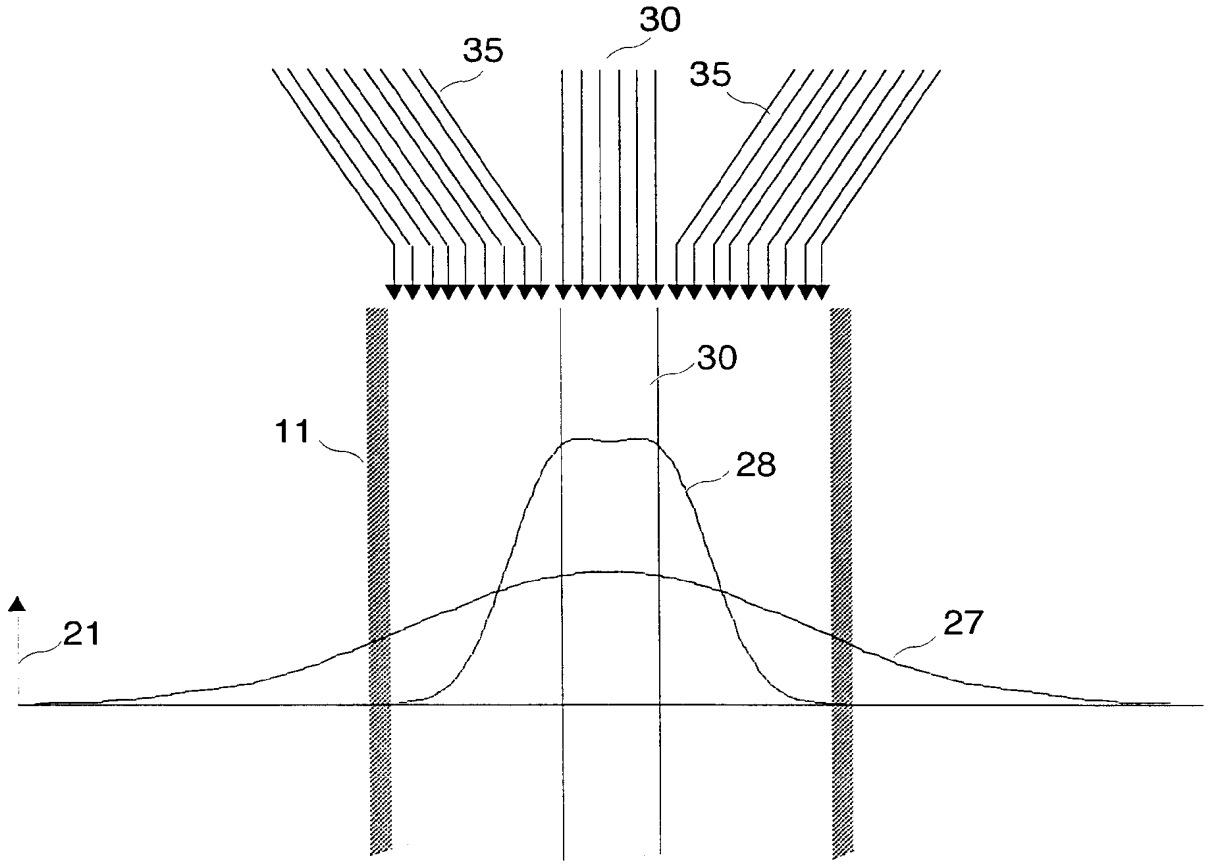


Fig. 6

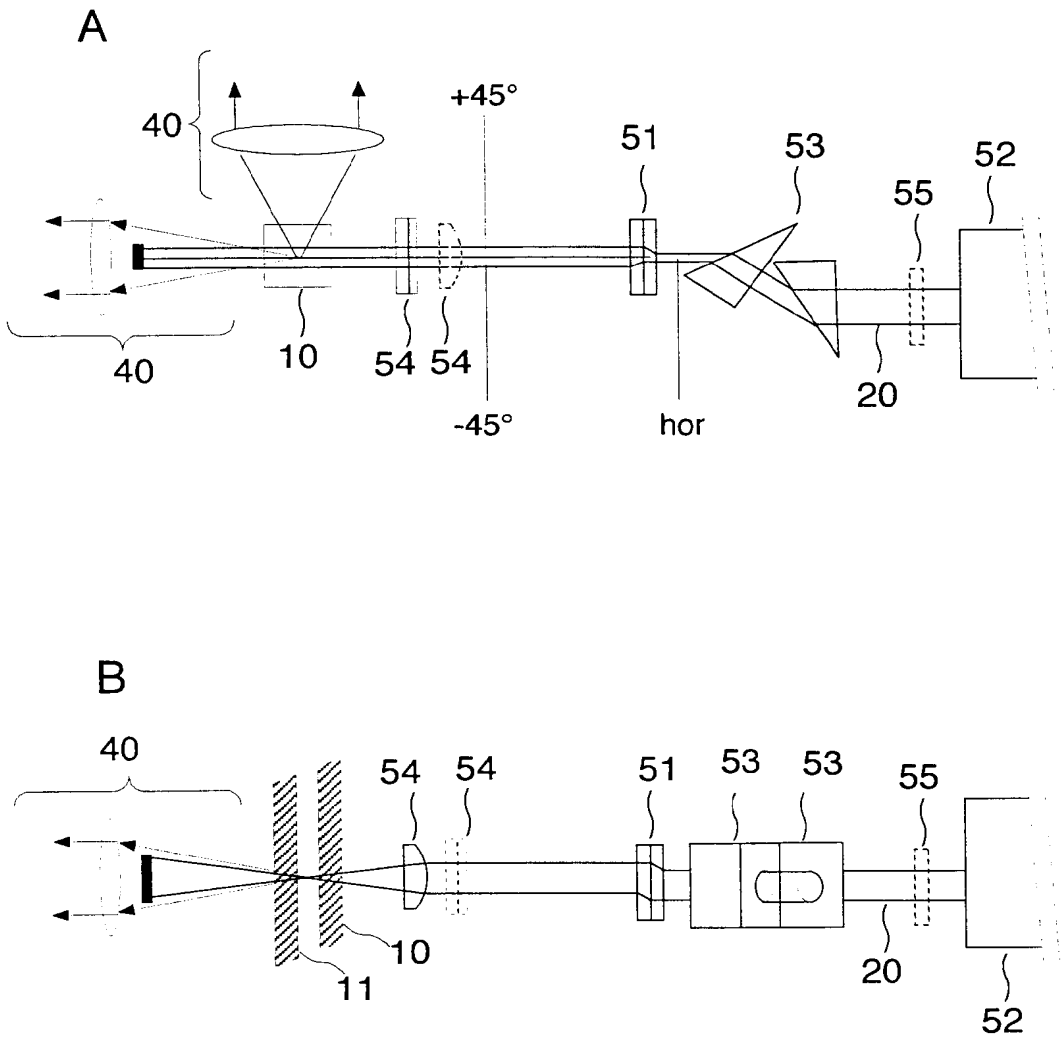

A
40
+45°
51
53
52
55
40
10
54
54
20
-45°
hor
B
40
54
54
51
53
53
55
10
11
20
52

Fig. 7

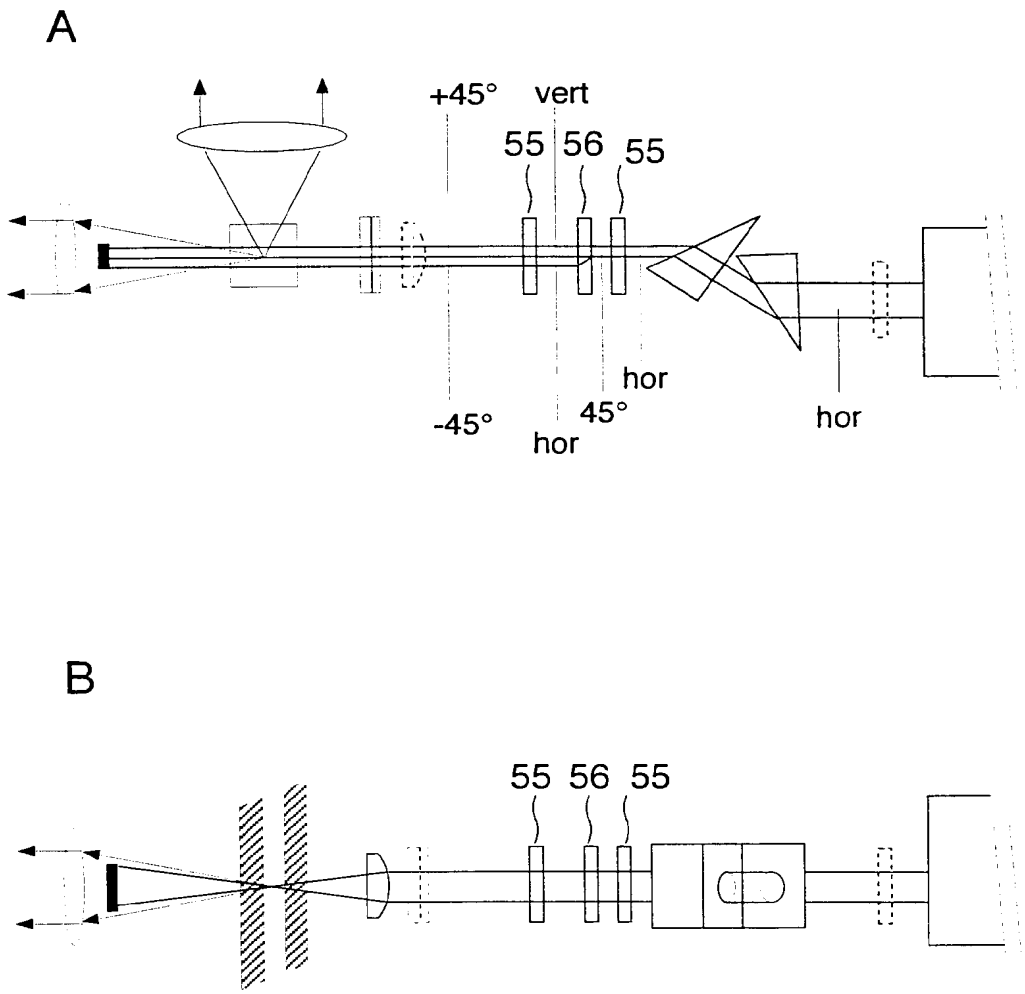


Fig. 8

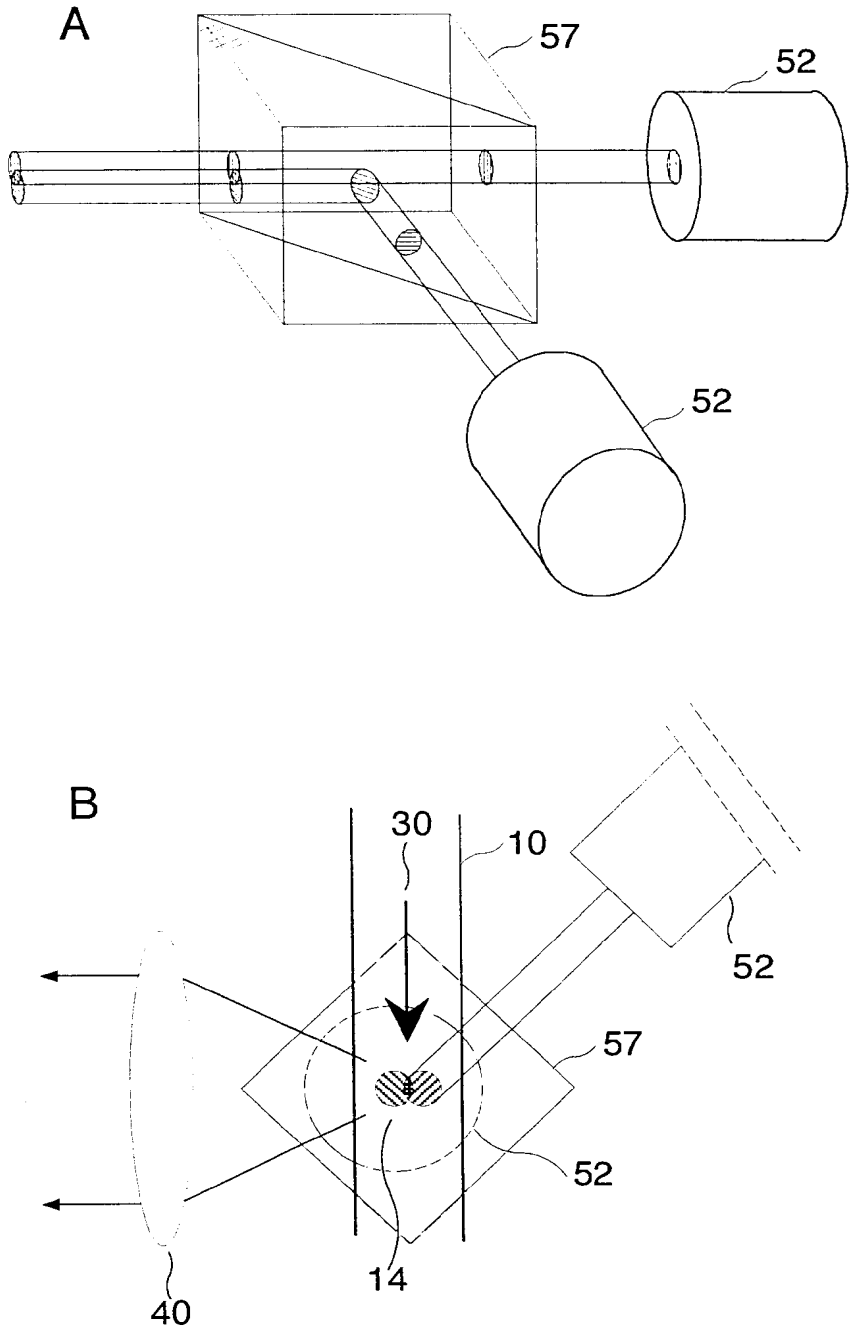


Fig. 9

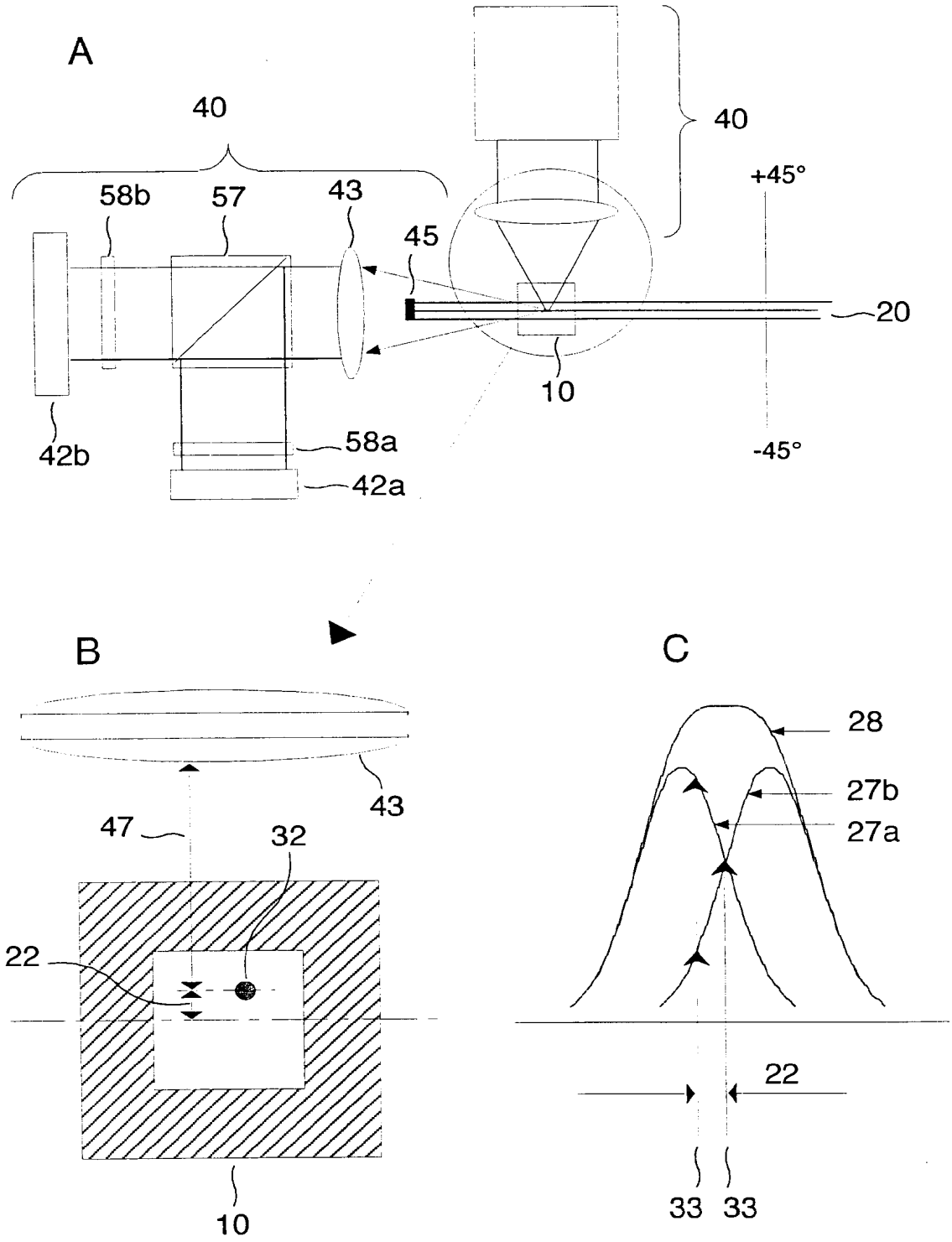

A
40
58b
57
43
45
42b
58a
42a
40
+45°
20
10
-45°
B
43
47
32
22
10
C
28
27b
27a
22
33
33

Fig. 10

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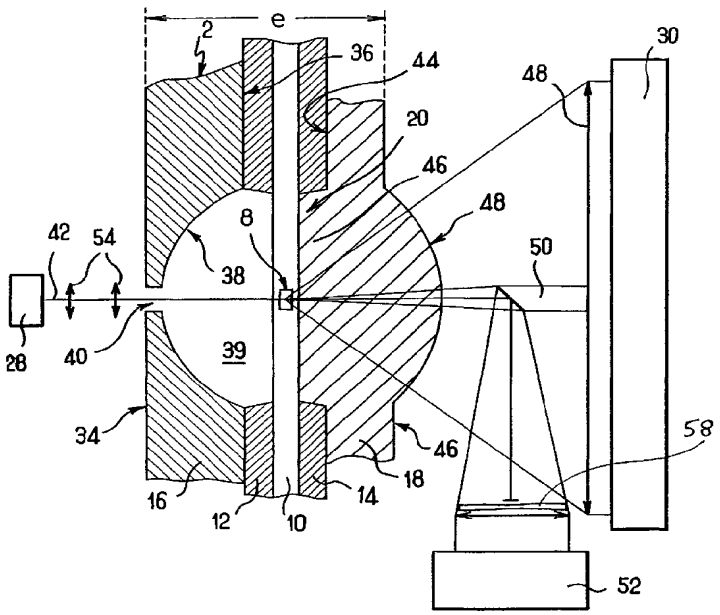
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[Suite sur la page suivante]

(54) **Title:** DEVICE FOR ANALYSING A SAMPLE IN PARTICULAR BY FLOW CYTOMETRY

(54) **Titre :** DISPOSITIF POUR L'ANALYSE D'UN ECHANTILLON NOTAMMENT PAR CYTOMETRIE DE FLUX



(57) **Abstract:** The invention concerns a device for analysing a sample comprising a sample receptacle (8) and a mirror (38), the mirror (38) comprising a break (40) such that a light beam (42) can pass through the mirror to reach the receptacle (8). The invention also concerns an assembly for analysing a sample comprising a device (2) and an equipment having a removable housing for receiving the device and a light source, the source being adapted to emit a light beam (42) passing through the mirror (38) to reach the receptacle (8) when the device is in the housing.

[Suite sur la page suivante]

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En ce qui concerne les codes à deux lettres et autres abréviations, se référer aux "Notes explicatives relatives aux codes et abréviations" figurant au début de chaque numéro ordinaire de la Gazette du PCT.

(57) Abrégé : L'invention concerne un dispositif pour l'analyse d'un échantillon comprenant un réceptacle d'échantillon (8) et un miroir, le miroir (38) présentant une discontinuité (40) de sorte qu'un rayon lumineux (42) peut traverser le miroir pour arriver jusqu'au réceptacle (8). L'invention concerne également un ensemble pour l'analyse d'un échantillon comprenant un dispositif (2) et un appareillage présentant un logement de réception amovible du dispositif et une source de lumière, la source étant apte a émettre un rayon lumineux (42) traversant le miroir (38) pour arriver jusqu'au réceptacle (8) lorsque le dispositif est dans le logement.

« Dispositif pour l'analyse d'un échantillon notamment par cytométrie de flux»

L'invention concerne les dispositifs d'analyse d'un échantillon, notamment par cytométrie de flux.

La cytométrie de flux est aujourd'hui couramment utilisée dans des domaines variés (médecine, agroalimentaire, biotechnologies, environnement) pour l'analyse de différents composés tels que des cellules, des macromolécules, etc. La cytométrie de flux peut par exemple être mise en œuvre de la façon suivante.

Les cellules (particules) à analyser sont centrées par un système de veine liquide dans l'axe d'un jet liquide. Elles passent individuellement, à raison de quelques milliers par seconde, au travers d'un rayon laser focalisé sur l'axe du jet et induisent ainsi un certain nombre de signaux lumineux.

Des systèmes optiques appropriés récupèrent la lumière laser diffusée dans un angle solide entre 0,5° et 15° (scatter axial) et la lumière émise perpendiculairement au jet et au rayon laser (scatter perpendiculaire, fluorescences). Un ensemble de miroirs et de filtres optiques permet de décomposer les signaux collectés selon leur longueur d'onde (par exemple fluorescences verte et rouge de l'orangé d'acridine). Les signaux optiques transformés en signaux électriques par des photodétecteurs sont traités dans des circuits électroniques spécialisés qui assignent à chaque cellule les intensités des signaux émis. Des distributions de fréquence mono- ou multi-dimensionnelles (histogrammes) donnent la répartition de la population de cellules analysée.

Un dispositif de ce type est par exemple décrit dans le document FR-2 325 038.

En vue de rendre plus souple l'utilisation de cette technique, il existe maintenant des dispositifs jetables ayant presque le format d'une carte de crédit et adaptés à recevoir un échantillon à analyser. Un tel dispositif peut être reçu de façon amovible dans un appareillage qui comprend la source lumineuse, les organes optiques et les moyens d'analyse des rayons lumineux émis par l'échantillon, ainsi que le système de fluide apte à

entraîner l'échantillon. Le système se connecte à la carte pour entraîner l'échantillon à travers une fenêtre d'analyse de celle-ci.

Certaines de ces cartes amovibles ont toutefois pour inconvénient que la quantité de la lumière collectée pour l'analyse est très inférieure à celle collectée dans un dispositif classique. En effet, dans ce dernier, les organes optiques tels que les lentilles sont généralement agencées très près de la veine d'analyse afin de collecter la plus grande quantité de lumière possible, c'est-à-dire suivant un angle solide le plus grand possible autour du point de la veine traversé par le rayon incident. Dans une carte amovible, en revanche, le caractère amovible oblige à prévoir un espacement minimal entre la veine et la lentille, ce qui entraîne une réduction de l'angle solide de collecte et donc une collecte moins importante.

Pour palier cet inconvénient, le document EP-1 058 939 propose d'intégrer à la paroi de la carte un organe optique tel qu'une lentille de collecte. Cette lentille pouvant être disposée très près de la veine, la quantité de lumière collectée se trouve accrue. Ce document propose également d'intégrer à la carte un élément réfléchissant. Un tel élément peut contribuer à augmenter la collecte de la lumière.

Un but de l'invention est d'accroître encore la quantité de la lumière pouvant être collectée dans le cadre d'un dispositif d'analyse.

A cet effet, on prévoit selon l'invention un dispositif pour l'analyse d'un échantillon au moyen d'un rayon lumineux comprenant un réceptacle d'échantillon et un miroir, le miroir présentant une discontinuité de sorte qu'un rayon lumineux peut traverser le miroir pour arriver jusqu'au réceptacle.

Ainsi, en ménageant dans le miroir une discontinuité pour le passage du rayon incident, il est possible d'étendre le miroir sur une grande superficie notamment de part et d'autre du rayon afin d'accroître encore la collecte de la lumière. Cet avantage est particulièrement utile dans les cartes d'analyse par cytométrie de flux, notamment si le miroir est intégré à la carte. Il est

également utile dans les systèmes de cytométrie sans élément amovible ou même d'autres domaines que l'analyse par cytométrie.

Le dispositif selon l'invention pourra présenter en outre au moins l'une des caractéristiques suivantes :

- le miroir est fixé au réceptacle,
- le dispositif comprend une paroi externe définissant le miroir,
- le miroir est rapporté sur une paroi externe du dispositif,
- le dispositif a une forme essentiellement plate,
- le réceptacle s'étend parallèlement à une face principale du dispositif, le miroir s'étendant au droit du réceptacle suivant une épaisseur e du dispositif,
- la discontinuité se trouve au droit du réceptacle suivant une épaisseur e du dispositif,
- la discontinuité est disposée de sorte que le rayon lumineux est incliné par rapport à une épaisseur e du dispositif,
- le dispositif est agencé pour qu'un fluide s'écoule dans le réceptacle dans un sens prédéterminé, la discontinuité se trouvant dans une moitié amont du miroir par référence au sens d'écoulement,
- le dispositif comprend un substrat ayant une face formant le miroir, cette face étant en contact avec un milieu différent de celui du substrat et s'étendant entre le substrat et le réceptacle,
- la face forme une cavité interne du dispositif,
- le dispositif comprend un substrat ayant une face formant le miroir et s'étendant en contact avec un milieu différent de celui du substrat, le substrat s'étendant entre la face et le réceptacle,
- le milieu est un gaz,
- le miroir a une forme en portion de sphère,
- le dispositif est agencé pour que le rayon émerge du dispositif d'un côté du réceptacle opposé au miroir,
- le dispositif présente en outre un organe optique fixé au réceptacle,
- le réceptacle s'étend entre le miroir et l'organe optique,

- le dispositif comprend une paroi externe définissant l'organe optique,

- le réceptacle a une forme allongée,

- le dispositif comprend un réservoir de fluide,

- l'échantillon comprend un fluide, et

- il s'agit d'un dispositif d'analyse par cytométrie de flux.

On prévoit également selon l'invention un ensemble pour l'analyse d'un échantillon comprenant un dispositif et un appareillage présentant un logement de réception amovible du dispositif et une source de lumière, la source étant apte à émettre un rayon lumineux traversant le miroir pour arriver jusqu'au réceptacle lorsque le dispositif est dans le logement.

Cet agencement peut permettre de vérifier le bon positionnement de la carte dans son logement en mesurant l'intensité du rayon incident transmis à travers la carte, qui doit être maximale, et l'intensité du rayon réfléchi par le pourtour de l'orifice en direction de la source, qui doit être minimale.

Avantageusement, l'appareillage comprend des moyens d'analyse du rayon disposés de sorte que le dispositif s'étend entre la source et les moyens d'analyse lorsque le dispositif est dans le logement.

Avantageusement, l'ensemble comprend des moyens pour analyser un rayonnement provenant directement ou indirectement de la source de façon à déterminer si le dispositif est reçu à une position prédéterminée dans le logement.

D'autres caractéristiques et avantages de l'invention apparaîtront encore dans la description suivante de trois modes préférés de réalisation donnés à titre d'exemples non limitatifs. Aux dessins annexés :

- la figure 1 est une vue en perspective de principe d'un dispositif selon l'invention ;

- la figure 2 est une vue schématique d'un ensemble selon l'invention apte à recevoir le dispositif de la figure 1 ;

- la figure 3 est une vue en coupe transversale montrant la tranche du dispositif de la figure 1 et illustrant le premier mode préféré de réalisation ;

- la figure 4 est une vue analogue à la figure 3 dans laquelle les éléments identiques n'ont pas été représentés et illustrant le deuxième mode préféré de réalisation ;

- la figure 5 est une vue analogue à la figure 3 illustrant le troisième mode préféré de réalisation ;

- la figure 6 est une vue à plus grande échelle du détail D de la figure 5 ; et

- la figure 7 est une vue de l'organe 52 de la figure 3.

On a illustré à la figure 1 un dispositif selon l'invention sous la forme d'une carte 2. Cette carte a une forme en plan généralement rectangulaire et présente une longueur et une largeur analogues à celles d'une carte de crédit, chacune de l'ordre de quelques centimètres. Elle présente une épaisseur e de l'ordre de quelques millimètres, par exemple 3 mm. Cette carte est de préférence transparente. Elle présente dans son épaisseur différents conduits 4 ainsi qu'un ou plusieurs compartiments 6, un ou plusieurs réservoirs 6, et/ou un ou plusieurs orifices 6 agencés en communication de fluide mutuelle au moyen des conduits 4. La carte peut ainsi comprendre un ou plusieurs liquides stockés ou en circulation à l'intérieur de la carte. Le ou les orifices de la carte permettent d'agir sur l'intérieur de celle-ci pour introduire un liquide dans la carte, extraire un liquide de la carte, ou faire circuler un liquide dans la carte. La carte comprend un conduit 8 sous la forme d'un capillaire en communication avec les autres conduits 4.

La carte comprend en référence à la figure 3 une paroi interne 10 et deux parois intermédiaires avant 12 et arrière 14 s'étendant de part et d'autre de la paroi interne 10. Cette dernière est donc prise en sandwich entre les parois intermédiaires. La carte comprend en outre des parois externes avant 16 et arrière 18 s'étendant de part et d'autre du groupe des

trois parois 10, 12 et 14. Les cinq parois 10 à 18 ont des formes sensiblement identiques entre elles et correspondant généralement à la forme de la carte.

En regard du capillaire 8, les parois intermédiaires 12 et 14 présentent des découpes identiques et en regard l'une de l'autre formant une fenêtre 20 dans l'épaisseur de la carte.

D'une façon classique et qui ne sera détaillée ici, les parois 10, 12 et 14 sont formées par l'empilement de plusieurs feuilles présentant chacune des découpes internes adaptées pour que l'empilement des différentes feuilles définisse à l'intérieur de la carte les éléments 4, 6, 8 et 20 précités. Une telle carte est connue dans son principe.

La carte 2 est destinée à être reçue de façon amovible dans un appareillage 22 tel que celui illustré schématiquement à la figure 2. A cette fin, l'appareillage présente un logement 24 formé par des éléments adaptés 26. De façon classique et connue en soi dans son principe, cet appareillage comprend au moins une source de lumière 28 telle qu'une source de rayonnement laser, et des moyens d'analyse et de traitement 30, 52 du rayonnement émanant de la carte. Ces moyens comprennent classiquement différents organes optiques tels que des lentilles, des filtres, des miroirs, etc. ainsi que des organes aptes à convertir ces rayonnements sous forme électronique en vue de leur traitement. L'appareillage comprend également des moyens 32 nécessaires pour assurer une circulation d'un ou plusieurs fluides à l'intérieur de la carte. Ces moyens pourront notamment comprendre une ou plusieurs pompes. Une fois la carte reçue dans son logement, ces moyens sont aptes à entrer en communication de fluide avec l'intérieur de la carte au moyen du ou des orifices qu'elle présente.

En référence à la figure 3 qui est une section perpendiculaire au plan général de la carte, la paroi avant 16 de la carte 2 comprend une face externe 34 de forme plane destinée à s'étendre en regard de la source 28 lorsque la carte est reçue dans le logement 24. La face interne 36 constituant la deuxième face principale de cette paroi a également une

forme plane sur la plus grande partie de sa surface mais présente une portion sphérique 38 en creux ménageant une cavité 39.

Cette portion sphérique 38 s'étend en regard du capillaire 8 et en regard de la fenêtre 20. Cette portion est recouverte par un revêtement adapté tel qu'un revêtement en métal de façon à former un miroir. Ce miroir constitue donc le dioptre formant la jonction entre le matériau de la paroi avant 16 et l'air emplissant la cavité 39. Le miroir est formé de sorte que le point C du capillaire s'étendant au centre de la fenêtre 20 occupe le centre de la sphère.

Dans le présent exemple, un orifice 40 est formé dans la paroi avant 16, dans la zone du miroir 38 s'étendant au droit du capillaire 8 suivant l'épaisseur e. Compte tenu de la forme du miroir, cette zone est celle de la paroi 16 où son épaisseur est la plus faible. Dans l'appareillage 22, la source 28 est positionnée de sorte que, lorsque la carte 2 est reçue dans le logement 24, un rayon 42 émis par la source traverse la paroi 16 au niveau de l'orifice 40 pour venir intercepter le capillaire 8 au centre C de la fenêtre 20 c'est-à-dire au foyer du miroir 38.

La paroi arrière 18 présente une face interne 44 présentant une protubérance 46 apte à remplir la découpe de la paroi intermédiaire arrière 14 correspondant à la fenêtre 20. La paroi arrière 18 présente en outre une face externe arrière 46. La plus grande partie de cette face est formée par une zone plane. Elle comprend en outre une zone sphérique bombée 48 s'étendant en saillie de la zone plane. Cette zone 48 forme une lentille s'étendant en regard de la fenêtre et dont le foyer se trouve au point du capillaire 8 apte à recevoir le rayon incident 42. Le capillaire 8 est donc interposé entre le miroir 38 d'une part et la lentille 48 d'autre part. La lentille 48 est une lentille convergente. Dans le mode de réalisation de la figure 3, le rayon incident 42 est essentiellement perpendiculaire au plan de la carte.

L'appareillage comprend également des moyens tels qu'un miroir 50 disposés au voisinage des moyens de traitement 30, dans le trajet du rayon incident 42 émis par la source 28, et aptes à dévier vers un dispositif adapté

non illustré la fraction du rayon incident ayant traversé le capillaire sans avoir été sensiblement déviée par celui-ci. En effet, cette fraction que nous appellerons fraction transmise ne comprend pratiquement aucune information exploitable et doit donc être évacuée sans gêner l'analyse des autres fractions.

En fonctionnement, lorsque la carte 2 est reçue dans le logement, le rayon incident 42 de la source 28 traverse l'orifice 40 et vient frapper le capillaire 8 contenant l'échantillon en cours d'analyse au point qui correspond au centre du miroir 38 et au foyer de la lentille 48.

La fraction transmise de ce rayon traverse la carte et est évacuée par le miroir 50.

Une autre partie de ce rayon est difractée lors des interaction avec les particules de l'échantillon durant son passage dans le capillaire. Cette diffraction a lieu suivant un angle solide de quelques stéradians de part et d'autre du faisceau transmis. Cette partie contient des informations lumineuses relatives à la taille des particules dans l'échantillon et peut donc être utilement analysée. Cette fraction traverse la paroi arrière 18. Les rayons qui la constituent sont initialement divergents. Leur orientation est modifiée au passage de la lentille 48 qui transforme par exemple cette fraction en un rayonnement parallèle ou de divergence réduite. Sur la figure 3, la fonction de la lentille 48 a également été illustrée par la flèche 48 conformément à la symbolisation classique. L'ensemble du faisceau diffracté initialement suivant un angle solide faible (typiquement compris entre 0,5 et 15 stéradians) est donc collecté pour être envoyé aux moyens de traitement 52 via le miroir 50.

On a illustré à la figure 7 une vue de la face 53 de l'organe 52 recevant le rayonnement.. Cette face a une forme générale circulaire. Elle présente à mi-hauteur une barre 55 d'obscuration laser s'étendant sur toute la largeur de la face. Cette barre comprend des détecteurs 57 d'intensité de lumière laser directe, ici au nombre de trois, espacés les uns de autres.

Une fraction du rayon incident 42 est également diffusée suivant un grand angle solide autour du point d'incidence du rayon sur le capillaire, et même le plus souvent dans tout l'espace, c'est-à-dire sur un angle solide de 4¶ stéradians. Une partie de cette fraction diffusée vers la lentille émerge de la carte à travers la lentille 48 de sorte qu'elle est également collectée et envoyée vers les moyens de traitement 30. Une autre partie de cette fraction diffusée est orientée en direction du miroir 38 qui la réfléchit au centre du capillaire, ce qui entraîne sa collecte par la lentille 48 puis par les moyens de traitement 30. Une très grande partie du faisceau diffusé se trouve donc envoyée directement ou indirectement vers les moyens de traitement 30.

Les composantes formant le faisceau diffusé pourront être séparées les unes des autres et mesurées par exemple à la longueur d'onde du laser et à la ou aux longueurs de la fluorescence caractéristiques des marqueurs de fluorescence utilisés dans le cadre de l'analyse et résultant du passage du rayon dans le capillaire.

L'ensemble comprenant l'appareillage 22 et la carte 2 pourra comprendre des organes optiques 54 disposés entre la source 28 et l'orifice 40, par exemple des lentilles convergentes aptes à focaliser le rayon incident sur le capillaire. Ces organes pourront être ou bien fixés à demeure sur l'appareillage ou bien fixés à demeure sur la carte 2 en étant intégrés à celle-ci.

La séparation des différentes composantes des faisceaux diffractés et diffusés pourra se faire au moyen d'organes classiques intégrés à l'appareillage tels que miroirs séparateurs, filtres, réseaux ou prismes. La quantification de chaque composante pourra s'effectuer au moyen de photodétecteurs de sensibilité appropriée.

Le miroir 38 permet de recueillir la lumière rétro diffusée et de la renvoyer en superposition de la lumière diffusée directement vers la lentille, vers l'avant ce qui permet de doubler l'intensité du flux.

L'orifice 40 permet de donner une grande surface au miroir sans que le rayon incident ne constitue un obstacle. La présence de l'orifice 40 dans le

miroir 38 permet également de vérifier que le positionnement de la carte 2 dans son logement 24 est correct. En effet, on peut prévoir par exemple entre le miroir 50 et les moyens d'analyse 52 tel qu'illustré (ou encore au sein des moyens 52 eux-mêmes) des moyens 58 pour mesurer l'intensité du flux lumineux réfléchi par le miroir 50 et reçu par cet organe. On peut également prévoir au niveau de la source 28 des moyens pour mesurer l'intensité d'un flux lumineux constitué par la fraction du rayon incident 42 éventuellement réfléchie par le pourtour de l'orifice 40. Lorsque le positionnement de la carte dans son logement est correct, le flux lumineux reçu par l'organe 58 devra être maximal tandis que le flux lumineux réfléchi par le pourtour de l'orifice sur la source 28 sera minimal. En revanche, si le positionnement de la carte est erroné, l'orifice 40 ne se trouve pas précisément en regard de la source 28. Dans ces conditions, l'intensité lumineuse reçue par l'organe 58 n'est pas maximale tandis que l'intensité réfléchie par le pourtour de l'orifice vers la source n'est pas minimale. Le contrôle du positionnement de la carte dans l'appareillage peut naturellement être automatisé pour servir à un système d'auto optimisation.

En l'espèce, le rayon laser a au niveau du point d'illumination c en section une forme d'ellipse aplatie ayant un grand axe (inscrit dans le plan de la figure 3) compris entre 60 et 100 micromètres, voire entre 20 et 100 µm, et un petit axe (perpendiculaire au plan de la figure) compris entre 10 et 20 micromètres, voire entre 3 et 20 µm. Les cellules ou les particules faisant l'objet de l'analyse par cytométrie pourront avoir des dimensions comprises entre 0,1 et 20 micromètres.

La plus grande dimension transversale du capillaire 8 traversé par le rayon, éventuellement son diamètre s'il a une section transversale circulaire, pourra être de l'ordre de 100 micromètres. Il pourra plus généralement être compris entre 50 micromètres et 1 millimètre. Tel qu'illustré à la figure 3, ce conduit 8 ici présente une section rectangulaire dans un plan perpendiculaire à son axe longitudinal. Cette section a ici une largeur comprise entre 20 et 200 µm et une longueur comprise entre 50 µm et 1 mm.

Les feuilles formant l'intérieur de la carte notamment formant la paroi 10 pourront être constituées dans le matériau connu sous l'appellation mylar. Les parois intermédiaires et les parois externes pourront être formées en verre ou en matière plastique telle que du PMMA ou du polycarbonate.

La source 28 pourra être de faible puissance (par exemple comprise entre 10 milliwatts et 100 milliwatts, ou encore entre 1 mW et 25 mW), ce qui permet d'en réduire le coût. La faible puissance a également pour avantage de simplifier les moyens à mettre en œuvre pour dévier et absorber le rayon transmis (organes 50 et 58) ainsi que pour éliminer les rayons parasites éventuels.

Dans une variante de réalisation, on pourra envisager de remplir la cavité 39 avec un matériau présentant un indice de réfraction différent de l'indice de l'air. Ce matériau pourra par exemple être un gel connu en lui-même pour son indice avantageux. Un tel matériau permet de favoriser l'orientation des rayons lors de leurs réflexions dans la cavité.

On a illustré à la figure 4 un deuxième mode préféré de réalisation de l'invention. L 'appareillage est identique à celui du premier mode. Seul est modifiée la carte 102. La paroi arrière 18 a la même forme que dans le mode de la figure 3. La paroi avant 116 a une forme généralement symétrique de celle de la paroi arrière 18 par rapport au plan médian s'étendant dans l'épaisseur de la carte. En d'autres termes, la face interne 136 présente cette fois une protubérance venant occuper la fenêtre 20. De plus, la face externe 134 présente une portion sphérique 138 s'étendant en saillie de la zone plane de cette face. La portion 138 est recouverte d'un revêtement tel qu'une métallisation apte à lui conférer des propriétés réfléchissantes de façon à en faire un miroir. Le miroir 138 est donc formé par le dioptre constituant la jonction entre la paroi 116 et l'air ambiant. Toutefois, à l'inverse de l'agencement du mode de réalisation de la figure 3, la paroi 116 est interposée entre l'air ambiant et le capillaire 8 tandis que dans le précédent mode la cavité remplie d'air était interposée entre la paroi et le capillaire. Ce mode de réalisation diffère donc du précédent notamment en ce que le

revêtement 138 s'étend cette fois à l'extérieur de la carte et non plus à l'intérieur

Le revêtement 138 présente une discontinuité 140 au droit du point C du capillaire 8 occupant le centre de la fenêtre 20, suivant l'épaisseur de la carte. Cette discontinuité s'étend au même endroit que l'orifice 40 dans le mode de réalisation de la figure 3.

Le fonctionnement est sensiblement inchangé par rapport au mode précédent. Ainsi le rayon émanant de la source 28 pénètre dans la carte à travers la zone 140 de la paroi 116 non couverte de revêtement pour venir frapper le capillaire 8. La fraction axiale est transmise comme précédemment vers les organes 52 et 58. La fraction diffractée aux petits angles passe à travers la lentille puis est traitée par les moyens 52. Enfin, la fraction diffusée aux grands angles passe par la lentille et est traitée par les moyens 30 tandis qu'une fraction rétrodiffusée traverse la paroi 116 et est réfléchie par le miroir 138 qui comme celui du premier mode est centré sur le capillaire. Les rayons sont donc réfléchis vers le foyer de la lentille, collectés par celle-ci, puis traités par les moyens 30.

Ces deux modes de réalisation ont toutefois pour inconvénient que la fraction transmise suit le même trajet qu'une partie significative de la fraction diffusée aux grands angles. Par conséquent, éliminer la fraction transmise pour ne pas perturber l'analyse entraîne l'élimination concomitante de cette partie de la fraction diffusée. De plus, des réflexions parasites du rayon incident sur les parois du capillaire et les parois de la carte demeurent dans le trajet des rayons mesurés et peuvent perturber l'analyse.

Le mode de réalisation qui va être décrit maintenant en référence aux figures 5 et 6 est à cet égard plus avantageux.

Ce mode de réalisation est très proche du mode de la figure 4 dans lequel la discontinuité sur le miroir est formée par une interruption du revêtement formant le miroir externe. Dans ce troisième mode de réalisation, la discontinuité 240 de la carte 202 ne s'étend au droit du centre C de fenêtre suivant l'épaisseur e mais est décalée vers un bord du miroir comme

le montre la figure 5. Ainsi, la droite s'étendant depuis cette discontinuité 240 jusqu'au centre C de la fenêtre occupé par le capillaire est inclinée suivant un angle aigu par rapport à la direction longitudinale du capillaire.

Dans l'appareillage, la position de la source 28 est modifiée en conséquence. Cette fois, l'axe de la source est sécant avec l'axe 31 des moyens de traitement 30 au centre c de la fenêtre. La source ne s'étend plus directement en regard des moyens 30 ni coaxialement à ceux-ci. La source est disposée de sorte que, lorsque la carte est convenablement positionnée dans son logement, le rayon 42 traverse la discontinuité 240 pour arriver au centre C de la fenêtre dans le capillaire. De même, l'organe 52 destiné à recevoir une fraction du rayon diffracté ne s'étend plus en regard des moyens 30 coaxialement à ceux-ci mais se trouve dans l'alignement de la source, de la discontinuité 240 et du centre C de la fenêtre.

Une zone centrale de la lentille de très grande surface se trouve donc en regard des moyens 30 sans aucun obstacle entre ceux-ci de sorte que tous les rayons traversant cette zone peuvent être recueillis pour être traités. Cet agencement permet de faire en sorte que l'angle solide 260 correspondant à la fraction du rayon recueillie par les organes 52 et 58 s'étend totalement en dehors de l'angle solide 262 correspondant à la fraction du rayonnement (grands angles) recueillie par les moyens 30.

La figure 5 illustre une section de la carte dans un plan perpendiculaire au plan général de la carte. Le capillaire s'étend dans le plan de la section, contrairement aux cas des figures 3 et 4. On remarque sur cette figure que la source 28 est disposée de sorte que le rayon 42 s'étend dans ce plan. Il n'est pas strictement nécessaire que le rayon s'étende dans ce plan. Ainsi, on pourra obtenir la séparation des angles solides 260 et 262 en disposant la source 28 en dehors du plan de la figure 5 tout en prévoyant la discontinuité 240 au voisinage d'un bord du miroir 238. Toutefois, la disposition de la source 28 dans ce plan permet de recueillir une fraction importante et très significative du faisceau diffusé et diffracté.

De plus, il est avantageux que l'angle a illustré sur la figure 6, formé entre le rayon incident 42 et l'axe 64 du capillaire, soit sensiblement inférieur à 90° pour faire en sorte qu'au moins une partie importante 66 du faisceau transmis 68 demeure piégée dans le capillaire 8 par réflexion sur la face interne du capillaire, celui-ci formant ainsi un guide d'ondes. Il s'ensuit que la fraction 70 du faisceau transmis émergeant effectivement de la carte a une intensité d'autant plus réduite et est donc d'autant plus facile à éliminer. Cet agencement permet également de réduire sensiblement le rayonnement parasite généré au cours de l'analyse. Dans le mode de réalisation de la figure 5, l'angle a est égal à 45°. Il sera avantageusement compris entre 30 et 50°.

De surcroît, il est préférable de rapprocher la source 28 d'une partie amont du capillaire 8 comme illustré sur la figure 5 plutôt que d'une partie aval de ce dernier par référence au sens d'écoulement du fluide dans le capillaire illustré par la flèche 72. En effet, de cette façon le rayonnement piégé dans le capillaire est envoyé vers une fraction de fluide aval qui a déjà été analysée.

Dans l'agencement contraire, une partie du rayonnement serait envoyée vers les fractions amont et risquerait de réagir par avance avec les fluorochromes présents dans le liquide ou autres substances destinées à interagir avec le rayonnement incident et par suite risquerait de fausser l'analyse.

Des particules à analyser telles que des cellules ou des macromolécules ont été illustrées sur la figure 6 sous la forme de billes défilant l'une après l'autre dans le capillaire 8 conformément au principe de la cytométrie de flux.

Ce mode de réalisation fonctionne sensiblement comme les précédents. Le rayonnement incident 42 pénètre dans la carte à travers la discontinuité 240 et arrive dans le capillaire au centre de la fenêtre. Une fraction 66 du rayonnement transmis 68 reste piégée dans le capillaire tandis qu'une fraction 70 est traitée et éliminée par les organes 52 et 58.. Une

fraction diffractée 260 du rayonnement est collectée par la lentille 48 et reçue par les moyens de traitement 52. Une fraction du rayonnement diffusée vers la lentille est reçue par les moyens 30. La fraction diffusée vers le miroir est réfléchie par le miroir 238 au centre de la fenêtre et collectée par la lentille 48 de la même façon.

Le dispositif 2 pourra être jetable et à usage unique. Il pourra être fabriqué en grande série. Le capillaire pourra être formé par la superposition des différentes feuilles se trouvant au centre de la carte. Alternativement, il pourra s'agir d'un capillaire individuel, par exemple en verre ou en quartz.

La carte pourra comprendre un ou plusieurs organes optiques tels que miroirs, lentilles, prismes, réseaux etc.... Un ou plusieurs de ces éléments pourront être d'une seule pièce avec l'une des parois externes ou internes de la carte. Un ou plusieurs de ces éléments pourront être rapportés sur la carte à demeure. Dans une variante du mode de réalisation de la figure 5, on pourra prévoir un miroir adjacent à une cavité comme dans le mode de la figure 3.

Le dispositif selon l'invention permet de collecter une grande quantité de la lumière émergeant du capillaire. Le miroir et la lentille étant fixés à demeure sur la carte dans les modes de réalisation ci-dessus, leur centrage par rapport au capillaire est effectué, une bonne fois pour toutes, au montage.

Dans les modes de réalisation des figures 4 et 5, et en partie dans le mode de réalisation de la figure 3, le confinement des organes optiques autour du capillaire sans présence d'air permet de réduire les problèmes liés à la présence de poussière parasite.

Bien entendu, on pourra apporter à l'invention de nombreuses modifications sans sortir du cadre de celle-ci.

L'invention pourra être mise en œuvre lors d'analyses menées au moyen de techniques autres que la cytométrie de flux. Elle pourra par exemple être mise en œuvre dans le cadre d'une technique d'électrophorèse.

Bien que l'on ait décrit les modes de réalisation ci-dessus en référence à une source laser, le rayonnement incident pourra être de natures variées. Il pourra s'agir de lumière ordinaire, de lumière non laser, de lumière non cohérente, de lumière non monochromatique, de lumière fluorescente, ultraviolette, ou d'une onde de nature non lumineuse voire non électromagnétique (onde sonore, rayonnement gamma, rayon x, ...) etc.....

L'invention pourra être mise en œuvre dans un dispositif recevant les échantillons de façon fixe et non pas amovible. Le fluide pourra être statique plutôt que mobile. Le fluide pourra être un fluide non aqueux tel qu'un solvant ou une huile. On pourra mettre en œuvre l'invention dans certaines circonstances sans présence d'un fluide. Ainsi l'invention pourra être utilisée avec un capillaire comprenant un principe actif solide.

On pourra également prévoir dans une variante du mode de réalisation de la figure 3 que l'orifice 40 se trouve à proximité d'une zone amont du capillaire suivant un agencement de la source 28 semblable à celui de la figure 5.

Indépendamment de la présence de la discontinuité, on pourra prévoir un dispositif tel qu'une carte, comprenant un réceptacle et un miroir (par exemple de forme sphérique) fixé au dispositif. Ce dispositif pourra comprendre en outre un organe optique tel qu'une lentille disposé de sorte que le réceptacle s'étend entre le miroir et la lentille conformément aux modes de réalisation illustrés.

REVENDICATIONS

1. Dispositif (2 ;102 ;202) pour l'analyse d'un échantillon au moyen d'un rayon lumineux comprenant un réceptacle d'échantillon (8) et un miroir, caractérisé en ce que le miroir (38 ;138 ;238) présente une discontinuité (40 ;140 ;240) de sorte qu'un rayon lumineux (42) peut traverser le miroir pour arriver jusqu'au réceptacle (8).

2. Dispositif selon la revendication 1, caractérisé en ce que le miroir (38 ;138 ;238) est fixé au réceptacle.

3. Dispositif selon l'une quelconque des revendications 1 ou 2, caractérisé en ce qu'il comprend une paroi externe (16 ;116) définissant le miroir.

4. Dispositif selon l'une quelconque des revendications 1 ou 2, caractérisé en ce que le miroir est rapporté sur une paroi externe du dispositif.

5. Dispositif selon l'une quelconque des revendications 1 à 4, caractérisé en ce qu'il a une forme essentiellement plate.

6. Dispositif selon l'une quelconque des revendications 1 à 5, caractérisé en ce que le réceptacle (8) s'étend parallèlement à une face principale (34 ;134) du dispositif, le miroir (38 ; 138 ;238) s'étendant au droit du réceptacle suivant une épaisseur (e) du dispositif.

7. Dispositif selon l'une quelconque des revendications 1 à 6, caractérisé en ce que la discontinuité (40 ;140) se trouve au droit du réceptacle suivant une épaisseur (e) du dispositif.

8. Dispositif selon l'une quelconque des revendications 1 à 6, caractérisé en ce que la discontinuité (240) est disposée de sorte que le rayon lumineux (42) est incliné par rapport à une épaisseur (e) du dispositif.

9. Dispositif selon la revendication 8, caractérisé en ce qu'il est agencé pour qu'un fluide s'écoule dans le réceptacle dans un sens prédéterminé (72), la discontinuité (240) se trouvant dans une moitié amont du miroir (238) par référence au sens d'écoulement.

10. Dispositif selon l'une quelconque des revendications 1 à 9, caractérisé en ce qu'il comprend un substrat (16) ayant une face (38) formant le miroir, cette face étant en contact avec un milieu différent de celui du substrat et s'étendant entre le substrat (16) et le réceptacle (8).

11. Dispositif selon la revendication 10, caractérisé en ce que la face (38) forme une cavité interne (39) du dispositif.

12. Dispositif selon l'une quelconque des revendications 1 à 9, caractérisé en ce qu'il comprend un substrat ayant une face (138 ;238) formant le miroir et s'étendant en contact avec un milieu différent de celui du substrat, le substrat (116) s'étendant entre la face (138 ;238) et le réceptacle (8).

13. Dispositif selon l'une quelconque des revendications 10 ou 12, caractérisé en ce que le milieu est un gaz.

14. Dispositif selon l'une quelconque des revendications 1 à 13, caractérisé en ce que le miroir (38 ; 138 ;238) a une forme en portion de sphère.

15. Dispositif selon l'une quelconque des revendications 1 à 14, caractérisé en ce qu'il est agencé pour que le rayon (42) émerge du dispositif d'un côté du réceptacle (8) opposé au miroir (38 ;138 ;238).

16. Dispositif selon l'une quelconque des revendications 1 à 15, caractérisé en ce qu'il présente en outre un organe optique (48) fixé au réceptacle.

17. Dispositif selon la revendication 16, caractérisé en ce que le réceptacle (8) s'étend entre le miroir (38 ;138 ;238) et l'organe optique.

18. Dispositif selon l'une quelconque des revendications 16 ou 17, caractérisé en ce qu'il comprend une paroi externe (18) définissant l'organe optique (48).

19. Dispositif selon l'une quelconque des revendications 1 à 18, caractérisé en ce que le réceptacle (8) a une forme allongée.

20. Dispositif selon l'une quelconque des revendications 1 à 19, caractérisé en ce qu'il comprend un réservoir de fluide (6).

21. Dispositif selon l'une quelconque des revendications 1 à 20, caractérisé en ce que l'échantillon comprend un fluide.

22. Dispositif selon l'une quelconque des revendications 1 à 21, caractérisé en ce qu'il s'agit d'un dispositif d'analyse par cytométrie de flux.

23. Dispositif selon l'une quelconque des revendications 1 à 22, caractérisé en ce qu'il s'agit d'un dispositif microfluidique.

24. Ensemble pour l'analyse d'un échantillon comprenant un dispositif (2 ;102 ;202) et un appareillage (22) présentant un logement (24) de réception amovible du dispositif et une source de lumière (28), caractérisé en ce que le dispositif est conforme à l'une quelconque des revendications 1 à 23, la source étant apte à émettre un rayon lumineux (42) traversant le miroir (38 ;138 ;238) pour arriver jusqu'au réceptacle (8) lorsque le dispositif est dans le logement.

25. Ensemble selon la revendication 24, caractérisé en ce que l'appareillage comprend des moyens d'analyse (30, 52) du rayon (42) disposés de sorte que le dispositif (2) s'étend entre la source (28) et les moyens d'analyse lorsque le dispositif est dans le logement (24).

26. Ensemble selon l'une quelconque des revendications 24 à 25, caractérisé en ce qu'il comprend des moyens pour analyser un rayonnement provenant directement ou indirectement de la source de façon à déterminer si le dispositif est reçu à une position prédéterminée dans le logement.

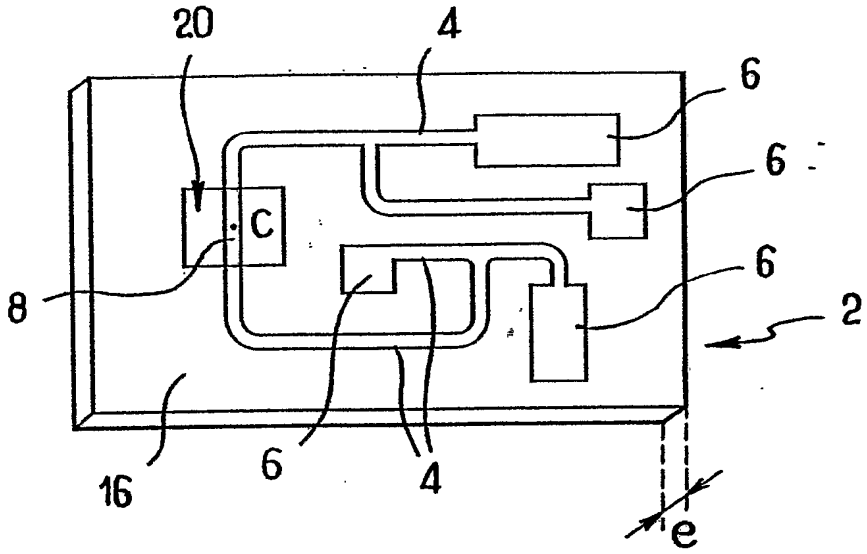


FIG_1

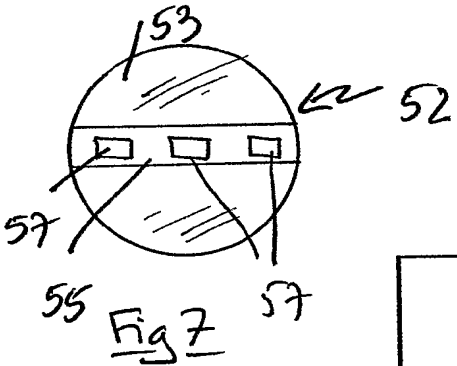


Fig 7

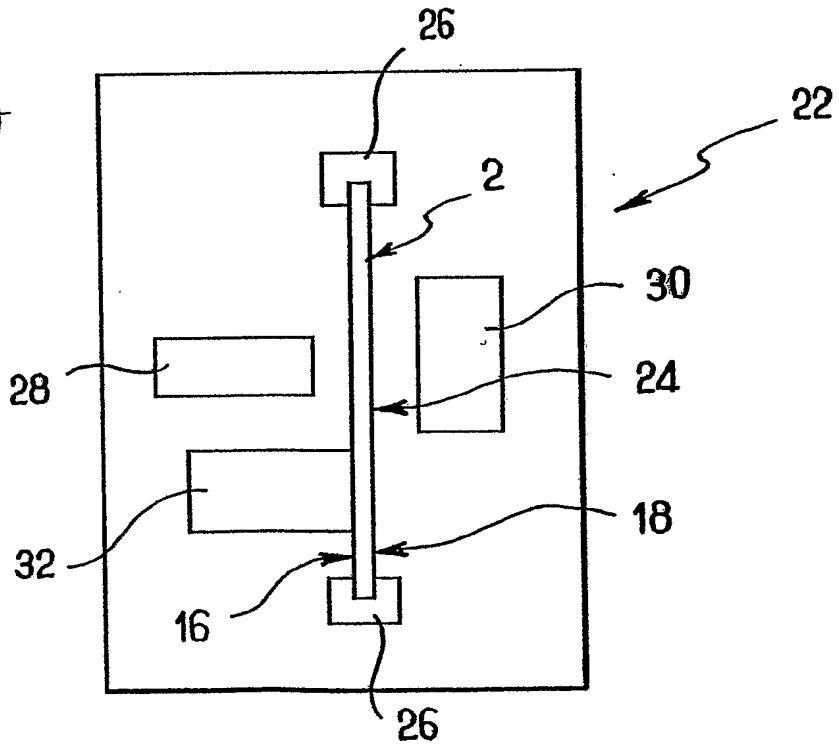


FIG_2

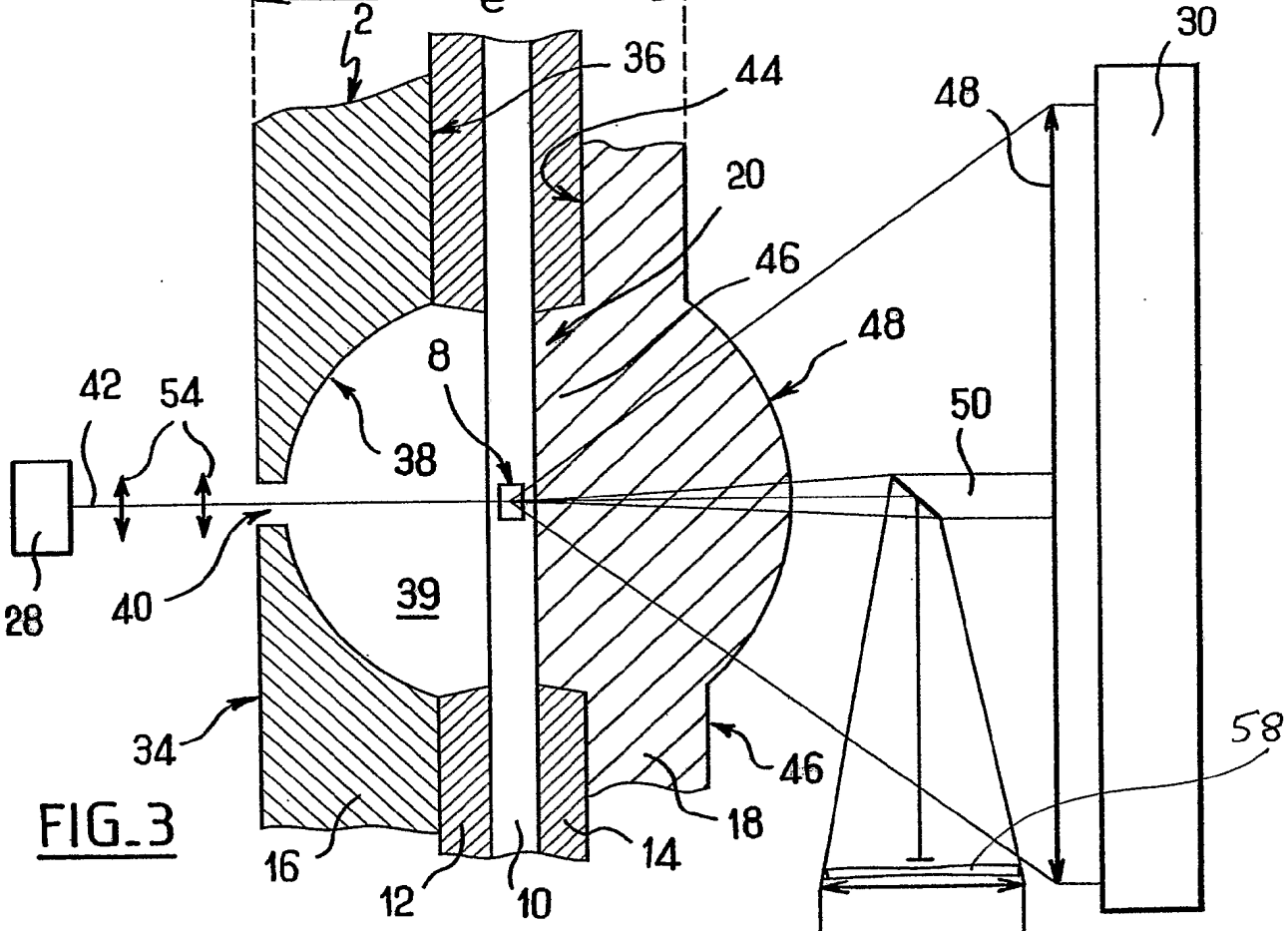

e
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52

FIG_3

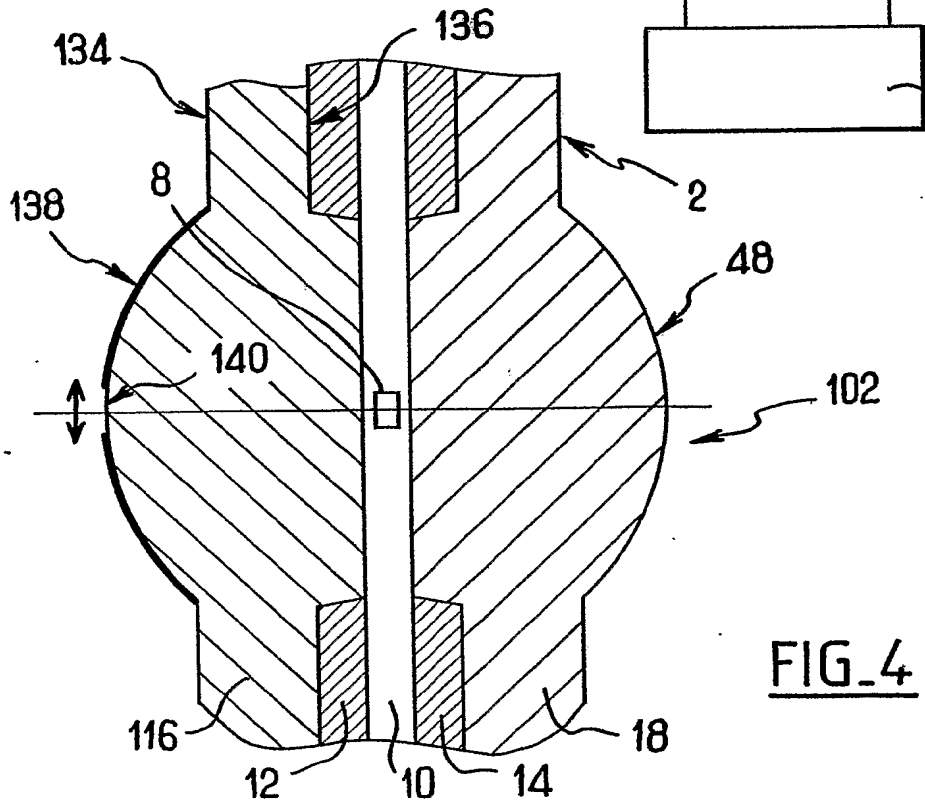

134
136
8
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102
116
18
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FIG_4

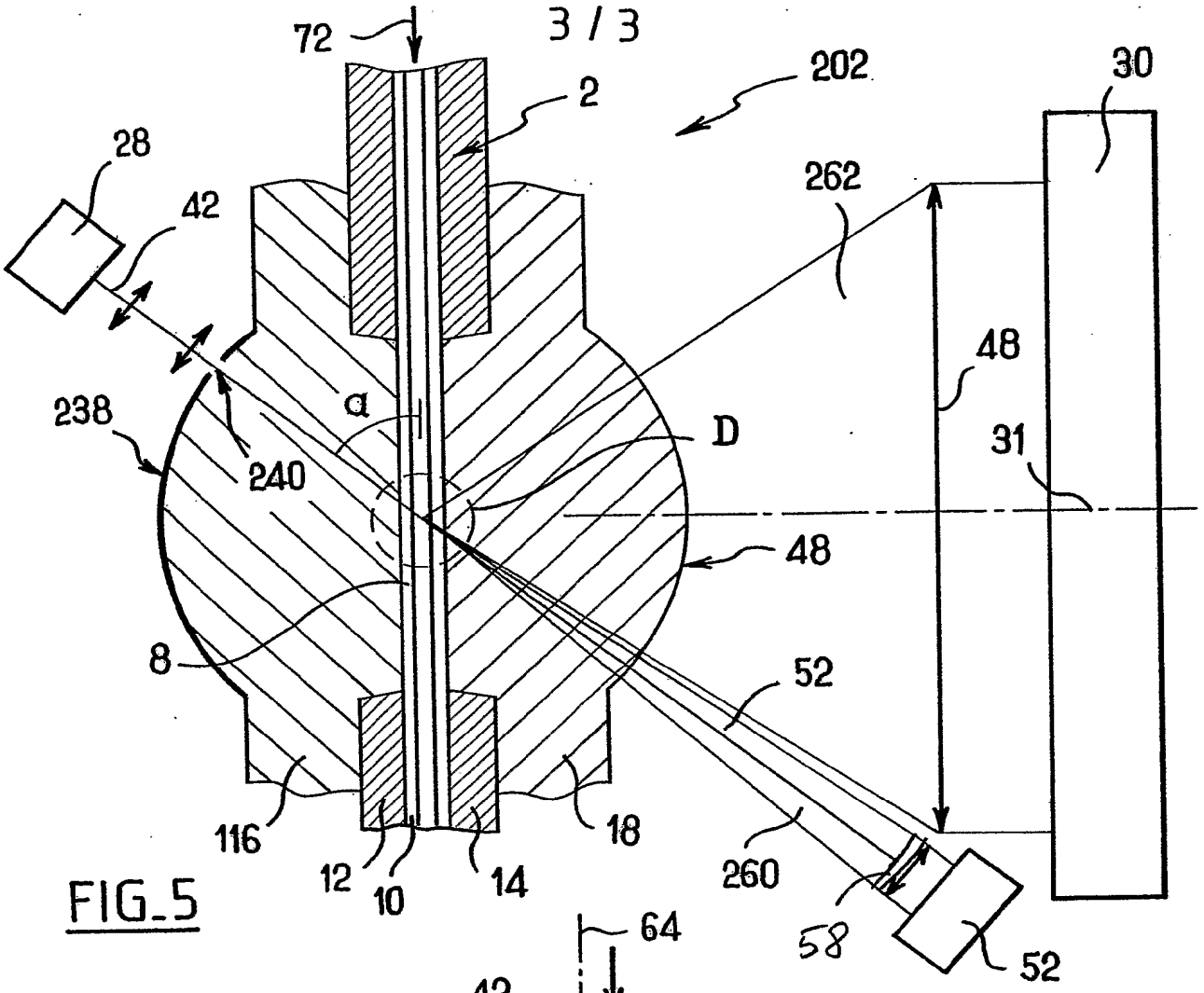

72
202
30
2
28
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262
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238
240
31
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116
18
12
10
14
260
58
52

FIG_5

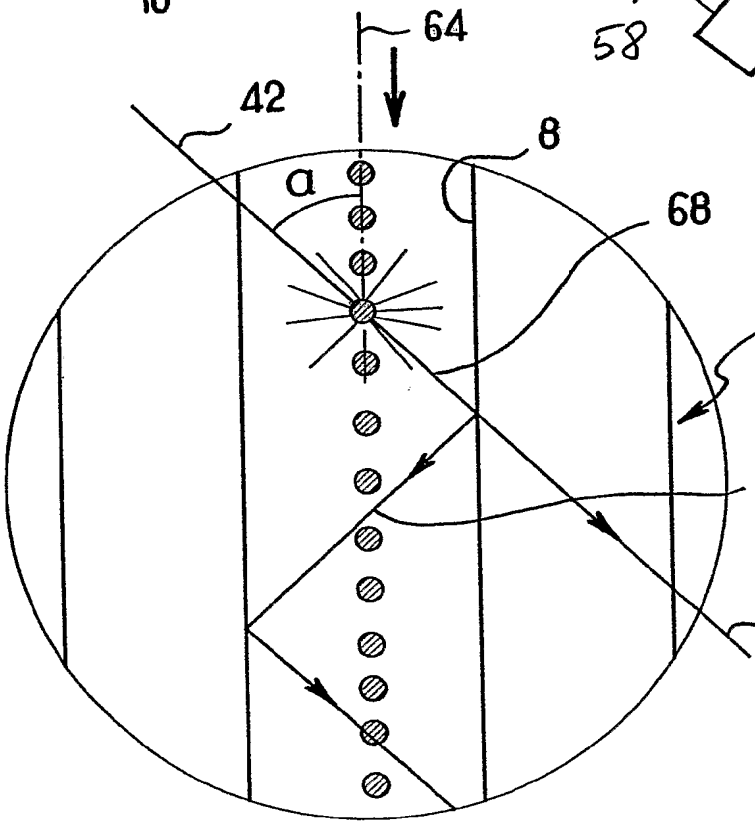

64
42
8
a
68
2
66
70

FIG_6

INTERNATIONAL SEARCH REPORT

National Application No
PCT/FR PCT/FR02/02791

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N15/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N G02B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 4 702 598 A (BOEHMER RALPH M) 27 October 1987 (1987-10-27) column 1, line 5 -column 1, line 24 column 2, line 38 -column 3, line 12 column 3, line 44 -column 4, line 22 figures 1-6 ---	1-4,6,7, 19-22,24 5,10-14, 16,18, 23,25
X Y	US 4 189 236 A (BRUNSTING ALBERT ET AL) 19 February 1980 (1980-02-19) column 2, line 59 -column 2, line 68 column 5, line 21 -column 7, line 20 figures 1-10 --- -/--	1-4 12,13, 16,18,25

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
7 January 2003	14/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Koch, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 1 058 939 A (CALIPER TECHN CORP) 13 December 2000 (2000-12-13) cited in the application	5,10-13, 23
Y	& US 6 100 541 A (NAGLE ET AL.) 8 August 2000 (2000-08-08) column 5, line 55 -column 6, line 54 figures 1-4	5,10-13, 23
Y	US 3 989 381 A (FULWYLER MACK J) 2 November 1976 (1976-11-02) column 3, line 38 -column 3, line 61 column 4, line 45 -column 4, line 59 figures 1,2	14

INTERNATIONAL SEARCH REPORT

Information on patent family members

ernational Application No
PCT/FR PCT/FR02/02791

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 4702598	A	27-10-1987	NONE		
US 4189236	A	19-02-1980	CA	1126977 A1	06-07-1982
			DE	2910030 A1	04-10-1979
			FR	2420752 A1	19-10-1979
			GB	2016734 A	26-09-1979
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			EP	1058939 A1	13-12-2000
			US	2002092973 A1	18-07-2002
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			US	6316781 B1	13-11-2001
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			FR	2325038 A1	15-04-1977
			JP	51136475 A	25-11-1976

RAPPORT DE RECHERCHE INTERNATIONALE

ande Internationale No
PCT/FR 02/02791

A. CLASSEMENT DE L'OBJET DE LA DEMANDE
CIB 7 G01N15/14

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

B. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée (système de classification suivi des symboles de classement)
CIB 7 G01N G02B

Documentation consultée autre que la documentation minimale dans la mesure où ces documents relèvent des domaines sur lesquels a porté la recherche

Base de données électronique consultée au cours de la recherche internationale (nom de la base de données, et si réalisable, termes de recherche utilisés)
EPO-Internal, WPI Data, PAJ

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Catégorie °	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
X Y	US 4 702 598 A (BOEHMER RALPH M) 27 octobre 1987 (1987-10-27) colonne 1, ligne 5 -colonne 1, ligne 24 colonne 2, ligne 38 -colonne 3, ligne 12 colonne 3, ligne 44 -colonne 4, ligne 22 figures 1-6 ---	1-4,6,7, 19-22,24 5,10-14, 16,18, 23,25
X Y	US 4 189 236 A (BRUNSTING ALBERT ET AL) 19 février 1980 (1980-02-19) colonne 2, ligne 59 -colonne 2, ligne 68 colonne 5, ligne 21 -colonne 7, ligne 20 figures 1-10 --- -/--	1-4 12,13, 16,18,25

[X] Voir la suite du cadre C pour la fin de la liste des documents

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7 janvier 2003	14/01/2003

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Formulaire PCT/ISA/210 (deuxième feuille) (juillet 1992)

mande Internationale No
PCT/FR 02/02791

C.(suite). DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie °	Identification des documents cités, avec,le cas échéant, l'indicationdes passages pertinents	no. des revendications visées
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Y	& US 6 100 541 A (NAGLE ET AL.) 8 août 2000 (2000-08-08) colonne 5, ligne 55 -colonne 6, ligne 54 figures 1-4	5,10-13, 23
Y	US 3 989 381 A (FULWYLER MACK J) 2 novembre 1976 (1976-11-02) colonne 3, ligne 38 -colonne 3, ligne 61 colonne 4, ligne 45 -colonne 4, ligne 59 figures 1,2	14

Document brevet cité au rapport de recherche		Date de publication	Membre(s) de la famille de brevet(s)		Date de publication
US 4702598	A	27-10-1987	AUCUN		
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			AU	747642 B2	16-05-2002
			AU	2873899 A	15-09-1999
			CA	2321718 A1	02-09-1999
			EP	1058939 A1	13-12-2000
			US	2002092973 A1	18-07-2002
			WO	9944217 A1	02-09-1999
			US	6316781 B1	13-11-2001
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			FR	2325038 A1	15-04-1977
			JP	51136475 A	25-11-1976

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:	WO 93/07471
G01N 21/64, 15/14, G01J 1/04	A1	(43) International Publication Date:	15 April 1993 (15.04.93)

(21) International Application Number: PCT/US92/08274

(22) International Filing Date: 29 September 1992 (29.09.92)

(30) Priority data:

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(71) Applicant: BECKMAN INSTRUMENTS, INC. [US/US]; 2500 Harbor Boulevard, Fullerton, CA 92634 (US).

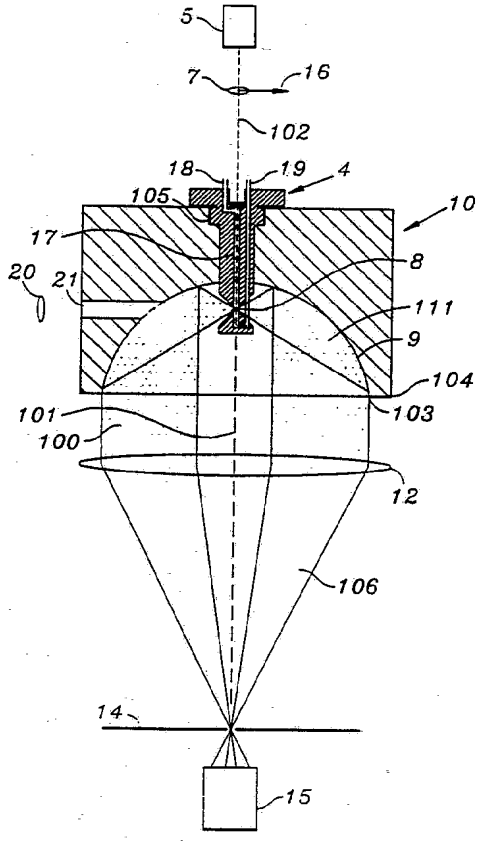
(72) Inventor: KAYE, Wilbur, I. ; 4679 Emmalani Road, P.O. Box 3034, Princeville, HI 96722 (US).

(74) Agents: MAY, William, H. et al.; Beckman Instruments, Inc., 2500 Harbor Boulevard, Fullerton, CA 92634 (US).

(54) Title: DETECTING A RADIATION SIGNAL

(57) Abstract

Focusing a generated radiation signal on a detector is effected by a curvilinear surface. The radiation beam signal from the reflected surface is collimated or focused on the detector. Light scattered, Raman scattered, fluorescence, chemiluminescence, phosphorescence radiation signals from particles as a result of a chemical procedure or reaction is enhanced through this focusing technique. The enhanced signal which is detected is subsequently measured through different detection techniques.



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DETECTING A RADIATION SIGNAL

RELATED APPLICATION

This application is a continuation-in-part of Serial No. 07/772,823 filed October 8, 1991 and entitled "Enhanced Fluorescence Detection of Samples in Capillary Column." The contents thereof are incorporated by reference.

BACKGROUND

This invention relates to the efficient collection and detection of low levels of radiation arising within microliter volumes of sample.

The radiation may be in the form of luminescence from a chemical reaction or result from the interaction of an intense light source and the sample. Alternatively, the processes of light scattering, Raman scattering, fluorescence and phosphorescence may be used.

The technique of light scattering is particularly useful in the detection of particles, and two broad classifications of instruments can be identified. Particles of diameters significantly smaller than the wavelength of probe light scatter isotropically, i.e. equally in all directions. This type of scattering is usually called Rayleigh scattering. Particles of diameters significantly larger than the wavelength of probe light scatter primarily in the forward direction and, often, the intensity of scatter is a complicated function of scattering angle. This type of scattering is

usually called Mie scattering. The intensity of forward scattered light varies as the sixth power of the particle diameter; hence, special precautions must be taken when attempting to measure the light scattered from very small particles.

The present invention permits for the determination of relative particle size and/or concentration of very small particles, particularly in a flowing system.

Intense light sources such as lasers are used for studying small particles. The use of such sources introduces problems associated with discrimination of light scattered by instrumental components from that scattered by the sample. The intensity of light scattered from small particles may be less than one millionth the intensity of laser radiation. To measure such low intensities, it is necessary to collect as much of the light scattered by the sample as possible, yet reject that scattered by the instrument. In general, it becomes increasingly difficult to eliminate instrumental light scattering as the angle of detected scattering decreases. Consequently, when measuring the light scattered by particles significantly smaller than the wavelength of probe light, it is best to avoid detection of the small angle scattering, while collecting as large a solid angle of scattered light as possible.

When measuring the light scattered by small particles, considerable attention must be paid to the overwhelming level of light scattered from unwanted foreign particles of large size. For this reason, it is advantageous to keep the size of the scattering volume (volume of sample simultaneously illuminated and detected) as small as possible. In a flowing sample,

these particles will be evidenced as signal "spikes" abruptly rising from a base signal. The base signal represents the signal from the more numerous small particles.

Large angle scattered light instruments described in the prior art have used large scattering volumes and require extensive sample treatment to minimize the detection of large foreign particles. Furthermore, sample volume has to be large to avoid detection of light scattered from the instrument. These prior art instruments have usually used lenses and apertures to define the scattering angle and, of necessity, capture a small solid angle of scattered light.

Moreover, most of the prior art has addressed the detection of relative large biological cells (cytometry). The signals from these large particles are much greater than those provided by the small particles of particular interest in the present invention.

Raman scattering is used for the chemical characterization of the samples rather than particle size. Like Rayleigh scattering, the signal intensities are very low and it is important to capture as large a solid angle of Raman scattered light as possible while also using very intense sources of incident radiation. The Raman scattered signals are of different wavelength from the incident light. Chemical characterization of the sample is determined by the difference between the frequency of the Raman signal and the frequency of the exciting radiation. It is customary to use a blocking filter to reject the incident radiation followed by a monochromator to determine the frequency of the Raman

signal. While this frequency difference helps in the discrimination between exciting and Raman signals, the Raman bands are often narrow; hence, the signals are very weak. It is important to use whatever geometric means are available to minimize the detection of the incident radiation and maximize the detection of the weak Raman radiation from the sample.

Fluorescence techniques are used when the sample or some component(s) of the sample can be tagged with a fluorescing dye. The sample is then excited by an intense beam of exciting radiation, usually from a laser. The wavelength of the exciting radiation is chosen to correspond to the wavelength of maximum sample excitation. The wavelength of the fluorescing radiation will usually be greater than the exciting wavelength, and a filter or monochromator will be chosen to pass only that radiation corresponding to the wavelength of maximum fluorescence emission. When the sample concentration is low, great care is needed in the choice of filters or monochromators in order to discriminate between exciting and fluorescing wavelengths. No filters provide a perfect discrimination, and it is desirable to use whatever geometric means are available to minimize the detection of exciting rays scattering from the instrument. Under ideal conditions, tagged molecules can be detected at extremely low concentration.

Phosphorescence techniques are employed where the sample, itself, or the tagged sample continues to emit radiation a significant time after excitation. Time as well as wavelength can then be used to discriminate between exciting and emitting radiation.

Luminescence techniques are used when the sample or some component of the sample emits radiation as a result of a chemical reaction of the sample with a reagent. No external exciting radiation is needed; hence, all of the radiated light may be detected without recourse to filtering. However, the luminescence signals are usually very weak and depend on the number of reacting molecules within the detected sample volume. A compromise must usually be made between detected sample volume and concentration of reacting molecules. The efficient collection and detection of the radiation emitted within the detected sample volume is of great importance.

There is a need to provide a system for radiation collection and detection which provides significant advances over the prior art.

SUMMARY

By this invention, there is provided a system for detecting a radiation signal in a manner which minimizes disadvantages of the prior art.

According to the invention, a generated radiation signal from an excited sample is collected over a large solid angle by means of a reflecting curvilinear surface of revolution and is directed to a detector. The angle should preferably be between about 35° and 145°.

The sample enters a detection volume in a flowing stream and the axis of sample flow is collinear with the axis of revolution of the reflecting curvilinear surface of revolution. The scattering volume, which is

part of the detection volume, is retained relatively small.

In one preferred form, the radiation signal after the above interaction has the same wavelength as the radiation signal incident on the sample. This type of interaction is called "light scattering." The incident radiation is of high intensity and is usually produced by a laser. There are means for directing this laser beam along an axis of the curvilinear reflection means.

In particular, small volumes of sample flow past the point of interaction. The axis of sample flow is coaxial with the axis of a reflective light collector. There are also means for directing a sample flow in a tube along an axis collinear with the laser beam in such a way that the beam impacts the sample without impacting the sample tube wall. In this manner, the three axes are collinear.

The scattered light ray output is the generated signal at the focus of the reflecting means.

The output reflection signal from the reflective means can be a collimated beam which is directed to a detector directly. The reflecting curvilinear surface of revolution producing a collimated beam is selectively a paraboloid. The collimated beam may also be focused onto a detector by a focusing lens. Alternatively, the curvilinear means can reflect the generated signal directly to a remote focal point. The curvilinear means for doing this is preferably an ellipsoid.

In different forms of the invention, the generated radiation signal may arise selectively from light scattering, Raman scattering, fluorescence, phosphorescence or luminescence.

The invention is further described with reference to the accompanying drawings.

DRAWINGS

Figure 1 shows the invention used to monitor some property of a sample as it changes in time as a result of passage through a separation means. The sample may be mixed with a reagent, and the geometric axes in the vicinity of the point of radiation generation are illustrated.

Figures 2A and 2B are respectively a side diagrammatic view and end diagrammatic view of the system for detection of scattered light.

Figure 3 shows the details of the containment vessel insert fitting into the curvilinear reflecting surface.

Figure 4 shows an alternate design of the insert in a configuration optimized for the detection of sample luminescence.

Figure 5 is a diagrammatic view of an embodiment wherein an annular aperture determines the detected scattering angle of a scattering signal.

Figure 6 is a diagrammatic view of a monochromator suitable for addition to any of the above

light collection systems. A Fery prism is used to disperse the radiation onto one or more detectors. An aperture plate in the focal surface and in front of the detector(s) determines the portion of the spectrum detected. Alternatively, an array detector may be located at the focal surface of the prism and the spectrum measured.

Figure 7 is an embodiment illustrating an ellipsoidal version of the reflective surface, and is similar to Figures 2A and 2B.

DESCRIPTION

Figure 1 identifies the major components of the system. A sample processed by some type of separation system 1, such as a chromatograph column, mixes with a reagent from a delivery system 2 at point 3. The mixture enters a sample containment vessel 4. An intense light source 5 such as a laser produces a beam 102 which is filtered by a filter 6 and focused by a lens 7 onto a point of radiation generation 8. This generation point 8 corresponds to a focal point of a curvilinear surface of revolution 9 milled into a block 10.

In this illustration, the curvilinear surface 9 is a paraboloid. The surface 9 of this paraboloid is reflecting and collimates the radiation generated at the point 8. From the point 8, there is generated a beam 111 of incident radiation which is directed through a large solid angle to reflective surface 9. The collimated beam 100 of reflected radiation is filtered by a filter 11 and focused by a lens 12 onto an aperture 13 in a plate 14. Radiation passing through the aperture 13 is detected by a detector 15.

An imaginary straight line 101 from the intense light source 5 to the aperture 13 defines the axis of the curvilinear surface 9 and also the axis of sample flow in the vicinity of the point of generation 8. Where pertinent, this imaginary line 101 defines the axis of the exciting light beam 102 in the vicinity of the point 8 of radiation generation.

Figure 2A shows an embodiment of the invention specifically for the detection of scattered light. The light beam 102 from a laser 5 is focused by the lens 7 and directed by means of a two-axis adjusting device 16 down the axis of a channel 17 in the sample containment vessel 4 which screws into the block 10 of the paraboloid. The sample from point 3 enters the containment vessel 4 at 18 and exits at 19.

The laser beam 102 must not strike the walls of the channel 17 in the sample containment vessel 4. Alignment is accomplished by viewing the generation point 8 by means of viewing lens 20 and a hole 21 in block 10 into which the curvilinear surface 9 is formed.

The lens 12 and the aperture in plate 14 serve as a spatial filter and allow only the radiation generated at 8 from passing to the detector 15. In some applications, this spatial filter is unnecessary, but the photosensitive area of the detector 15 must then be as large as the mouth of the paraboloid.

Figure 2B is an end view of the apparatus looking down the axis 101 of the curvilinear surface 9. Looking into the open end of the paraboloid, one "sees" the end of the end cap 27 of the containment vessel. The

inner edge 103 and outer edge 104 of the paraboloid mouth as well as the outer edge of the lens 12 are visible.

Figure 3 shows the details of the sample containment vessel 4 and the end cap 27. The parts are made of a material such as black "Delrin." The containment vessel 4 is threaded at 105 to fit into the paraboloid block 10 which has mating threads and position the generation point 8 at the focal point of the paraboloid surface 9.

The sample enters at 18 through a stainless steel tube 22 tightly inserted in a hole 23 drilled into the black plastic. At a point below the stainless steel tube 22, a second hole 24, at 90° to the first hole 23, is drilled into the black plastic and conveys the sample mixture to channel 107 running down the axis of the containment vessel. The second hole 24 is closed by plug 25 to keep the sample mixture from escaping from the desired channel 24. A glass tube 26 fits tightly into bore 107 in the sample containment vessel 4 and extends into the second black plastic end cap 27.

A glass plate 28 serves as a window to allow laser radiation 102 to pass into the containment vessel while keeping the sample in its channel 24. The glass plate 28 is held against an "O ring" 29 by means of retainer 30. The glass tube 26 does not come in optical contact with the glass plate 28. Thereby, the light scattered at the surface of the glass plate 28 does not channel into the glass tube 26. Were a significant fraction of the light scattered by glass plate 28 to enter tube 26, some unwanted radiation could escape from tube 26 in the vicinity of the generation point 8.

The end cap 27 allows the sample to exit the system through hole 108 and also acts as a light trap for the excess incident radiation from laser beam 102. A plate 31 of polished black glass acts as the primary light trap. This plate 31 is angled to send the weak reflected beam to the end wall 109 at the end of hole 108. The black glass plate fits against the wall of the black plastic by means of a threaded plug 32. Another "O-ring" 33 seals against escape of the sample stream. A second plug 25 again confines the sample to the desired stream.

A short piece of stainless steel tubing 34 fitted into a hole 110 conveys the sample stream to a piece of Teflon tubing 35 which mates with still another piece 36 of stainless steel tubing by which the sample stream exits the containment vessel at 19. The plastic tubing 35 blocks only a negligible portion of the scattered rays from falling on the paraboloid.

The end cap 27 is dimensioned to slip through the hole in the paraboloid block 10 at its base.

The solid angle 112 of incident radiation is between about 45° and 135° relative to the axis 101. The end face 113 of the vessel 4 defines one limit of the angle 112. The end face 114 of the end cap 27 can define a second limit of the angle 112. Alternatively to the end face 114, the limit 103 of the parabaloid surface 9 can limit the extent of the angle, as illustrated in Figures 1 and 2.

Figure 4 shows another embodiment of the containment vessel 4 and its end cap 27. In this case, the sample mixture is introduced to the generation point

by means of a capillary tubing 41. A chemiluminescence reagent is introduced through entry 18. This embodiment is primarily intended for use with chemiluminescent samples. Exciting light source is not necessary in this application. It is presumed that the luminescence resulting from the chemical reaction is of short lifetime, hence, has a maximum intensity at the point of reaction which in this case occurs at the generation point.

Figure 5 is similar to Figure 2A and is useful in certain types of light scattering measurements. Plate 43 with an annular aperture 42 is inserted into the apparatus where the beam from the sample is collimated. The inner and outer diameters of this annular aperture determine the solid angle 112 of detected scattered light.

Figure 6 is an extension of Figure 2A and shows the focused radiation 106 passing through the aperture 13 in plate 14 and on to a Fery prism 44 which disperses the radiation 116 and focuses a spectrum on a curved plate 45. The location and size of the aperture 47 in plate 45 determines the wavelength and wavelength interval of radiation detected by detector 46. A multiplicity of apertures and detectors may be used when it is desired to monitor different spectral intervals simultaneously. Similarly, means other than a Fery prism may be used to disperse radiation 106.

Alternately, an array detector may be positioned in place of the aperture plate. The signal from the array of detectors 46 may be processed in the usual manner to provide the dynamic changing spectra as samples flow through the system. It should be apparent

that any type of dispersing system may be employed in Figure 6. However, the Fery prism 44 affords a high efficiency along with low stray light.

When there is no need to filter the light from the sample (by means of filter 11 shown in Figure 1), surface 9 of block 10 may be in the shape of an ellipsoid. As shown in Figure 7, the beam 106 is then focused directly onto aperture plate 14 and lens 12 is not needed.

Applications

Description of a few applications for the above equipment may help to understand the invention.

The apparatus in its light scattering configuration is useful in the study of antigen-antibody reactions. When the antigen specific to an antibody mixes with a solution containing the antibody, there is a reaction producing aggregates of rapidly increasing size. If the concentration of antibody and antigens is sufficiently high, the aggregates may become large enough to see with the naked eye. However, at low concentration, the limiting aggregate size may be submicroscopic and detectable only by very sensitive light scattering techniques.

The above invention is applicable to the screening of antibody-antigen reactions wherein a separation technique isolates fractions of either antibodies or antigens. As the components elute from the separations means 1 and mix with a potential conjugate steadily flowing from 2, the sample generates an elevated scattered light signal, indicative of an aggregating

pair. The detector means 15 can be set up to respond to a spatial or temporal signal from the generating point 8.

Raman spectra often characterize particular classes of molecular structure. A detector can sense when sample fractions having that particular class of structure elute from a chromatograph column 11.

Fluorescence and phosphorescence techniques provide for extremely sensitive detection of classes of samples eluting from a separation system when certain classes of effluent can be tagged with a fluorescent dye or set of dyes. For example, DNA fragments of different size may be chromatographed to separate fragments by molecular weight. At the same time, there are only four possible end groups and these can be dyed with fluorophores specific to the end groups. The above apparatus can identify the end group of the eluting fractions.

Certain oxidation-reduction reactions can be sensitively detected by chemiluminescence. The above apparatus can provide a good detector when an eluting sample triggers a chemiluminescence reaction.

Many more examples of the invention exist, each differing from the other in matters of detail only. The invention is to be determined solely by the appended claims.

CLAIMS

1. Apparatus for directing a radiation signal to a detector comprising means for generating an incident radiation signal caused by the interaction of a flowing sample with an exciting radiation source, a reflecting surface of revolution having an axis and having a focal point, the reflecting means being means for receiving the generated incident radiation signal and for reflecting the incident signal as an output reflection signal, and means for generating the incident signal at substantially the focal point of the reflection means, the reflection signal output is directed to the detector, and wherein at least one of the directions of the flowing sample or the direction of the exciting radiation is substantially collinear with the axes of the reflecting surface at least at the focal point.

2. Apparatus as claimed in claim 1 wherein the reflection means outputs a reflected collimated signal.

3. Apparatus as claimed in claim 1 wherein the reflection means outputs a reflected signal converging at a remote focus location.

4. Apparatus as claimed in claim 1 wherein the reflection means includes a parabolic surface.

5. Apparatus as claimed in claim 1 wherein the reflection means includes an ellipsoidal surface.

6. Apparatus as claimed in claim 1 including lens means between a reflection means and the detector.

7. Apparatus as claimed in claim 1 including filter means between the reflection means and the detector.

8. Apparatus as claimed in claim 1 including grating monochromator means between the reflection means and the detector.

9. Apparatus as claimed in claim 1 including an annular aperture between the reflection means and the detector, the annular aperture being located in a plate spaced from the reflection means and wherein the center of the annular aperture is located on an axis of the reflection means.

10. Apparatus as claimed in claim 9 wherein the annular aperture has a selected diameter, the selected diameter being determined in accordance with a selected incident radiation signal and a reflected signal from the reflection means.

11. Apparatus as claimed in claim 1 including means at the focal point for generating the incident light permitting for a solid angle of incident light in the range between about 45° and about 135° relative to the axis of revolution.

12. Apparatus as claimed in claim 1 wherein the generated incident radiation signal is selectively at least one of a light scattered signal, Raman scattering fluorescence, phosphorescence, or luminescence signal, the incident radiation signal being generated by the impact of a laser radiation source directed on particles in the sample at the focal point.

13. Apparatus as claimed in claim 1 wherein the signal is selectively at least one of a light scattering signal, Raman scattering, fluorescence or phosphorescence and wherein the light beam laser radiation directed axially relative to the reflecting means and towards the focal point of the reflecting means, and wherein the sample flows axially substantially parallel with the laser radiation, and the axis of revolution.

14. Apparatus as claimed in claim 1 including an elongated tube for the sample flow substantially along the axis of revolution, and wherein the tube includes a side wall, wherein the light beam is laser radiation, and including focus means for the laser radiation such that the radiation essentially travels wholly within the tube and is free of contacting the tube side wall, and the focus means directs the radiation into contact with the sample at substantially the focal point.

15. Apparatus for directing a light scattered signal to a detector comprising a reflecting surface of revolution for receiving an incident light scattered signal, a focal point for the reflecting surface, a detector spaced from the reflecting surface, the reflecting means being for reflecting the light scattered signal to the detector, means for generating a laser radiation beam along an axis of the reflecting surface, means for directing a sample coaxially with the reflecting surface, such that the laser radiation beam and the sample impact at least at the focal point, means for directing the light scattered signal from the focus of the reflecting surface such that the incident light scattered signal is directed from the reflecting surface towards the detector.

16. Apparatus as claimed in claim 15 including a tube axially directed relative to the reflecting surface, the tube being directed to receive the laser radiation beam axially and to permit flow of the sample axially through the tube, and means optically occluding the tube selectively around its perimeter and permitting the incident point light scattered signal to exit from the tube at the focal point.

17. Apparatus as claimed in claim 16 including a cap on a downstream end of the tube optically occluding a light scattered signal, and a window on an upstream side of the tube, the window being optically isolated from the tube.

18. Apparatus for directing a radiation signal to a detector comprising a reflecting surface of revolution having an axis and a focal point for receiving a radiation signal, a detector spaced from the reflecting surface, the reflecting surface being for reflecting the radiation signal to the detector, means for generating an excitation signal beam, means for directing the excitation beam to impact along the axis of radiation at the focal point for generating the incident radiation signal substantially at the focus of the reflecting surface such that the radiation signal is directed from the focal point over a solid angle in the range of about 45° to 135° relative to the axis of revolution.

19. Apparatus as claimed in claim 18 wherein an excitation beam source is located adjacent the reflecting means, and including filter means downstream the reflecting means, the reflecting means generating a reflected signal from the incident signal generated at the focal point, the filtration means being for removing

an excitation signal and for permitting passage of the reflected signal.

20. Apparatus as claimed in claim 19 including lens means downstream the filter means, the lens means focusing a reflected fluorescence signal on the detector.

21. Apparatus as claimed in claim 20 including a monochromator located between the reflecting surface and the detector, and including a diffraction mirror between the monochromator and the detector, the diffraction mirror focusing the reflected signal on the detector.

22. Apparatus for directing a radiation signal to a detector comprising a substantially curvilinear reflecting surface having a focal point and an axis of revolution, a detector, the reflecting surface reflecting an output signal to the detector, means for generating an incident radiation signal by the interaction of a sample directed along the axis of revolution with an excitation signal at the focal point, means for directing the generated signal to the reflecting surface and the detector and including means for responding selectively to a spatial or temporal sorted signal, selectively, a Raman scatter signal.

23. Apparatus for directing a radiation light scattered signal to a detector comprising a curvilinear reflecting surface having a focal point and an axis of revolution, the reflecting surface being for receiving a generated incident radiation signal, means for directing a flowing sample axially relative to the axis of revolution towards the focal point, a detector, the reflecting surface transmitting a reflected signal to the

detector, means for generating an incident radiation signal by the interaction of a sample with an excitation signal directed axially relative to the axis of revolution, including means for selecting the excitation radiation signal to have a wavelength greater than a particle size in the sample whereby the incident radiation reflected from the particles is isotropic.

24. Apparatus as claimed in claim 23 wherein the incident radiation signal disperses radiation over a solid angle range selected between about 45° and about 135° relative to the axis of the reflecting surface.

25. A method for directing a radiation signal to a detector comprising generating an incident radiation signal by the interaction of a sample with an excitation source, the sample being directed coaxially relative a curvilinear reflecting surface of revolution source, receiving the generated incident radiation signal from a focal point of reflecting surface, generating an output reflected signal from the reflecting surface, and directing the reflected signal to a detector.

26. A method as claimed in claim 25 wherein the reflected output signal is a collimated signal.

27. A method as claimed in claim 25 wherein the reflected output signal converges to a remote focus location.

28. A method as claimed in claim 25 wherein the generated radiation defines a solid angle in the range of about 45° to 135° relative to the axis of revolution.

29. A method as claimed in claim 25 wherein the curvilinear means selectively includes an ellipsoidal or parabolic surface.

30. A method as claimed in claim 25 including directing an excitation source axially relative to the reflection means.

31. A method for directing a light scattered signal to a detector comprising generating a laser radiation beam along an axis of a curvilinear reflecting surface having a focal point, directing a sample axially to the focal point whereby the laser radiation beam impacts the sample at the focal point, and directing a light scattered generated signal from an impact at the focal point to the curvilinear reflecting surface and reflecting the incident light scattered signal towards a detector.

32. A method for directing a radiation signal to a detector, the signal being selectively a light scattered signal, Raman scattering, luminescence, phosphorescence or fluorescent signal, comprising generating an excitation signal beam and directing the excitation signal beam to a sample to generate an incident radiation signal, receiving a radiation signal on a curvilinear reflecting surface, the curvilinear means having an axis of revolution and a focal point and being for reflecting the incident radiation signal from the focal point towards a detector, the sample and the excitation signal being directed towards each other at the focal point at a selected angle relative to each other, and wherein the generated radiation signal from the impact is generated at the focal point that a

generated radiation signal is directed to the curvilinear surface and in turn, towards the detector.

33. A method for directing a radiation signal to a detector comprising generating the radiation signal by the interaction of a sample with an excitation signal at a focal point of curvilinear reflecting means, directing the generated signal as an incident radiation signal to the reflecting surface, reflecting the incident signal to a detector and including having the detector responding selectively to a spatially or temporally sorted reflected signal.

34. A method as claimed in claim 25 wherein the radiation signal has a wavelength greater than a particle size in the sample such that the radiation reflected from the particles is isotropic.

35. A method as claimed in claim 25 wherein the signal from the sample reflects radiation over a solid angle range selected between about 45° and about 135° relative to the axis of the surface of revolution.

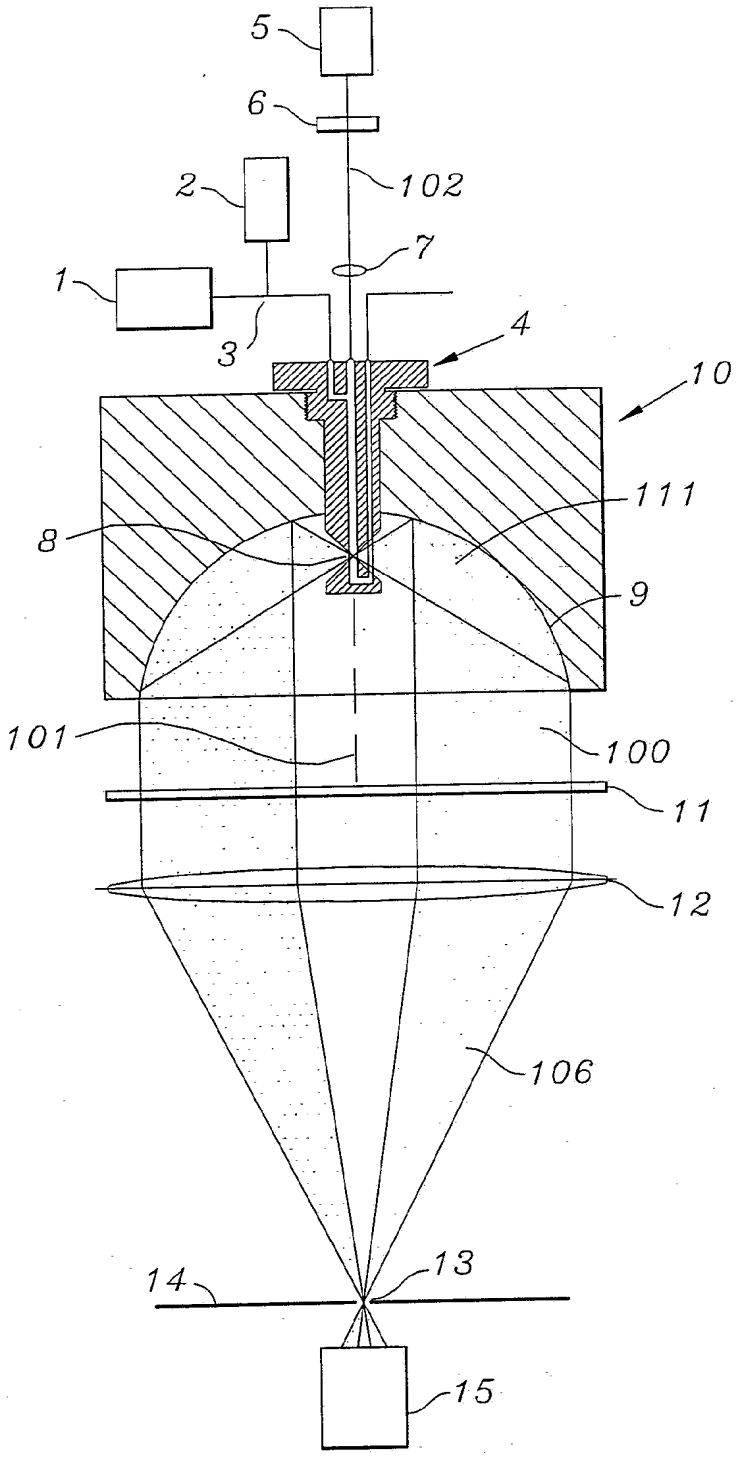


FIG. 1

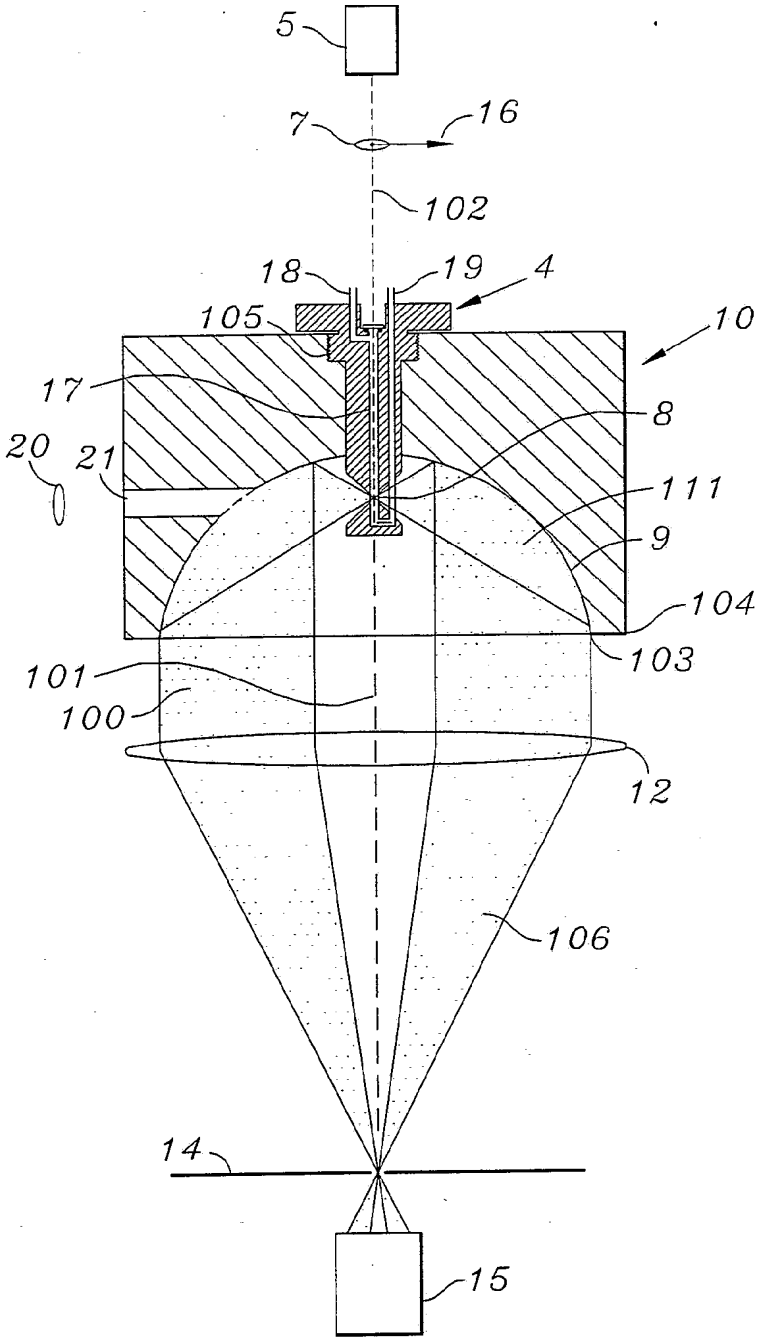


FIG. 2A

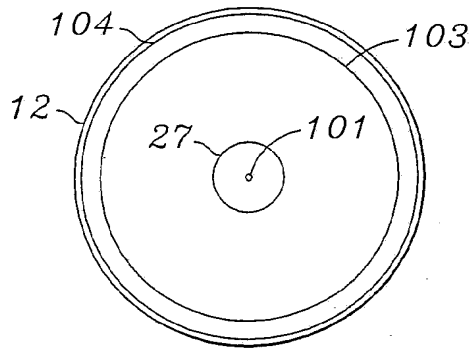

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FIG. 2B

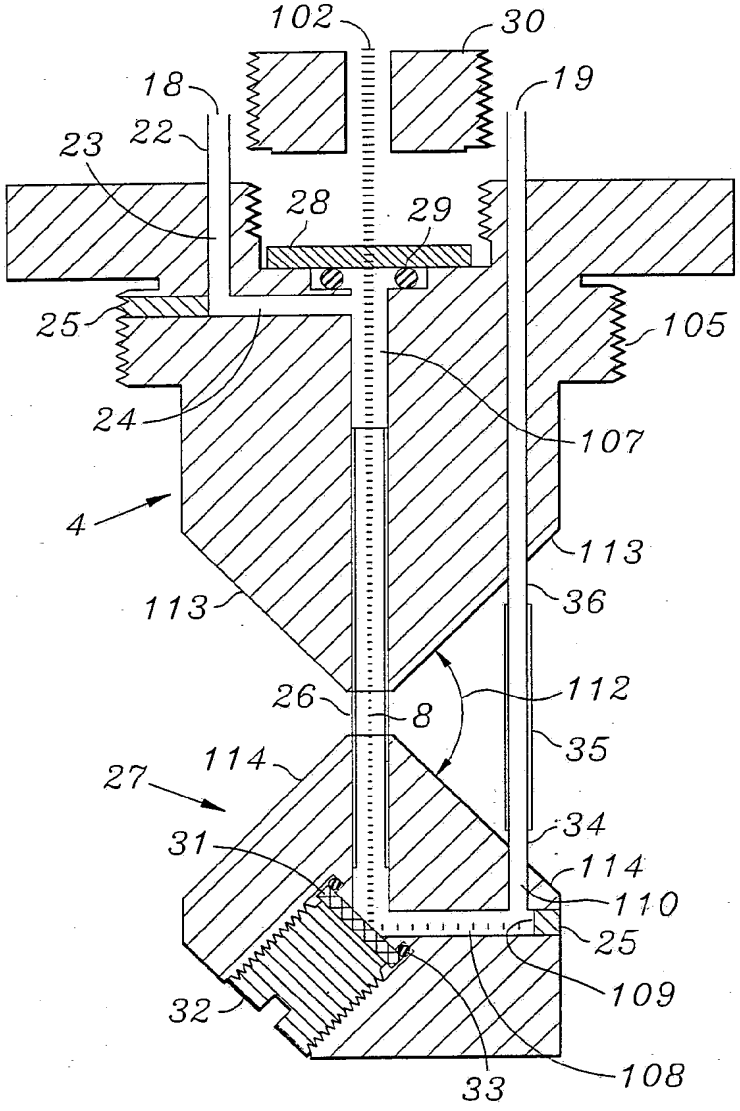

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FIG. 3

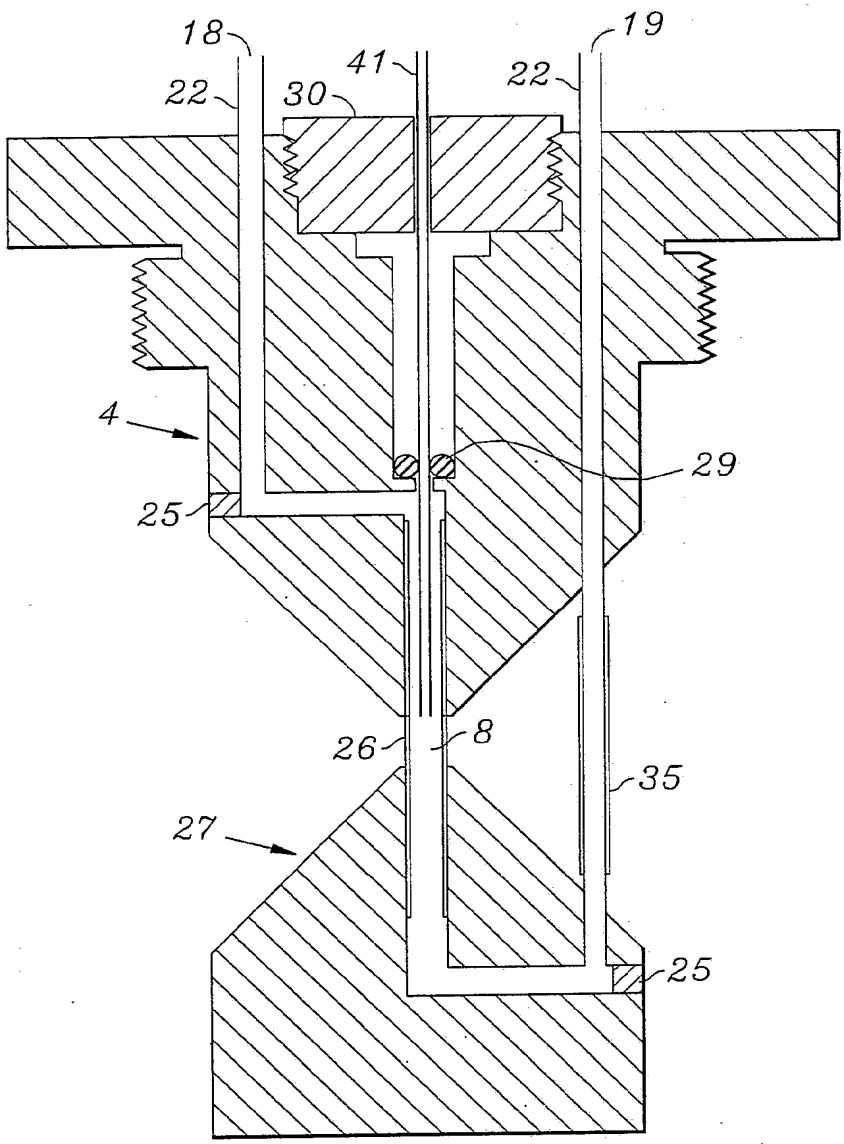


FIG. 4

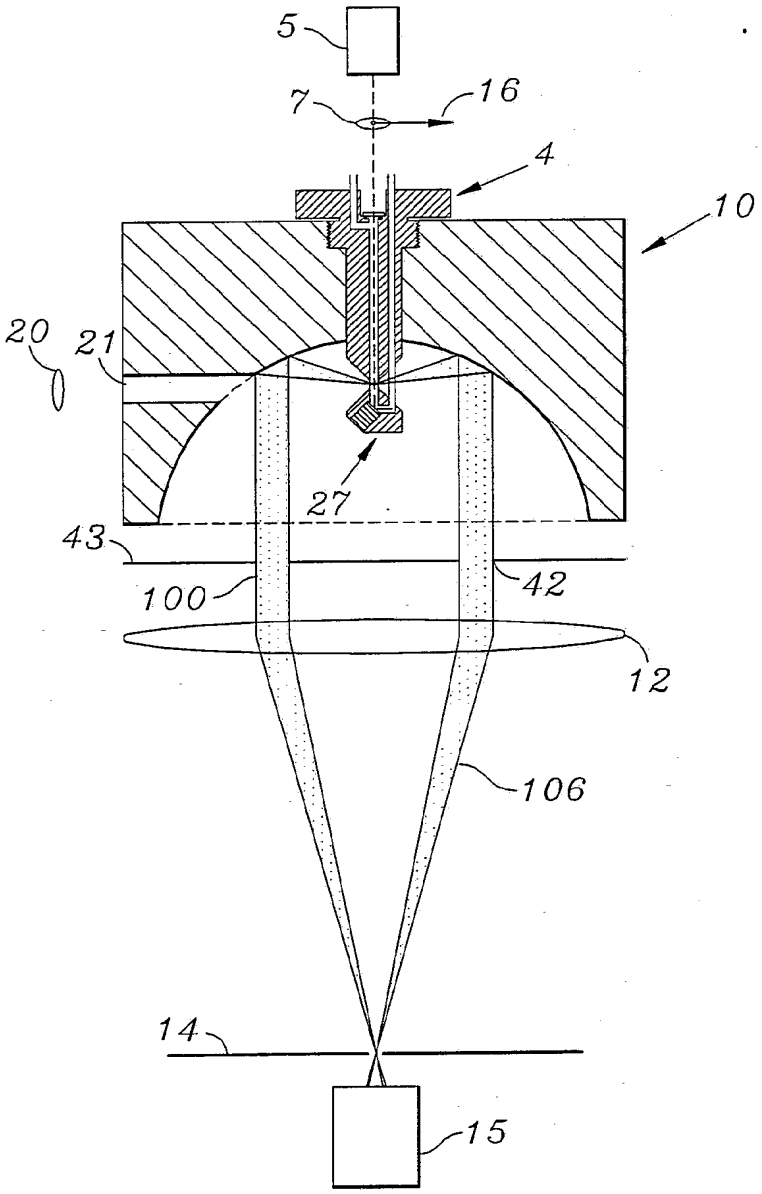


FIG. 5

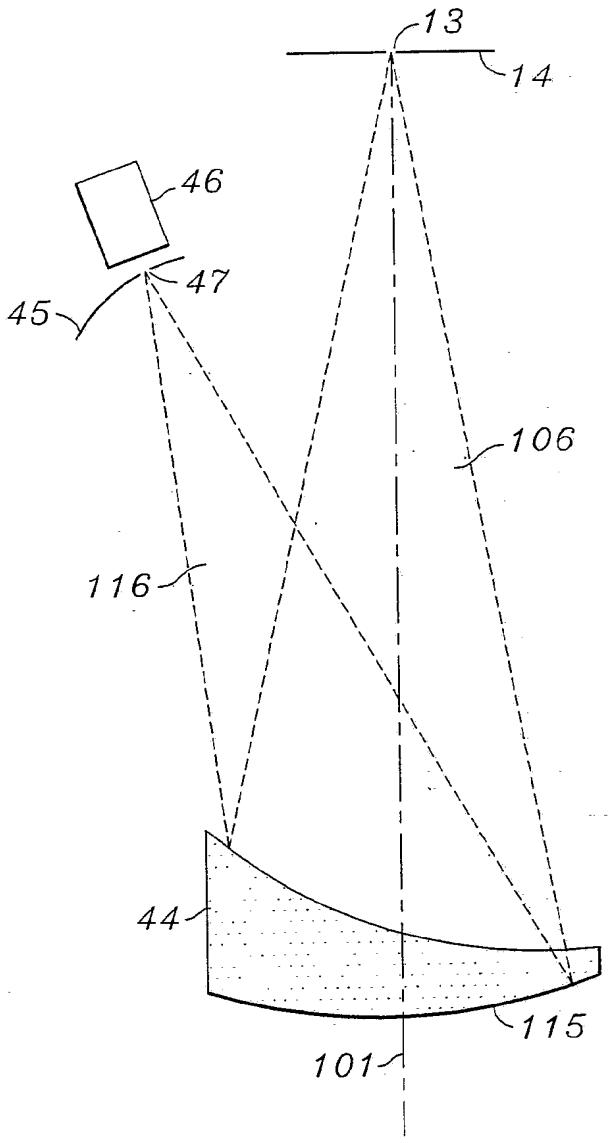


FIG. 6

SUBSTITUTE SHEET

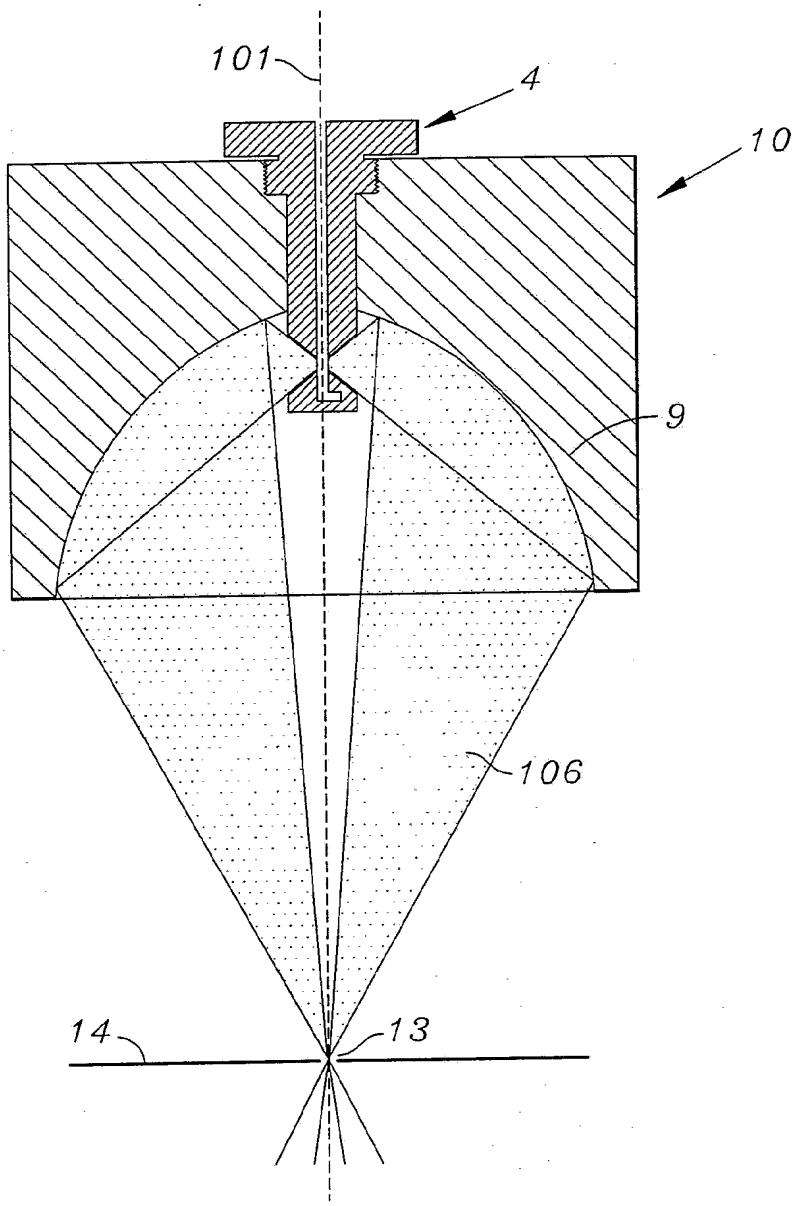


FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/08274

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N21/64; G01N15/14; G01J1/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
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Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	PATENT ABSTRACTS OF JAPAN vol. 14, no. 534 (P-1135)26 November 1990 & JP,A,22 27 637 (SHIMADZU) 10 September 1990 see abstract	1,3,5-7, 11, 13-20, 25, 27-32,35
A	--- -/--	12

° Special categories of cited documents :[10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07 JANUARY 1993	03. 02. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	THOMAS R.M.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	US,A,4 088 407 (SCHOEFFEL) 9 May 1978 see column 1, paragraph 2 see column 1, line 66 - column 2, line 7 see column 3, line 3 - line 41	1-5,11, 18,25, 26, 28-30, 32,35
Y	see figures 3,4	1,8,12, 22,33
A		9,10, 13-17,31

Y	US,A,4 200 802 (SALZMAN) 29 April 1980 see abstract see column 2, line 11 - line 35 see column 2, line 46 - line 63 see column 3, line 1 - line 20; figures	1,8,12, 22,33

X	US,A,4 606 636 (MONIN) 19 August 1986 see abstract see column 2, line 28 - line 35 see column 2, line 47 - line 52 see column 2, line 61 - line 67 see column 3, line 27 - line 30 see column 3, line 56 - line 60 see column 4, line 30 - line 36 see figures 1,2	1,2,4, 11,12, 25,26, 28,29, 32,35
A		9,10,23, 24

X	US,A,3 248 551 (FROMMER) 26 April 1966 see column 1, line 9 - line 15 see column 2, line 29 - line 40 see column 2, penultimate line - column 3, line 20; figure 1	1,3,5,9, 10,12,32
A		17,18

X	US,A,2 852 693 (HUGHES) 16 September 1958 see column 1, line 53 - line 58 see column 2, line 25 - line 52 see column 3, prepenultimate line - column 4, line 2; figures	1,6,7,32

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	EP,A,0 421 156 (PACKARD) 10 April 1991 see column 8; figure 3	18,19
X	US,A,2 551 542 (MARSH) 1 May 1951 see column 3, line 50 - line 51 see column 4, line 49 - line 57; figure 1	18,19

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9208274 SA 65539

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		DE-A,B,C 2415049	18-09-75
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		JP-A- 55131750	13-10-80
US-A-4606636	19-08-86	None	
US-A-3248551		None	
US-A-2852693		None	
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US-A-2551542		None	

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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(71) Applicant *(for all designated States except US)*: CAMBRIDGE CONSULTANTS LIMITED [GB/GB]; Science Park, Milton Road, Cambridge CB4 4DW (GB).

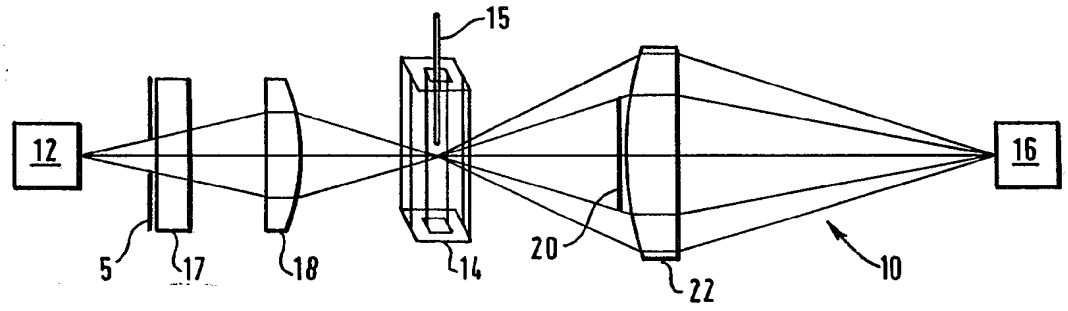
(72) Inventors; and
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(74) Agents: BERESFORD, Keith, Denis, Lewis et al.; Beresford & Co., 2-5 Warwick Court, High Holborn, London WC1R 5DJ (GB).

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(54) Title: PARTICLE MEASUREMENT SYSTEM



(57) Abstract

The invention concerns a method of and apparatus for measuring particles in a fluid medium in which the particles are moved relative to a structured light field preferably produced by a multi-facet laser light source. Light scattered by the particles is detected to generate an output signal which is analysed to give an indication either of particle size or particle velocity.

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PARTICLE MEASUREMENT SYSTEM

The present invention relates to the optical measurement of particles and particularly, though not exclusively, to the measurement of particle sizes.

Particle sizing by means of optical techniques using Doppler methods which measure the amount of light scattered by the particle as it passes through a light field is widely practised. For biological particles of diameter 1-10um and sub-micron particles the scattered light levels are low. Usually therefore particles are made to flow in a focused light field through a flow cell.

Particular sizing techniques based on Doppler methods require the interferometric combination of crossed laser beams to create a structured pattern. This requires the coherent laser light sources and precision lasers, or more recently, the use of diffraction gratings. The extent of the structured light field necessarily occupies a large part of the inspection volume and consequently requires quality optical components. These requirements are not

consistent with the manufacture of a low cost particle sizing equipment.

A problem of known techniques that rely purely on scattered light is that the region of focus is frequently subject to significant variations in radiant intensity when it occupies practical sample volumes. A given particle passing a region of high focal intensity can therefore scatter the same signal as a larger particle in a region of defocus. It has been proposed to overcome this problem by providing a composite light beam incorporating a concentric trigger of one wavelength through the centre of a surrounding analysis beam having a second wavelength. Only particles whose presence is indicated by the trigger beam are analysed. This occurs when the particle traverses the uniform region of the analysis beam. Measurements of the intensity of scattered light are thus made under repeatable conditions of particle illumination. This technique still requires high precision optics however and does not overcome the difficulty that the intensity of light scattered by particles is not a single valued function of the particle size, so that light intensity does not

necessarily provide a reliable indication of particle size.

For example in medical bacteria tests in urine, tests for bacteria (typically 1-4um) may not clearly be distinguished from residual red cells (5-8um) or even white cells (10-15um). Additionally still greater expense and complexity is involved.

The present invention is concerned with reducing the above mentioned disadvantages.

According to one aspect of the present invention a method for particle detection comprises passing particles in a fluid medium relative to a light source which generates a light field having a plurality of variations in intensity spaced along the relative direction of movement, and detecting variations in light intensity caused by the particles passing through the variations in the light field.

According to a second aspect the present invention comprises apparatus for particle measurement comprising apparatus for measuring particles

comprising a light source for generating a light field having a plurality of spaced variations in intensity, means for moving particles in a fluid medium relative to the light field so that the particles pass successively through the intensity variations, and means for detecting variations in light intensity caused by the passage of the particle relative to the light field.

Various embodiments of the invention will now be described by way of example and with reference to the following drawings in which:

Figures 1 and 1A illustrate the layout of optical components in one form of particle sizer according to the invention;

Figure 2 is a block diagram of a typical signal processing system for the analysis of a particle size distribution based on signals from the light detector of Figure 1;

Figure 3 shows a general form of an output signal generated by the embodiment of Figure 1;

Figure 4(a)-(d) shows experimental results of the voltage output signal from the detector of the embodiment of Figure 1;

Figure 5 shows a scatter plot derived from structured outputs showing distribution of particles in the range 9u to 3um; and

Figures 6A and B show alternative embodiments of light detection optics systems.

Referring now to Figure 1 of the accompanying drawings this shows the optical layout of one embodiment of apparatus according to the present invention for sizing particles in a fluid stream and is generally indicated at 10 for sizing particles in a fluid stream. A potential application of the apparatus is to detect the presence of bacteria in urine. However it will be appreciated that the apparatus could also be used in a similar manner to measure particles carried in a gaseous medium, and that the term "fluid

medium" as used in the present specification is intended to cover both gaseous and liquid media.

The apparatus 10 comprises a light source 12, a flow cell 14 and a light detector 16.

The light source 12 is a structured light source comprising an array of discrete light emitting elements uniformly separated. In a preferred form the structured light source 12 is realised by laser diodes having multiple facets. The source illustrated comprises three facets, but may in practice incorporate any suitable number of uniformly separated emitting elements.

The light source 12 is imaged by the aperture 15 and cylindrical lenses 17 and 18 into the inspection volume of the flow cell 14. In the preferred embodiment of the invention the structured light source comprises a laser diode, e.g. Sharp type LTO90MFO(TM) in which the three light emitting facets are strips approximately 3 microns by 1 micron and separated by 50 microns. For the detection of E coelli in urine the magnification of the lens systems

17 and 18 is chosen so that the separation of the intensity peaks in the focused image is approximately 5 microns. Thus in the embodiment being described the actual volume of fluid in the effective area of light is approximately 20 x 20 x 20 microns. It is possible for the fluid under test to be passed through a standard flow cell but it is preferred to inject a stream of fluid into a cell 14 by means of a narrow bore pipe 15 such as a hypodermic needle so that the stream passes through the focused light. In such a case the diameter of the bore of the needle is preferably between 100 to 300 microns. The fluid can be supplied under gravity or by a suitable pump.

Figure 1a shows a stream of fluid passing through the three focused facets of the light source 12. As shown in Figure 1a the image of the line source facets section the flow cell normal to the flow direction and extend beyond the width (w) of the flow cell. The depth of focus is ideally made greater than the cell depth although this is not a fundamental requirement. The magnification of the cylindrical lenses is also chosen so that the separation (q) of the image bars or

lines approximate to the range of particle size to be measured.

Light from the image volume in the flow cell is collected by lens 22 into the detector 16. A stop 20 is sized so that normally in the absence of particles or other scattering sites in the image column the detector 16 is not illuminated, whilst forward scattered light is collected and detected. The detector 16 can be a PIN diode or an avalanche photo-diode.

Particles that traverse the focused light field are thus exposed to light from each facet or focused variation in light intensity in turn. The intensity of light detected is thus modulated with a frequency q/u, where u is the particle traverse velocity and with intensity given by the convolution of the particle scattering cross section with the structured light image. A particle p of diameter d where d>>q effectively smears out the structure and an event of uniform intensity is displayed.

Particles for which d>q partially resolve the structure and thus partially modulate the signal intensity and particles for which d>>q fully resolve the structure and display full modulation with intensity limited only by the detection noise limit.

It will be appreciated that the embodiment being described is capable of measuring bacterial particles which are poor absorbers of light. If the apparatus is intended to measure particles which are good light absorbers then a simple obscuration method could be employed. In such a case the stop 20 could be omitted so that the output of detector 16 would normally be high and the passage of particles through the inspection volume would cause appropriate reductions in the measured light input. However with poorly absorbent particles the signals caused by particle obstruction are small compared to the noise level of the light sensor gemerated by the directly incident radiation and it is for this reason that a system which detects scattered light is preferred.

The processing of the output signal will now be described by reference to the block diagram illustrated in figure 2.

When a particle traverses the inspection volume of the flow cell light scattered in the structured light field by the particle is collected in the detector 16 to generate an output signal which is amplified by an amplifier 22, thresholded by a threshold circuit 24, sampled by an Analogue-to-Digital convertor 24 and analysed by a suitable microprocessor unit 26. A display unit is shown at 28 for displaying the results of the analysis and can take a wide number of different formats such as a CRT-based video system or hard copy device such as a plotter, whilst the microprocessor unit can be a PC.

A general form of the output signal 16 generated by the passage of a single particle is shown in Figure 3.

Considering this general form of signal the A-D detector typically generates samples from which mean values of the background voltage V_b, a set of three

peak voltages V_{p1}, V_{p2} and V_{p3} corresponding to each facet of the light source structure and two trough voltages V_{T1} and V_{T2} are generated.

System noise in general varies even during the period of a single pulse, as will be apparent from the experimental results illustrated in figure 4(a)-(f), generally due to thermal and flow effects.

Accordingly values of the mean peak voltages V_p and trough voltages V_p are corrected for the background voltage value by subtracting the latter as is shown in the following equations.

$$V_{pn'} = V_{pn} - V_b$$

$$V_{tn'} = V_{tn} - V_b$$

Further calculations for each particle sampled are then performed

$$\overline{V_p} = \frac{1}{3}\sum_{n=1}^{3} V_{pn'}$$

$$\overline{V_T} = \frac{1}{2}\sum_{n=1}^{2} V_{tn'}$$

and the visibility V is then calculated

$$\overline{V} = \left(\frac{\overline{V}_p - \overline{V}_T}{\overline{V}_p + \overline{V}_T}\right)$$

Figure 5 shows particularly clearly the effect of this calculation of the normalised peak-to-trough variation. Thus the layer, i.e. 9um particles shown in the left hand side of the figure are particles which have displayed the highest mean peak scatter level and smallest depth of modulation as defined by the mean visibility of the signal. Similarly the smallest, i.e. 3um particles which occupy the bulk of the right hand side of the figure display the lowest mean peak scatter level and highest depth of modulation.

Evidently for a source having a greater number of facets than 3, corresponding summations for the greater number of peak and trough events can be performed. These calculations are carried out in microprocessor unit 26 and the results displayed at 28. The particle size can be collected and displayed in histogram form or in other suitable form of display to illustrate the sizes of particles present. In

general the visibility is found to be a single valued function of the particle size, and calibration of the particle sizing instrument is therefore simplified. Figure 5 shows a scatter plot from experimental results for particles of 9um, 5um and 3um diameters.

The detection optics illustrated in Figure 1 places the detector 16 under dark field illumination when there is no particle present in the inspection volume, due to the stop 20. This has the advantage that the scattered light signal can be focused into a small area detector and all the light collected is employed to generate the output signal, thereby minimizing noise. However as already mentioned the stop may be removed, thereby normally exposing the detector 15 to continuous light illumination. When a particle passes through the inspection volume it partly shadows the detector, lowering the illumination. In that case the signal of figure 3 is in effect inverted, so that V_p and V_T are lower than V_b. However the same calculation is performed to extract the visibility and thus the size of the particle. It is normally to be expected however that the noise component of this

arrangement is greater than that described above, so that the accuracy of particle size denomination is lower and the least particle size that can be detected greater. The system without stop 20 in as accordingly less practicable where the particles to be measured have poor light absorbtion characteristics, such as bacteria and blood cells.

Alternative systems for collecting and detecting the scattered light are illustrated by reference to figure 6, in each case using forward scatter. Alternatively it is perfectly feasible to use either side or back scattered light and in fact the actual geometry chosen may be dictated by the practical constraints of the application.

One alternative light collection system in figure 6(a) comprises a detector 16' partially screened by stop 20' placed adjacent to the flow cell 14. In this case the detector collects the light without the benefit of a collection lens 22, and consequently the detector is somewhat larger in area. Such an arrangement may be built at less cost, but owing to the larger detection

area is subject to increased noise in the detection circuit.

A more compact arrangement is also obtained by incorporating the mirror 19 as well as lens 22'. Alternatively the mirror 19 can be appropriately curved so that it also acts as a lens so as to provide a simple component which collects and focuses scattered light from the flow cell 14.

In the prior art the structured light field has been generated interferometrically in the form of fringes by focusing light from a coherent laser source via two paths into the inspection volume. In the present invention a structured light field is developed using incoherent methods and may take a number of forms. One convenient form described above takes the form of a muli-faceted light emitting or laser diode which is focused into the inspection volume by means of suitable cylindrical lenses or an equivalent optical system.

However it will be evident that in general the source may be comprised of a number of structurally separate light sources optically combined by suitable beam splitters and cylindrical lenses to provide an equivalent structured image in the inspection volume; or alternatively may be obtained by an array of slits exposing a common light source suitably focused into the image plane; or alternatively may be obtained from a line source divided by a prism (or prisms) or diffraction grating into two or more line sources: and any other structured light field developed using incoherent imaging methods. The objective of the invention is to develop a structured light field having two or more basis of focused light having a predetermined separation (q) having a low manufacturing cost but providing high light intensity.

Although the invention has been described above with regard to particle size measurement it will be evident that the particle speed may also be extracted by monitoring the frequency of the voltage signals obtained. Additionally particle speed in more than one direction can be measured by having a second

structured light field and associated optical system. In such a case the facets of the second field could be orthogonal with respect to those of the first field. It will also be appreciated that apparatus of the types just described could be cascaded along the path of particle flow with the facets being separated by varying distances.

CLAIMS

1. A particle measurement method comprising passing particles in a fluid medium relative to a light source which generates a light field having a plurality of variations in intensity spaced along the relative direction of movement, and detecting variations in light intensity caused by the particles passing through the variations in the light field.

2. A method as claimed in claim 1, wherein the light detected is light scattered by the particles.

3. A method according to claim 1 or claim 2, wherein the light source generates three variations in intensity through which particles successively pair to generate pulses.

4. A method according to any one of the preceding claims wherein the size of a detected particle is measured by plotting the mean peak signal as a function of the normalised peak-to-trough variation in the output pulses generated by the passage of the particle through the light field.

5. A method according to any one of claims 1 to 3 wherein the velocity of a particle relative to the light source is measured by calculating the frequency content in output pulses generated by the passages of the particle through the light field.

6. Apparatus for measuring particles comprising a light source for generating a light field having a plurality of spaced variations in intensity, means for moving particles in a fluid medium relative to the light field so that the particles pass successively through the intensity variations, and means for detecting variations in light intensity caused by the passage of the particle relative to the light field.

7. Apparatus as claimed in claim 1, wherein the light source generates a light field having three spaced variations in intensity.

8. Apparatus according to either claim 6 or claim 7, wherein the means for detecting are arranged to detect light scattered by particles as they move relative to the light field.

9. Apparatus as claimed in any one of claims 6 to 8 wherein the light source comprises three spaced sources of light.

10. Apparatus according to claim 9, and including at least one lens arranged to focus light from the light source into the light field so that the spacing between the variations in intensity is approximately equal to the size of a particle to be measured.

11. Apparatus as claimed in any one of claims 6 to 10 in which the fluid medium carrying the particles is supplied to the light field in a stream in a narrow bore pipe.

12. Apparatus as claimed in claim 12, wherein the pipe has a diameter between approximately 100 and 300 microns.

13. Apparatus as claimed in any one of claims 6 to 12, and including a stop to prevent direct illumination of the detecting means.

14. Apparatus as claimed in claim 13, wherein the stop is located, in the optical path, before a lens which focuses light scattered by particles onto the light detector.

15. Apparatus as claimed in claim 13, wherein the stop is provided adjacent the light detector so that active area of the light detector only receives light scattered by particles.

16. Apparatus as claimed in claim 13, and including a mirror having a central aperture through which unscattered light emitted by the light source can pass, the mirror reflecting light scattered by particles onto a lens for focusing the scattered light onto the light detector.

17. Apparatus as claimed in claim 13, and including a mirror having a central aperture through which unscattered light emitted by the light source can pass, the mirror being curved to act as a lens to focus light scattered by particles onto the light detector.

18. Apparatus as claimed in any one of claims 6 to 17, and including means for deriving particle size by plotting the mean peak signal as a function of the normalised peak-to-trough variation in the output pulses generated by the passage of the particle through the light field.

FIG.1

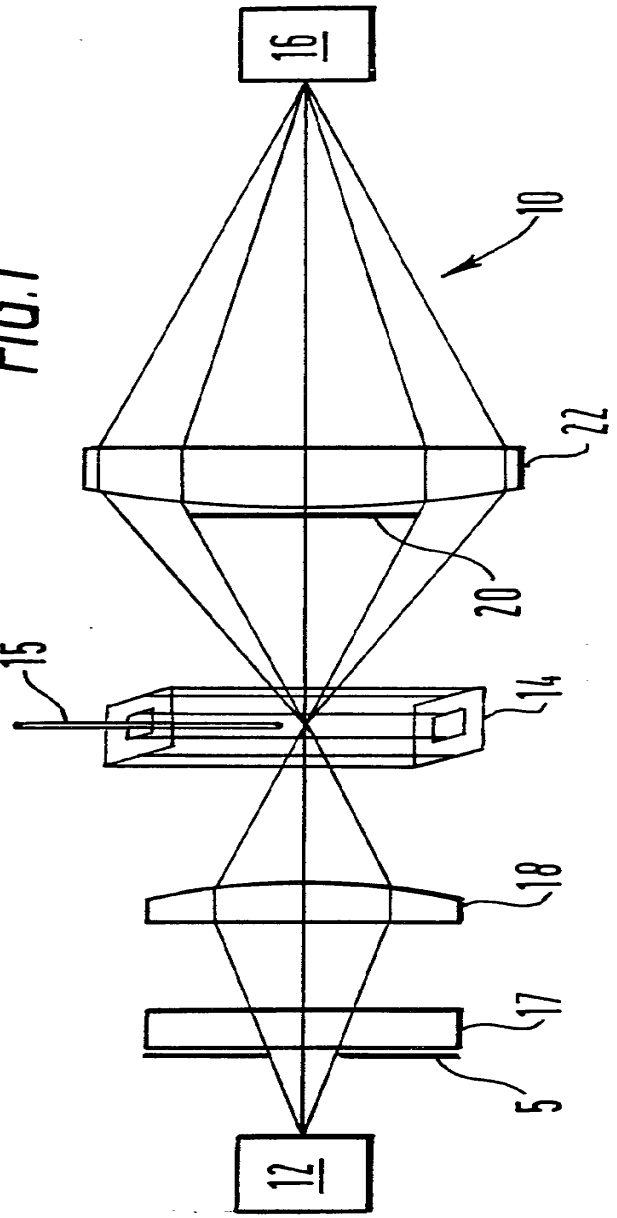


FIG.1A

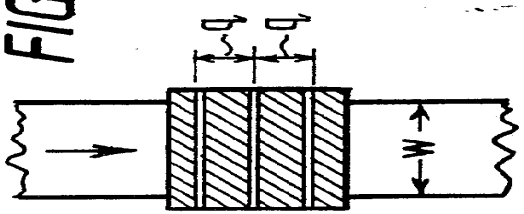


FIG.2

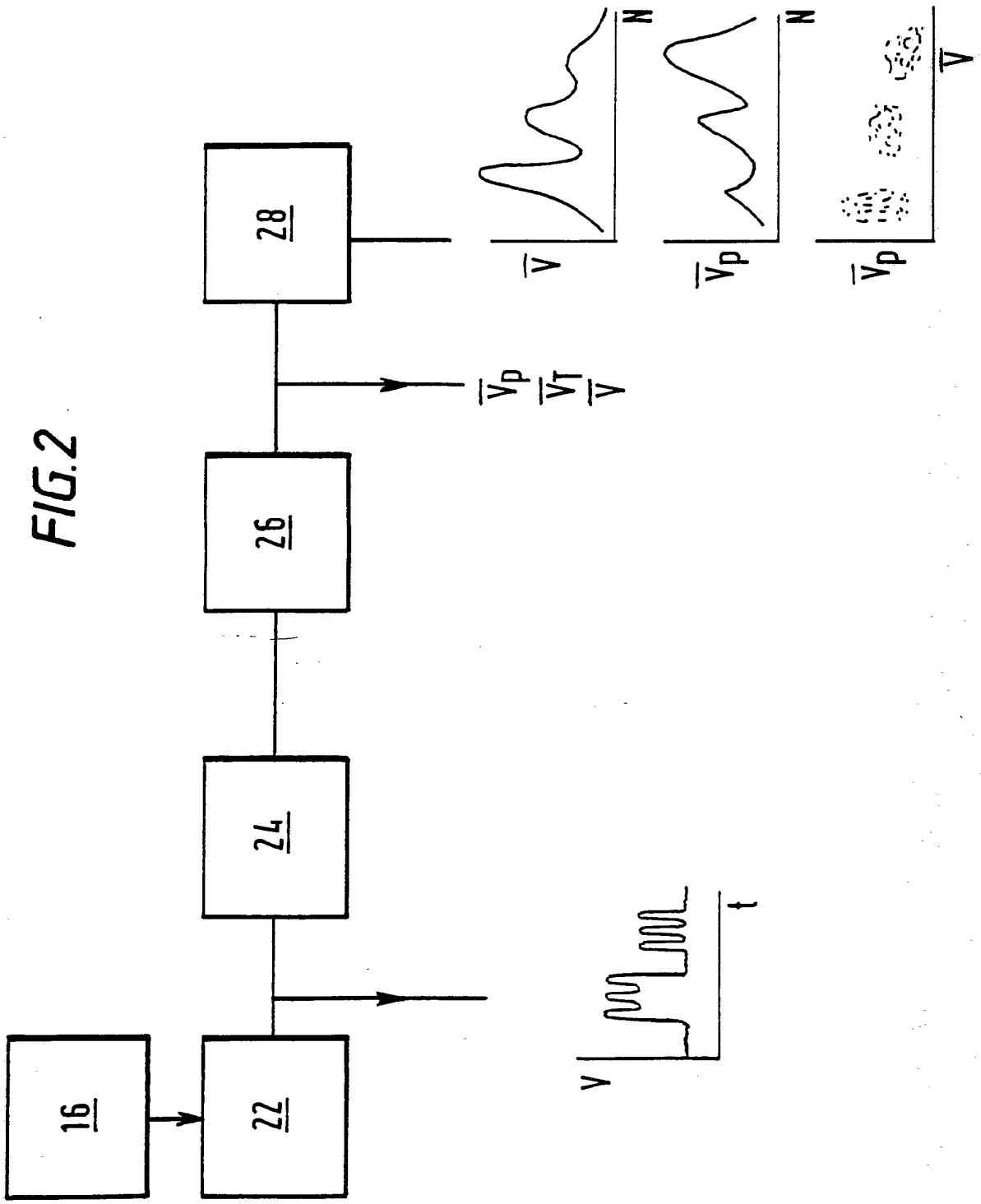


FIG. 3

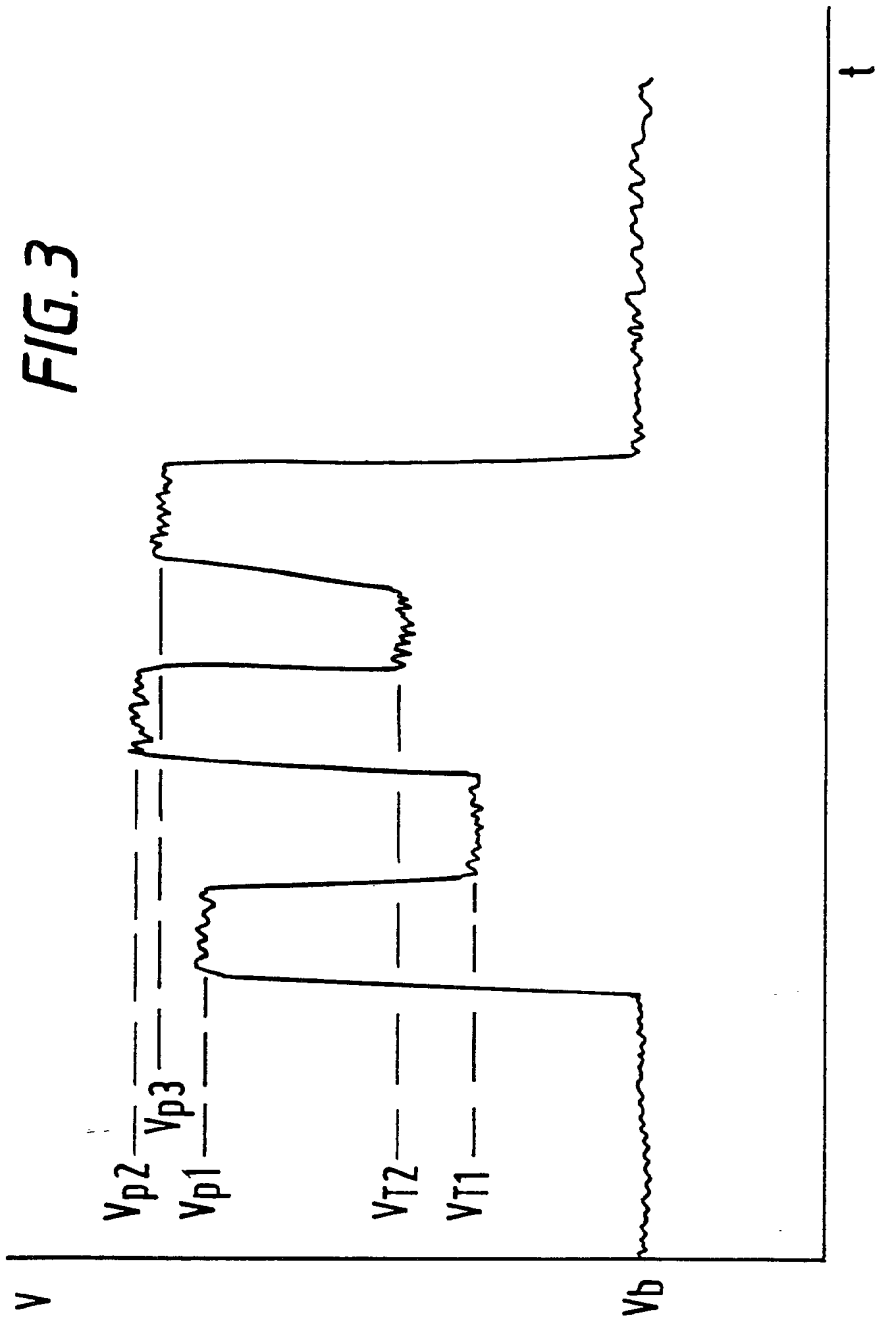
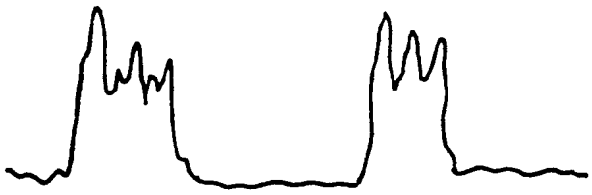


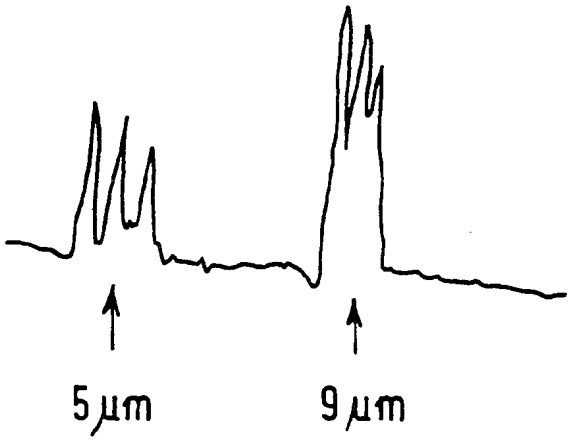
FIG. 4

(a)



2 × 9 μm particles

(b)



5 μm particle

9 μm particle

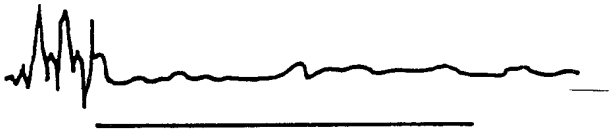
(c)



3μm particle

FIG. 4

(d)  3 μm particle

(e)  1 μm particle

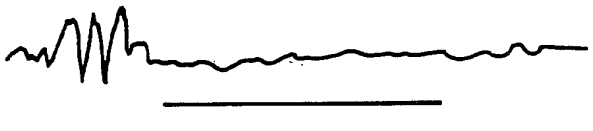
(f)  1 μm particle

FIG.5

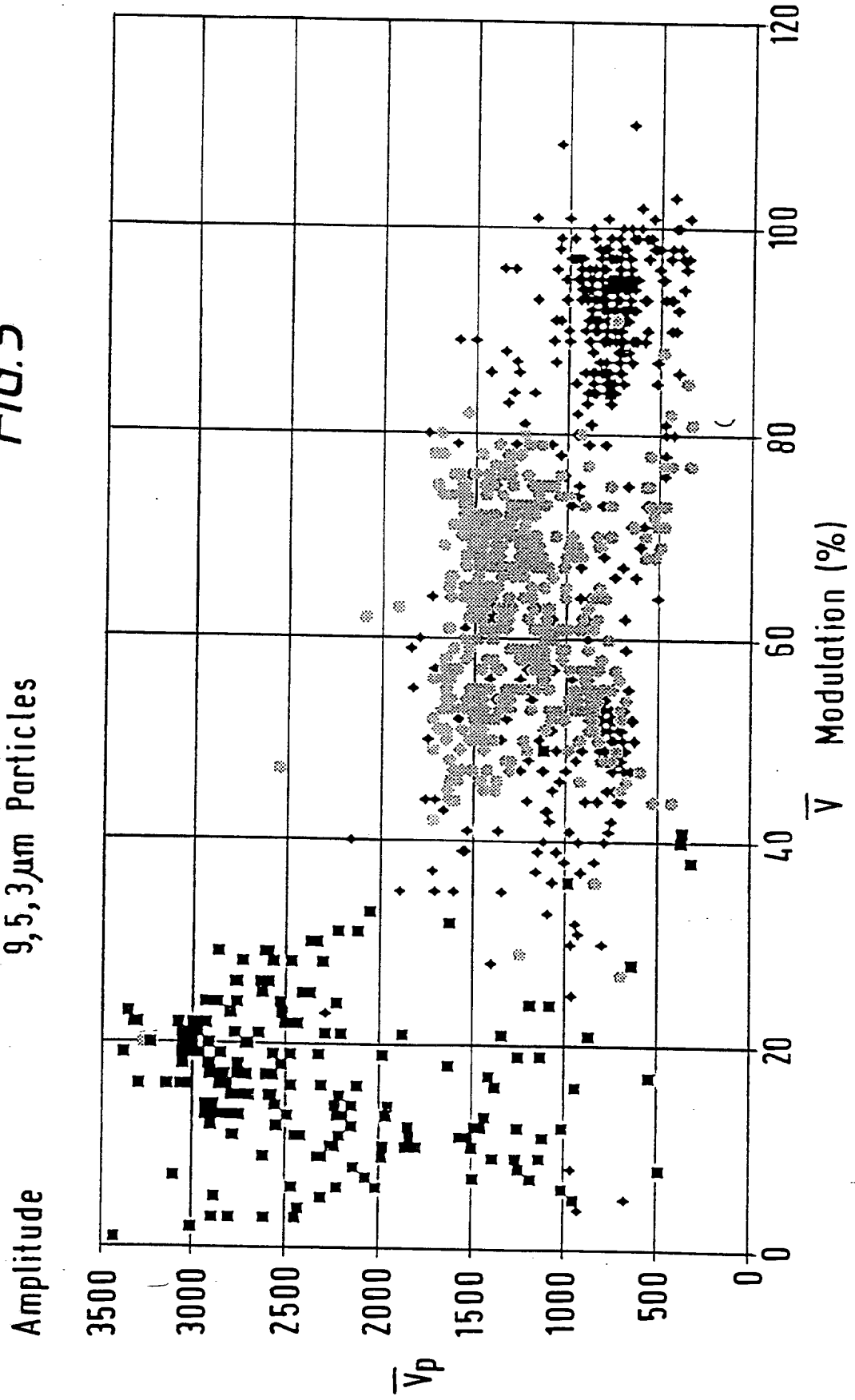
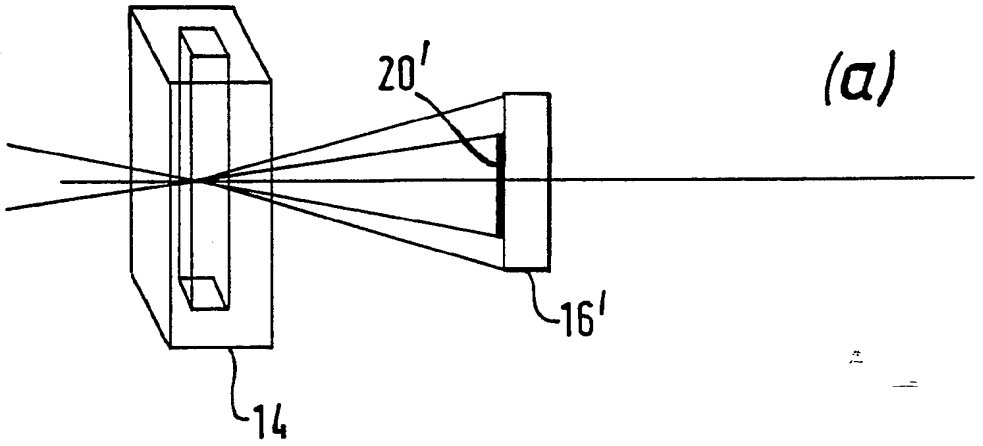
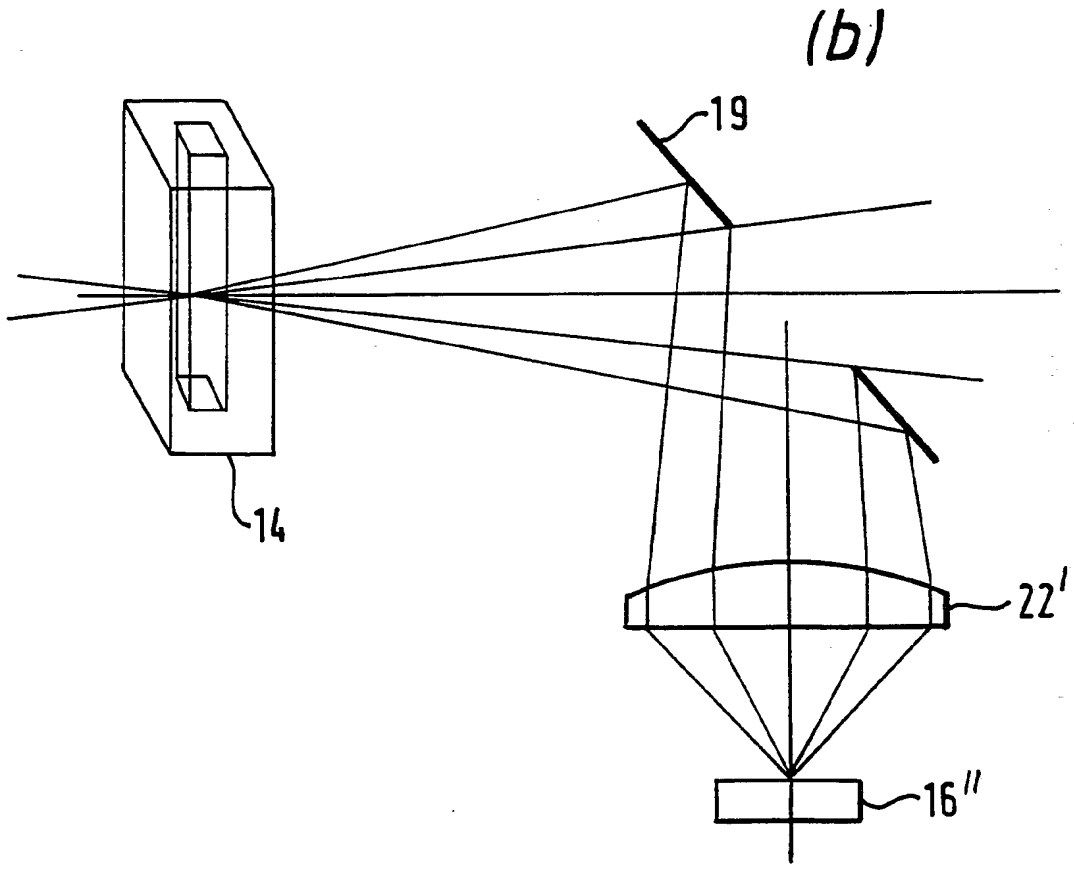

Amplitude
9,5,3μm Particles
3500
3000
2500
2000
1500
1000
500
0
$\overline{V_p}$
0
20
40
60
80
100
120
$\overline{V}$ Modulation (%)

FIG. 6





INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00289

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N15/14

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
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X	US,A,3 819 270 (T.HIRSCHFELD) 25 June 1974 see column 5 - column 9 ---	1-3,6-8, 10-11, 13-15
X	US,A,4 986 659 (W.BACHALO) 22 January 1991 see column 3 - column 8 ---	1-2,5-8
X	PATENT ABSTRACTS OF JAPAN vol. 12, no. 488 (P-803)(3335) 20 December 1988 & JP,A,63 201 554 (CANON) 19 August 1988 see abstract --- -/--	1-3, 6-11, 13-15

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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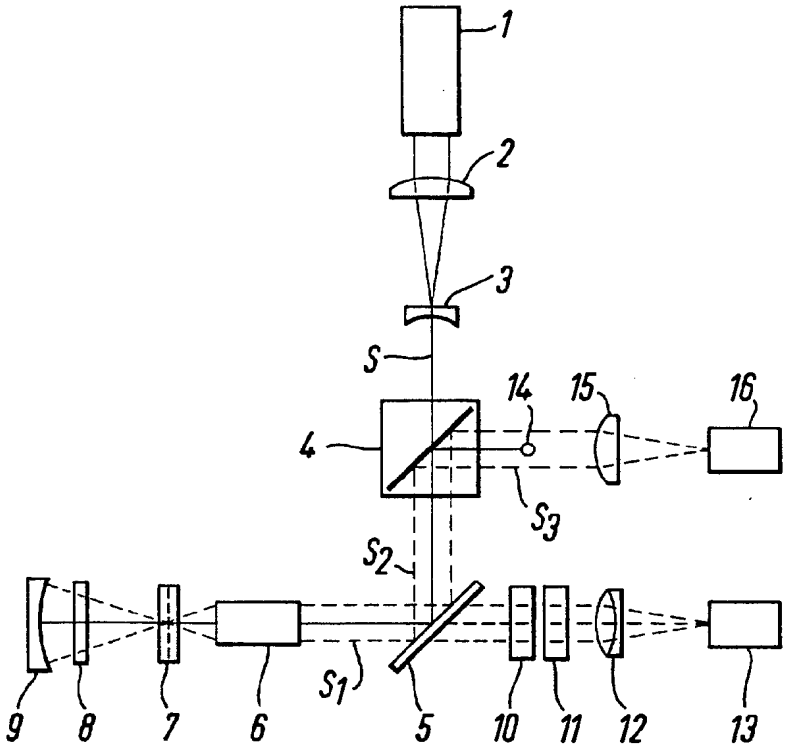
(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
In English translation (filed in Norwegian).

(54) Title: LIQUID FLOW CYTOMETER

(57) Abstract

A liquid flow cytometer for counting and classification of particles such as biological cells and other microscopic particles in liquids, where the cytometer comprises an optical system for irradiation and detection in a metering zone (7), thus enabling optical dispersion and fluorescence caused by the particles to be simultaneously detected when the particles pass the metering zone. The optical system comprises in a first beam path (S_1) a mirror reflector (9) with its focal point in the metering zone (7), a quarter-wave plate (8) between the metering zone (7) and the mirror reflector (9), a lens (6) with its focal point in the metering zone (7) and located on the opposite side of it together with a dichroic mirror (5) behind the lens and provided in such a manner that it gives reflection to a second beam path (S_2). In the second beam path (S_2) a beam splitter (4) is provided between the dichroic mirror (9) and a known per se light source (1), and produces a third beam path (S_3). The cytometer comprises a firt detector (13) provided in the first beam path (S_1) behind the dichroic mirror (5) and arranged for the detection of fluorescence emitted by the particles in the metering zone (7), together with a second detector (16) provided in the third beam path (S_3) and arranged for the detection of optical dispersion caused by the particles in the metering zone (7).



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Liquid flow cytometer

The invention concerns a liquid flow cytometer for counting and classification of particles such as biological cells or other microscopic particles in liquids, where the cytometer comprises an optical system for irradiation and detection in a metering zone, thus enabling optical dispersion and fluorescence caused by the particles to be simultaneously detected when the particles pass the metering zone.

The use of cytometers based on optical dispersion is known in the prior art for counting and classification of microscopic particles in a liquid. This prior art is illustrated schematically in figs. 1-3, where fig. 1 shows the flow system with a flow cell, fig. 2 shows a hydrodynamic focusing device assigned to the flow cell, and fig. 3 shows the detector device for optical dispersion caused by particles in the flow cell. In fig. 1 the sample is fed into a flow cell together with a transport liquid in the form of filtered water which is pumped into the flow cell from a container. The transport liquid and the sample, i.e. those particles which have to be counted and classified pass through the flow cell, a pump system and a filter which removes particles, after which the transport liquid, i.e. filtered water, is passed back to the container. In the flow cell there is provided a hydrodynamic focusing device in flow connection with the actual flow cell which is composed of a capillary tube. The hydrodynamic focusing device is in the form of a conical nozzle which tapers towards and leads into the capillary tube. The sample is passed into the nozzle through a thin tube in the centre of the nozzle and is carried along with the transport water in such a manner that the sample is accelerated towards the mouth of the nozzle and the capillary tube. By directing a focused beam of preferably monochromatic, coherent light onto the flow cell, the particles in the liquid will cause a scattering or dispersion of the light. The scattered light is focused by a lens device on to a photodetector and the intensity of the detected, scattered light will be a measure of the degree of dispersion which in turn is related to the number of particles in the metering zone and their size. In order to prevent the light from the laser beam from entering the detector there is positioned between the flow cell and the focusing device a beam stop which forms a shield against the direct radiation from the laser, but which substantially does not impair the scattered light.

A cytometer which is substantially based on the above-mentioned principles and is suitable for detection, counting and classification of biological cells and other microscopic particles in liquids is known from Norwegian patent application No. 91 424, which hereby is incorporated as reference.

There are also known cytometers and similar instruments for the detection of particles based on the use of fluorescence which is emitted by the particles when they are irradiated with light from a light source. One example of a detector of this kind is known from EP-A1-0539 743, in which there is disclosed a collimator in the form of a parabolic reflector 40 which permits a more efficient use of bandpass filters in order to block scattered radiation during detection.

The need for stable, reliable and sensitive analytical instruments for the determination of microbial and cellular content in liquids such as water, beverages, oils and bodily fluids is constantly increasing.

By means of liquid flow cytometry biological particles which are in suspension can be counted and classified in a rapid and reliable manner. As mentioned above, according to this method the particle sample is fed along with a transport liquid and care is normally taken to ensure that the particles pass substantially one at a time through the flow cell where they are subjected to the impact of an intense beam of light, the result of which is that the particles can be counted individually and measured with regard to light scatter and fluorescence. At present, liquid flow cytometry is routinely employed principally for the analysis of white blood cells (leukocytes), and in the field of bacteriology liquid flow cytometry has only been used for research purposes.

Cells such as leukocytes are very large in proportion to microbes such as, e.g., enterobacteria. Consequently the requirement for stability and sensitivity for a liquid flow cytometer which is to be routinely employed in the field of microbiology is therefore much greater than for a cytometer for use in haematology.

Thus a first object of the present invention is to provide a liquid flow cytometer which is sufficiently stable and sensitive to enable it to be generally employed in the field of microbiology, but which can naturally also be used in the known manner for counting and classification of blood cells in mammals.

A second object of the present invention is to provide a liquid flow cytometer which provides an improved irradiation of the particles and a better collection both of scattered light and fluorescent light caused by the particles, thus enabling the sensitivity to be increased.

A further object of the present invention is to provide a liquid flow cytometer which can be used simultaneously for the detection of both optical dispersion and fluorescence, without the need for a complicated optical system for this purpose.

Yet another object of the present invention is therefore to simplify the optical system and to integrate detectors and optical system, thus providing a liquid flow cytometer with fewer optical components and reduced physical dimensions, which in turn will help to improve the instrument's stability and also lead to lower production costs.

The above-mentioned and other objects are achieved according to the present invention with a liquid flow cytometer which is characterized in that the optical system in a first beam path comprises a mirror reflector with its focal point in the metering zone, a quarter-wave plate between the metering zone and the mirror reflector, a lens with its focal point in the metering zone and located on the opposite side of it, together with a dichroic mirror behind the lens and arranged in such a manner that it gives a partial reflection substantially orthogonally to the axis of the lens, the reflection direction being in a second beam path of the optical system, and in the second beam path a beam splitter located between the dichroic mirror and an as per se known light source, the beam splitter producing a third beam path substantially orthogonally to the second beam path, that the cytometer further comprises a first detector provided in the first beam path behind the dichroic mirror and arranged for the detection of fluorescence emitted by the particles in the metering zone, together with a second detector provided in the third beam path and arranged for the detection of optical dispersion caused by the particles in the metering zone.

Further features and advantages are presented in the attached independent claims.

The fluid flow cytometer according to the present invention will now be described in more detail in connection with an embodiment and with reference

to the drawing, where figs. 1-3 as mentioned above illustrate the prior art, while fig. 4 schematically illustrates an optical system for a fluid flow cytometer according to the present invention, and fig. 5 shows in rather more detail those components which form part of the fluid flow cytometer in fig. 4.

The optical system of a fluid flow cytometer according to the present invention is illustrated in fig. 4. Light from a laser 1 is focused and collimated to a beam S which passes through a beam splitter 4 in a beam path S_2 and is reflected by a dichroic filter or mirror 5 through the metering zone or flow cell 7 which here is in the form of a capillary tube and into an excitation amplifier. The capillary tube 7 and excitation amplifier 9 are located in an additional beam path S_2. The laser beam S passes through the flow cell 7 and returns from the excitation amplifier 9, since it is focused on the flow cell 7 by the excitation amplifier 9, and a lens 6 provided between the dichroic mirror and the flow cell respectively. Thus the laser beam passes twice through the flow cell and any particles in this will now cause a light scatter and possibly also fluoresce. The fluorescent light passes through the dichroic mirror and continues in the beam path S_1 to a fluorescence detector, while the scattered light is reflected back to the beam path S_2 and on to the beam splitter where it is exposed to a further reflection of yet another beam path S_3 and ends in a detector for light scatter.

With this embodiment the object is achieved that two different detection principles can be employed in one and the same optical system, while at the same time an intensification is achieved of the light which is used for the two detection mechanisms.

The liquid flow cytometer according to the present invention is illustrated in some more detail in fig. 5 where the three beam paths are indicated in the same way as in fig. 2. A light source 1, preferably a diode laser, emits a monochromatic, coherent beam S of polarized light and this beam S is focused and collimated by a lens device 2,3, which comprises a planoconvex lens 2 and planoconcave lens 3. The beam S passes through a polarizing beam splitter 4 which can be designed as a cubiform body and strikes a dichroic mirror or filter 5 which produces a beam path S_1, substantially orthogonally to the beam path S_2. The laser beam S reflected by the dichroic filter 5 is focused on the metering zone or flow cell 7 by a lens 6 which can be an ordinary microscope objective and thereafter passes a quarter-wave plate 8 located in the beam path S_1, which plate causes a polarizing rotation of the laser light by $\pi/4$. The light then strikes

an excitation amplifier in the form of the mirror reflector 9 which like the lens 6 has its focal point in the metering zone 7, the mirror reflector 9 which can preferably be a spherical concave mirror, once again reflecting the light through the quarter-wave plate 8, where it once more receives a polarizing rotation of $\pi/4$ and passes through the metering zone 7. The light from the laser 1 has thereby passed twice through the metering zone 7 and each time is scattered by the particles in the metering zone or causes these to emit fluorescent light. Both the scattered light and the fluorescent light pass through the lens 6 and strike the dichroic mirror 5. The scattered light which is rotated 90° in relation to the laser beam's polarization angle, is reflected by the dichroic mirror 5 and passed back along the beam path S_2 to the beam splitter 4 where due to the altered polarization plane it is reflected to a further beam path S_3. In the beam path S_3 there is provided a beam stop 14 which causes the narrow, collimated laser beam to be blocked, while the scattered light passes substantially unimpaired to a lens device 15, e.g. in the form of a planoconvex lens with a long focal length, which focuses the scattered light on a detector 16 for detection of the optical dispersion. A photodiode, for example a PIN diode, is preferably used as the detector 16.

If those particles which have to be detected are fluorescent, they will as mentioned also emit fluorescent light. If particles are not intrinsically fluorescent, they can be tagged with one or more fluorochromes which have an absorption corresponding to the wavelength of the light source and in both cases the particles will fluoresce when they pass through the metering zone 7 and are irradiated by the laser beam. The fluorescent light is intercepted directly by the lens 6 and indirectly via the mirror reflector 9. The fluorescent light will have a longer wavelength than the excitation light, i.e. the laser beam, and passes unreflected through the dichroic mirror 5 and continues on the beam path S_1 to a fluorescence detector 13 which preferably employs a photodiode and particularly a silicon avalanche diode. In order to eliminate any remains of scattered light which have passed through the dichroic mirror 5 and continued on the beam path S_1, there are provided optical filters 10,11 in the beam path S_1, between the dichroic mirror 5 and a lens device 12 which focuses the fluorescent light on the detector 13. The optical filters 10,11 have a passband which lets through the fluorescent light which has a longer wavelength than the laser light. The lens device 12 for focusing can preferably be an achromatic lens with a long focal length.

Since both the scattered light and the fluorescence from the particles which have to be detected are directly intercepted via the lens 6 and indirectly via the mirror reflector 9, thereby passing the metering zone 7 twice, the effective light collection is increased as well as the intensity of both the light dispersion and the fluorescence. It should be noted that the lens 6 will intercept both scattered and fluorescent light in two different polarization planes, the light which has passed through the quarter-wave plate 8 and is reflected back by the mirror reflector 9 being rotated by $\pi/2$ in relation to the laser beam's original polarization plane. This allows the detection to be performed in an optional polarization plane. As mentioned a photodiode in the form of an avalanche diode can be used in the detector 13, this being an advantage if the fluorescent light is weak in addition to making the use of a photomultiplier for light intensification superfluous. It should also be noted that in the design of the liquid flow cytometer according to the present invention, due to the double scattering in the metering zone a scatter signal of high intensity is achieved. Background noise is largely eliminated and a liquid flow cytometer is thus provided which is suitable for use in microbiology and which has the necessary stability, reliability and sensitivity.

Designed according to the present invention, the liquid flow cytometer has small dimensions and is simple to use. The analysis time is less than 10 s and the sample volume normally used is approximately 0.5 ml.

The liquid flow cytometer according to the present invention is suitable for analysis and counting of biological microparticles with diameters from 0.2-30μ in concentrations of between 10^3 and 10^7 pr. ml.

PATENT CLAIMS

1. A liquid flow cytometer for counting and classification of particles such as biological cells or other microscopic particles in liquids, where the cytometer comprises an optical system for irradiation and detection in a metering zone (7), thus enabling optical dispersion and fluorescence caused by the particles to be simultaneously detected when the particles pass the metering zone, characterized in that the optical system in a first beam path (S_1) comprises a mirror reflector (9) with its focal point in the metering zone (7), a quarter-wave plate (8) between the measuring zone (7) and the mirror reflector (9), a lens (6) with its focal point in the metering zone (7) and located on the opposite side of it together with a dichroic mirror (5) behind the lens and provided in such a manner that it gives a partial refection substantially orthogonally to the axis of the lens, the reflection direction being in a second beam path (S_2) of the optical system, and in the second beam path (S_2) a beam splitter (4) located between the dichroic mirror (5) and a known per se light source (1), the beam splitter (4) producing a third beam path (S_3) substantially orthogonally to the second beam path (S_2), that the cytometer further comprises a first detector (13) provided in the first beam path (S_1) behind the dichroic mirror (5) and arranged for the detection of fluorescence emitted by the particles in the metering zone (7), together with a second detector (16) provided in the third beam path (S_3) and arranged for the detection of optical dispersion caused by the particles in the metering zone (7).

2. A cytometer according to claim 1, characterized in that the mirror reflector (9) is a spherical concave mirror.

3. A cytometer according to claim 1, characterized in that the beam splitter (4) is a polarizing beam splitter.

4. A cytometer according to claim 3, characterized in that a suitable lens device (2,3) is provided in the second beam path (S_2) between the polarizing beam splitter (4) and the light source (1) for focusing of light from the light source (1) on to the beam splitter (4).

5. A cytometer according to claim 4, characterized in that the light source (1) is a diode laser provided in the second beam path (S_2).

6. A cytometer according to claim 5, characterized in that between the first detector (13) and the dichroic filter (5) there is provided an optical filter (10,11) together with a suitable lens device (12) for focusing of fluorescent light which passes the dichroic mirror (5) and on to the first detector (13).

7. A cytometer according to claim 6, characterized in that the first detector (13) comprises a photo diode, preferably in the form of an avalanche diode.

8. A cytometer according to claim 1, characterized in that between the second detector (16) and the beam splitter (4) there is provided a beam stop (14) and a suitable lens device (15) for focusing of scattered light reflected by the beam splitter (4) and on to the second detector (16).

9. A cytometer according to claim 7, characterized in that the second detector (16) comprises a photo diode, preferably in the form of a PIN diode.

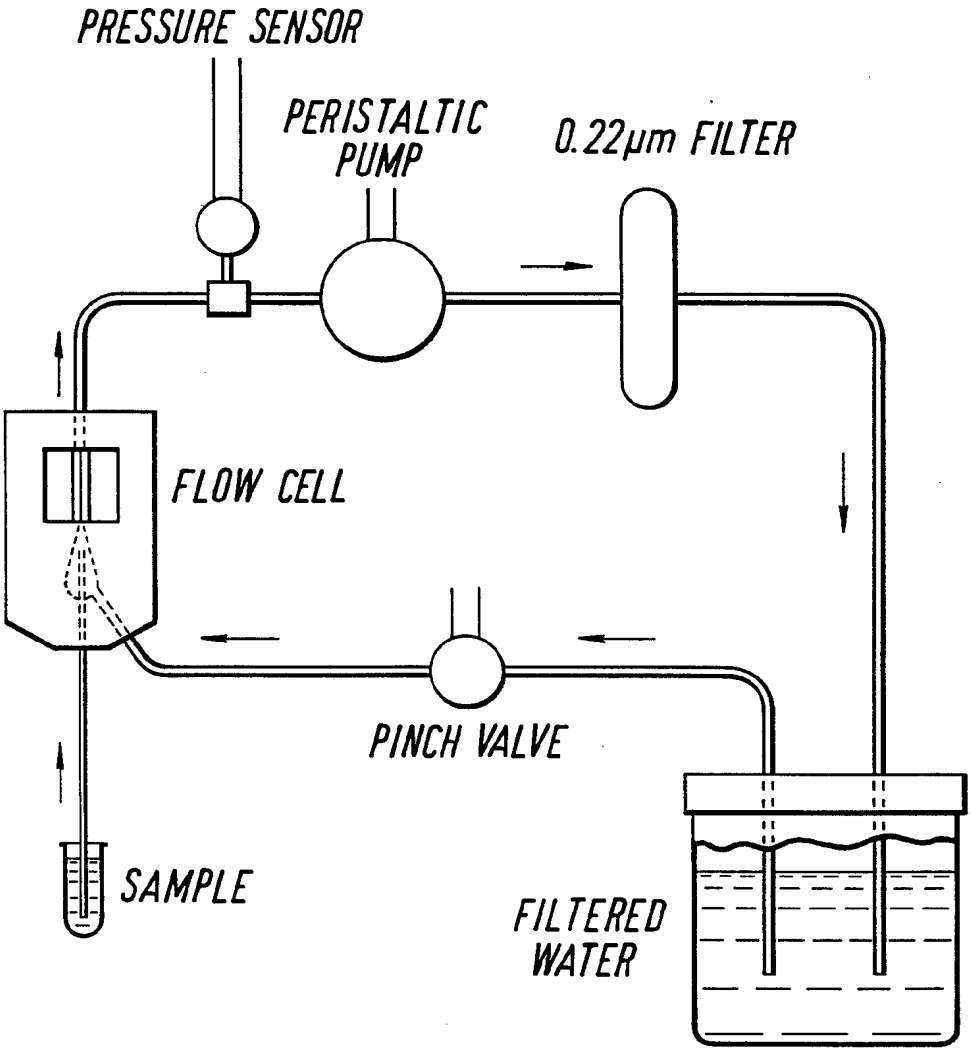


Fig. 1 (PRIOR ART)

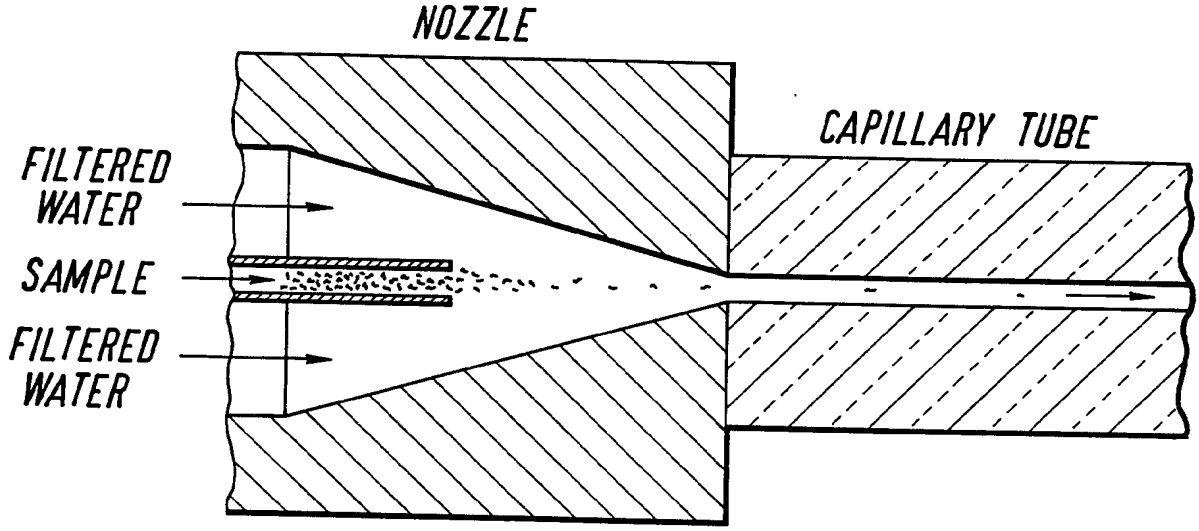


Fig.2 (PRIOR ART)

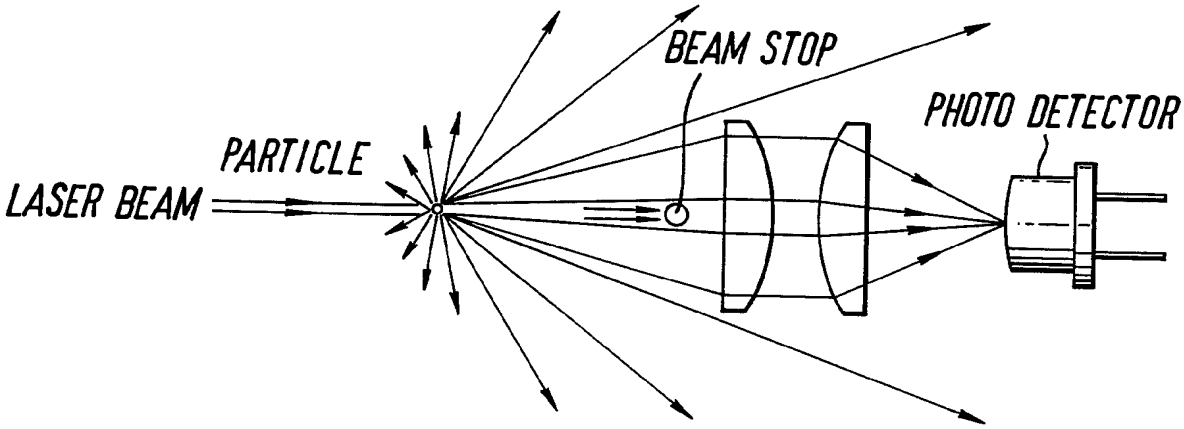


Fig.3 (PRIOR ART)

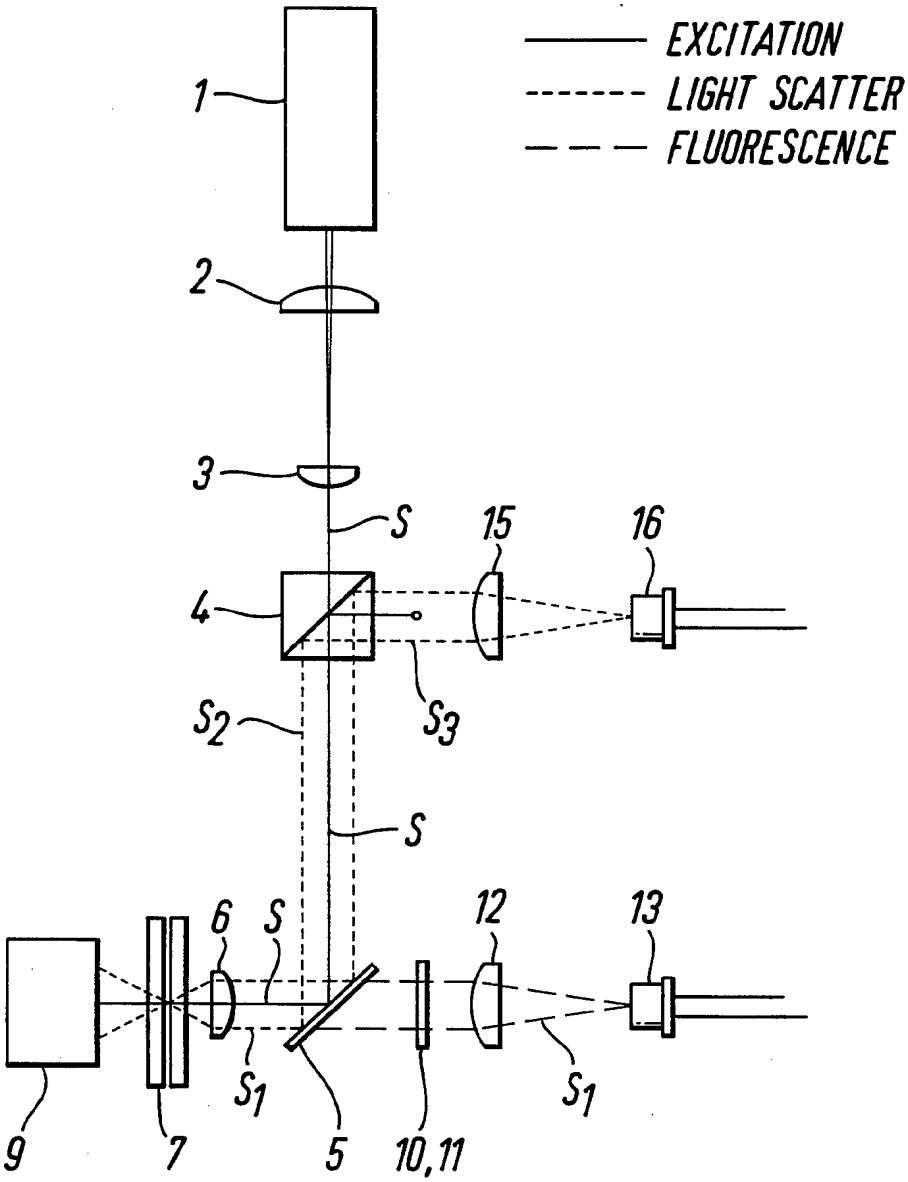

EXCITATION
LIGHT SCATTER
FLUORESCENCE
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S
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S
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S
12
13
S1
S1
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10,11

Fig. 4

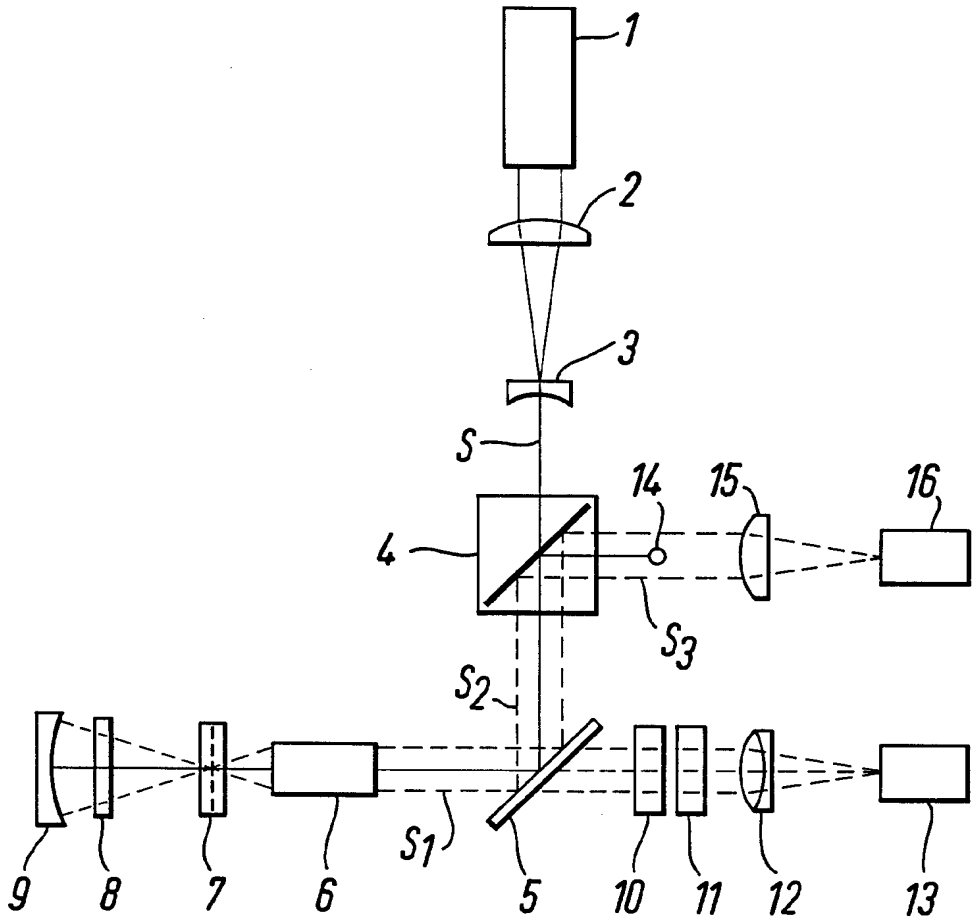


Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 94/00106

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: G01N 15/14, G01N 21/47, G01N 21/64
According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)

IPC5: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	EP, A2, 0538551 (TOA MEDICAL ELECTRONICS CO., LTD.), 28 April 1993 (28.04.93), column 5, line 1 - line 46 --	1-9
A	GB, A, 2238612 (SKATRON A/S), 5 June 1991 (05.06.91) --	1-9
A	EP, A1, 0442025 (TOA MEDICAL ELECTRONICS CO., LTD.), 21 August 1991 (21.08.91) --	1-9

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
26 Sept 1994	27 -09- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Bertil Dahl Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

27/08/94

International application No.
PCT/NO 94/00106

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GB-A- 2238612	05/06/91	BE-A- 1004550	08/12/92
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		JP-A- 5209832	20/08/93
		US-A- 5292483	08/03/94

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(71) Applicant: SDL, INC. [US/US]; 80 Rose Orchard Way, San Jose, CA 95134 (US).

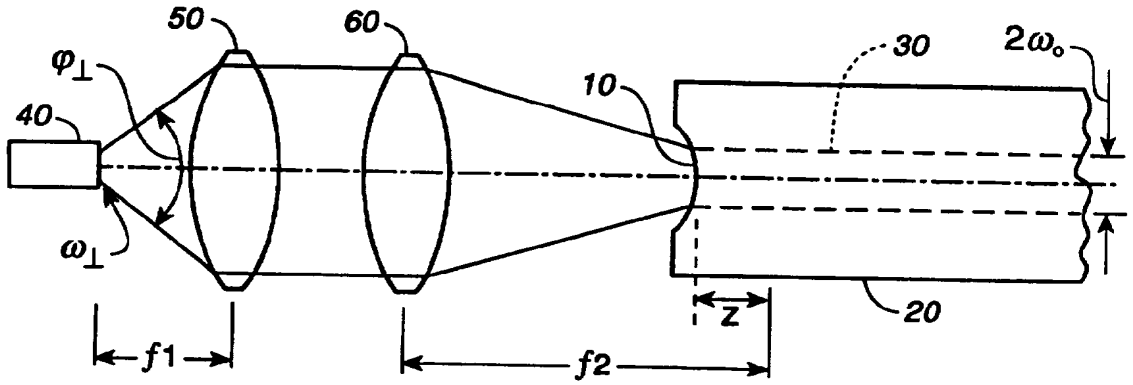
(72) Inventor: OLESKEVICH, Tanya; 89 Moss Street, Victoria, British Columbia V8V 4O2 (CA).

(74) Agent: CAROTHERS, W., Douglas, Jr.; SDL, Inc., 80 Rose Orchard Way, San Jose, CA 95134 (US).

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(54) Title: METHOD FOR IMPROVING THE COUPLING EFFICIENCY OF ELLIPTICAL LIGHT BEAMS INTO OPTICAL WAVEGUIDES



(57) Abstract

An optical coupling system for improving the coupling efficiency of an elliptical light beam into optical fibre (20) consists of a cylindrical concave microlens (10) on the end facet of the optical fibre (20) in conjunction with a pair of bulk optic asphere lenses (50 and 60). A method of producing a cylindrical concave microlens (10) according to the invention consists of translating a fine wire (100) across the end facet of an optical fibre (20) so as to create a cylindrical groove (10).

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METHOD FOR IMPROVING THE COUPLING EFFICIENCY OF ELLIPTICAL LIGHT BEAMS INTO OPTICAL WAVEGUIDES

TECHNICAL FIELD OF THE INVENTION

The present invention relates to improving the coupling efficiency of elliptical light beams to circular optical wave guides and specifically to an optical coupling system comprising a concave cylindrical microlens on the end facet of an optical fibre in conjunction with bulk optic lenses, and to a method of fabrication of the concave cylindrical microlens.

BACKGROUND ART

Efficient coupling between a laser diode source and an optical fibre is essential for optimal performance in optical communication, laser surgery and fibre optic sensing devices. Two techniques to launch the light from semiconductor diode lasers into optical fibre have been documented extensively in the literature: butt coupling, in which the optical fibre is brought in direct mechanical contact with the laser diode, and lensing, in which one or more lenses are used to collimate and focus the laser light into the fibre. Because the core diameter of a single-mode fibre is small (5 μm-10 μm) and the numerical aperture is low (typ. 0.14), and the laser diode has large beam divergence angle (30°) perpendicular to the junction plane, a high coupling efficiency between laser and fibre cannot be obtained by a butt joint. Further, the alignment tolerance of the laser relative to the fibre axis is as small as 1 μm in this configuration and difficult to control in a production environment.

Lensing systems provide significantly improved coupling as the beam parameters of the laser diode may be modified to better match those of the optical fibre. In a production environment, stable coupling efficiencies of 65% are typical for an optimized pair of high quality bulk optic aspheric lenses. The main cause for the 2 dB loss in coupling is due to symmetrical circular profile of the optical fibre. To address this issue a third, cylindrical lens is often introduced between the two aspheres. The increase in alignment complexity and mechanical tolerancing associated with this solution, however, prove to be uneconomical. A lens system design which corrects the modal mismatch between an elliptical light beam and the circular waveguide structure of an optical fibre is required if

substantial improvements in coupling efficiency from single lateral mode diode lasers into single-mode fibres is to be realized.

SUMMARY OF THE INVENTION

The present invention significantly improves the coupling efficiency of lensed systems for elliptical light beams into circular waveguides. The preferred embodiment of the invention relates to the coupling efficiency between laser diodes and optical fibre but the principles thereof are equally applicable to any elliptical beam and circular waveguide. In the preferred embodiment, a pair of bulk optic, rotationally symmetric, asphere lenses is supplemented by a third, concave, cylindrical microlens fabricated on the end facet of the optical fibre. The microlens is designed to correct the more highly divergent, or fast axis of the elliptical beam typically emitted by laser diodes. The difference in the divergence angle of the diode laser beam perpendicular ($\theta_{\perp}$) and parallel ($\theta_{||}$) to the junction plane is known as the aspect ratio of the beam and it is this ratio which dictates the optimum radius of curvature of the microlens. The purpose of the cylindrical microlens is to circularize the focused beam. The microlens collimates the fast axis of the laser beam at the point in the focal plane of the focusing asphere where the beam diameters of the fast ($\theta_{\perp}$) and slow ($\theta_{||}$) axes are matched. In effect, the elliptical beam is circularized upon entry into the optical fibre. The mode profiles of the light beam and the fibre waveguide are much better matched as a result, and the coupling efficiency into the optical fibre is substantially improved.

More particularly, the invention is an optical coupling system comprising a light source, a lens system and a circularly symmetric optical waveguide which has an end facet. The light source emits a substantially elliptical beam having a major and a minor axis. The light beam is more highly divergent in a first plane perpendicular to the major axis than in a second plane parallel to the major axis. The lens system comprises at least one rotationally symmetric bulk optic lens. A concave cylindrical microlens is fabricated on the end facet of the waveguide.

In another aspect, the invention is an optical coupling system as described in the previous paragraph wherein the elliptical light source is a laser diode emitting in a single elliptical spatial mode. The lens system comprises two rotationally symmetric built optic asphere lenses. The concave cylindrical

microlens has a spherical radius of curvature and the waveguide is an optical fibre which is single mode at the emission wavelength of the laser diode.

The method of fabricating the concave cylindrical microlens according to the invention comprises moving a fine wire in contact with and relative to the end facet, whereby friction between the wire and the end facet creates a concave, cylindrical groove in said end facet.

BRIEF DESCRIPTION OF DRAWINGS

The invention will be more fully appreciated by reference to the following detailed description and to the accompanying drawings, in which:

Figure 1 is a perspective view of a preferred embodiment of the cylindrical microlens according to the invention;

Figure 2A is an optical ray traced diagram for a laser diode emitting through a pair of built optic rotationally symmetric lenses;

Figure 2B illustrates the layout of a laser diode, a pair of bulk optic lenses and an optical fibre including a cylindrical microlens and the effect of the microlens on the optical rays coupled into the fibre; and,

Figure 3 is a side view of apparatus for fabricating a concave cylindrical microlens according to the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

A preferred embodiment of the optical fibre used in the invention is depicted in Figure 1 wherein a concave, cylindrical microlens 10 is polished directly into the end facet of an optical fibre 20. The microlens is centered about the core 30 of the fibre. The optical fibre 20 is provided as part of an optical coupling system or assembly which also includes a laser diode 40 and two asphere rotationally symmetric bulk optic lenses 50 and 60, illustrated in Figure 2B.

The optimum radius of curvature of the cylindrical lens is dictated by the aspect ratio of the elliptical light beam such as, for example, the beam emitted by a laser diode 50 (illustrated in Figure 2A), as well as discussed below. The theoretical derivation of the coupling efficiency which follows is based upon a single-frequency laser diode emitting in an elliptical single spatial mode and exhibiting very little astigmatism. The optical waveguide is that of a standard

telcom fibre with a step-index profile. Losses due to Fresnel reflection and angular or transversal misalignment of any of the components are neglected.

The laser beam emitted from the facet of the diode 40 is substantially elliptical with a major axis and a minor axis. The beam has a Gaussian intensity profile with spot sizes $\omega_{\parallel}$ and $\omega_{\perp}$ parallel and perpendicular to the junction plane, respectively, as shown in Figure 2A. These spot sizes are the beam waist radii ($1/e^2$) and $\omega_{\parallel} \neq \omega_{\perp}$. The ratio $\omega_{\parallel} / \omega_{\perp}$ is known as the aspect ratio and typica $\omega_{\perp}$ ranges from 1.2:1 to 10:1 for state of the art laser diodes. The elliptical beam emitted by the laser diode is more highly divergent in the plane perpendicular to the major axis of the ellipse (i.e., the "fast" axis) than in the plane parallel to the major axis (the "slow" axis).

A pair of rotationally symmetric bulk optic lenses with focal lengths f_1 and f_2, collimate (50) and then focus (60) the laser beam at some point z with transformed spot sizes $\omega_{\parallel}'$ and $\omega_{\perp}'$ and $\omega_{\parallel}' \neq \omega_{\perp}'$. The ray traces and beam waists of both the slow and fast axes of the laser diode, $\omega_{\parallel}$ and $\omega_{\perp}$, respectively, are superimposed in the plane of the page in Figure 2A and depicted in exaggerated scale for clarity. In Figure 2B the effect of the concave cylindrical microlens is illustrated, wherein the fast axis of the laser beam is incident upon the microlens at an axial distance z from the focal plane of the second bulk optic asphere lens. At this position z the beam diameter of the fast axis, $2\omega_{\perp}''$ and the beam diameter of the slow axis, $2\omega_{\parallel}'$, match the mode diameter of the optical fibre, $2\omega_0$.

The laser beam is coupled into a step-index optical fibre, defined as single-mode at the laser wavelength. The power distribution in a step-index single-mode fibre can be approximated by a Gaussian beam if the normalized frequency V of the fibre is in the range $1.9 \leq V \leq 2.4$ where V is given by

$$V = \frac{2\pi a}{\lambda}\sqrt{n_1^2 - n_2^2} \qquad (1)$$

where λ is the wavelength of the optical power, η_1 and η_2 are the indices of the core and cladding, respectively, and a is the radius of the fibre core. The fibre mode radius ω_0 of such a fibre is given by

$$\omega_0 = a\left(0.65 + \frac{1.619}{V^{3/2}} + \frac{2.879}{V^6}\right). \qquad (2)$$

If we position the fibre at the waist of the focused laser beam, then the power coupling efficiency η between the elliptical laser beam and the fundamental mode of the optical fibre is

$$\eta = \eta_{\parallel}\eta_{\perp} \tag{3}$$

where

$$\eta_{\parallel} = \frac{2\omega_{\parallel}'\omega_0}{\left(\omega_{\parallel}'^2 + \omega_0^2\right)}. \tag{4}$$

The same formula holds for $\eta_{\perp}$, if the superscript '$\parallel$' is replace by '$\perp$' in the last equation. The coupling efficiency depends upon the degree of overlap between the waist radii of the focused laser beam and the fibre mode. To maximize η, we match the focused beam waist of the slow axis to the fibre mode, $\omega_{\parallel}'=\omega_0$, by choosing an appropriate pair of lenses and then create a non-symmetric transformation upon the fast axis, $\omega_{\perp}'$, such that $\omega_{\perp}'=\omega_{\parallel}'=\omega_0$. This is achieved with the cylindrical microlens. The radius of curvature of the cylindrical microlens is chose to null the wavefront curvature of the beam at the point where $\omega_{\perp}''=\omega_0$.

The propagation of the focused gaussian laser beam is describe by

$$\omega_{\perp}''(z)^2 = \omega_{\perp}'^2\left[1+\left(\frac{\lambda z}{\pi\omega_{\perp}'^2}\right)^2\right]. \tag{5}$$

To optimize the coupling efficiency the mode fields must match, and so we impose the constraint that $\omega_{\perp}''=\omega_0$ and solve for z. $R(z)$, the radius of curvature of the beam wavefront at this point can then be calculated from

$$R(z) = z\left[1+\left(\frac{\pi\omega_{\perp}'^2}{\lambda z}\right)^2\right]. \tag{6}$$

If the focal length of the microlens is exactly matched, but opposite in sign, to the curvature of the optical beam, the curvature of the beam wavefront is nulled and thus matched to the wavefront of the fibre mode, which is planar, by definition, at the end facet of the fibre.

Classically, for a spherical lens, the radius of curvature and focal length are related by

$$f = \frac{R_L}{n-1} \tag{7}$$

where R_L is the radius of curvature of the microlens and n is the refractive index of the lens material. Optimum coupling will occur when the centre of curvature of the wavefront lies at the focal point of the lens, and we can thus calculate the optimum radius of curvature for the microlens. The optimum radius of curvature of the microlens is therefore given by

$$R_L = (n-1)z\left[1+\left(\frac{\pi\omega_{\perp}^{'2}}{\lambda z}\right)^2\right]. \qquad (8)$$

Effective radii of curvature of the microlens according to the invention are between 5 and 50 μm.

This method of improving the coupling efficiency is applicable to any fibre optic devices which require high coupling efficiency of an elliptical laser beam into optical fibre such as fibre lasers and fibre amplifiers. As an example, light from a 150 mW, single spatial mode diode laser was launched into standard telcom fibre, single mode at the laser wavelength. The aspect ratio of the emitting region of the laser diode was 2.2:1, and the radius of curvature of the microlens was 9 μm. 130 mW of light was captured by the fibre for a coupling efficiency of 87% (ex-facet). This constituted and increase in coupling of 20% relative to a traditional lensing system in which no correction for beam elipticity was provided.

METHOD OF FABRICATION

In the preferred embodiment, the cylindrical concave 10 microlens is polished directly into the polished or cleaved end facet of the optical fibre 20. The microlens is created by polishing the fibre facet with an extremely fine, high tensile strength wire which may vary in diameter from 10 μm to 100 μm with the apparatus illustrated schematically in Figure 3. In the preferred method of fabrication, the optical fibre 20 is fixed in a ceramic ferrule 140 such that the facet to be polished extends beyond the ferrule by approximately 500 μm. The ferrule is then mounted on a precision 3-axis translator 150. The wire 100 is spooled onto a drum 110 which is mounted onto a precision spindle 115. The wire is drawn through a series of guide pins 120 into which microgrooves have been machined to eliminate lateral motion of the wire during the polishing sequence. Tension is

applied to the wire by fixing a free hanging weight 130 to one end of the wire; this ensures a positive, loaded contact through the grooves on the guide pins.

The core of the fibre is carefully centered with respect to the wire (±2 μm tolerance). This alignment is verified visually with the aid of a stereoscope 160. As the wire is dragged across the end facet of the fibre, a cylindrical groove is created in the fibre with a radius of curvature equal to that of the wire. This cylindrical, spherical groove acts as a cylindrical microlens with a focal length equal to the diameter of the wire.

In an exemplary embodiment, a hard temper tungsten wire with a diameter of 50 μm was spooled onto a 2 cm drum on a precision spindle, alignment in the grooves on the guide pins, and tensioned with a 20-gram mass. A bi-directional translation of the wire parallel to its longitudinal axis, of 10 cm, repeated 75 times, produces a cylindrical groove in the end facet of the optical fibre (Corning 1060), typically 20 μm-30 μm in width and 3 μm-5 μm in depth. The translation of the wire is not limited to this translation sequence and those knowledgeable in the art will appreciate that any relative motion between the wire and the fibre, such as for example, bi-directional, axial, sonic, or spinning, or a combination thereof, which creates a concave cylindrical groove in the end facet of the fibre is within the spirit of the invention.

The translation of the wire may be automated. Polishing compounds may be used during the polishing sequence. The optical quality of the surface finish of the polished cylindrical microlens may be improved by flash heating the fibre end facet in the arc of an optical fusion splicer. The end facet of the fibre may be anti-reflection (AR) coated.

The concave cylindrical microlens may also be created in other ways. One method is to "stamp" or emboss the end facet of the fibre with a tool in which a convex ride with an appropriate radius of curvature has been machined. The fibre tip is then heated to just below the softening temperature of silica and the embossing tool is heated to a temperature approximately 100°C-200°C above this. Another method may involve preferential chemical etching of the fibre end facet in a pattern outlined by a mask. Yet another method may involve melting the fibre end facet with an extremely hot wire of the appropriate diameter.

What is claimed is:

1. An optical waveguide having an end facet a cylindrical microlens formed on said end facet.

2. The optical waveguide of claim 1 wherein a radius of curvature of said cylindrical microlens is in the range of about 5 μm to about 50 μm.

3. The optical waveguide of claim 1 wherein said waveguide comprises an optical fibre having a core, said cylindrical microlens is concaved and substantially centered relative to said core.

4. The optical waveguide of claim 1 further comprising a laser beam provided as input into said end facet, a radius of curvature of said cylindrical microlens chosen to null the wavefront curvature of the laser beam at a beam position where orthogonal transverse axes of said laser beam substantially match.

5. The optical waveguide of claim 4 wherein said radius of curvature is dictated by an aspect ratio of said laser beam.

6. The optical waveguide of claim 5 wherein said aspect ratio is in the range of about 1.2:1 to about 10:1 and the radius of curvature is in the range of about 5 μm to about 50 μm.

7. The optical waveguide of claim 4 further comprising a lens system positioned along an optical axis between a source for said laser beam and said microlens, said microlens having a radius of curvature given substantially by

$$R_L = (n-1)z\left[1+\left(\frac{\pi\omega_\perp'^2}{\lambda z}\right)^2\right]$$

where λ is the wavelength of the laser beam, n is the refractive index of the lens system, $\omega_\perp'$ is the beam radius in a first orthogonal transverse direction at the focal plane of said lens system and z is a position along the optical axis where beam diameter of said laser beam in both the first and in a second orthogonal transverse direction are substantially equal to one another.

8. The optical waveguide of claim 7 wherein said source comprises a semiconductor laser diode.

9. The optical waveguide of claim 7 wherein said lens system comprises at least one spherical lens.

10. The optical waveguide of claim 9 wherein said lens system comprises a spherical lens to collimate said beam and a spherical lens to focus said beam to said optical fibre end facet.

11. The optical waveguide of claim 1 wherein said waveguide is an optical fibre.

12. The optical waveguide of claim 11 wherein said optical fibre is a single mode fibre or a multimode fibre.

13. The optical waveguide of claim 1 wherein said laser beam is from a semiconductor laser diode.

14. The optical waveguide of claim 13 wherein said laser is a single mode or multimode laser.

15. An optical waveguide having an end facet for receiving input light, a concave lens formed in said facet end.

16. The optical waveguide of claim 15 wherein said lens is a concave cylindrical lens.

17. The optical waveguide of claim 16 wherein a radius of curvature of said cylindrical lens is in the range of about 5 μm to about 50 μm.

18. The optical waveguide of claim 16 wherein said waveguide comprises an optical fibre having a core, said cylindrical lens is concaved and substantially centered relative to said core.

19. The optical waveguide of claim 15 further comprising a laser beam providing said input light into said end facet, a radius of curvature of said cylindrical lens chosen to null the wavefront curvature of the laser beam at a beam position where orthogonal transverse axes of said laser beam substantially match.

20. The optical waveguide of claim 19 wherein said radius of curvature is dictated by an aspect ratio of said laser beam.

21. The optical waveguide of claim 20 wherein said aspect ratio is in the range of about 1.2:1 to about 10:1 and the radius of curvature is in the range of about 5 μm to about 50 μm.

22. The optical waveguide of claim 15 further comprising a lens system positioned along an optical axis between a source for said laser beam and said lens, said lens having a radius of curvature given substantially by

$$R_L = (n-1)z\left[1+\left(\frac{\pi\omega_\perp'^2}{\lambda z}\right)^2\right]$$

where λ is the wavelength of the laser beam, n is the refractive index of the lens system, $\omega_\perp'$ is the beam radius in a first orthogonal transverse direction at the focal plane of said lens system and z is a position along the optical axis where beam diameter of said laser beam in both the first and in a second orthogonal transverse direction are substantially equal to one another.

23. The optical waveguide of claim 22 wherein said source comprises a semiconductor laser diode.

24. The optical waveguide of claim 22 wherein said lens system comprises at least one spherical lens.

25. The optical waveguide of claim 24 wherein said lens system comprises a spherical lens to collimate said beam and a spherical lens to focus said beam to said optical fibre end facet.

26. The optical waveguide of claim 24 wherein said waveguide is an optical fibre.

27. The optical waveguide of claim 26 wherein said optical fibre is a single mode fibre or a multimode fibre.

28. The optical waveguide of claim 24 wherein said light input is a laser beam is from a semiconductor laser diode.

29. The optical waveguide of claim 28 wherein said laser is a single mode or multimode laser.

30. An optical coupling system for increasing the coupling efficiency between a semiconductor laser source emitting a laser beam and an optical waveguide having an end facet both positioned along a common optical path so that the waveguide end facet receives the laser beam as an input, comprising:

a lens formed on the end facet of said waveguide;

said facet lens positioned in said optical path where the orthogonal transverse axes of the laser beam are substantially the same.

31. The optical coupling system of claim 30 wherein said facet lens is concaved and its radius of curvature is determined by an aspect ratio of the laser beam.

32. The optical coupling system of claim 39 further comprising a lens system positioned along an optical axis between a source for said laser beam and said lens, said lens having a radius of curvature given substantially by

$$R_L = (n-1)z\left[1+\left(\frac{\pi\omega_\perp'^2}{\lambda z}\right)^2\right]$$

where λ is the wavelength of the laser beam, n is the refractive index of the lens system, $\omega_\perp'$ is the beam radius in a first orthogonal transverse direction at the focal plane of said lens system and z is a position along the optical axis where beam diameter of said laser beam in both the first and in a second orthogonal transverse direction are substantially equal to one another.

33. The optical coupling system of claim 30 wherein said facet lens is a cylindrical lens.

34. The optical coupling system of claim 33 wherein said cylindrical lens is a concave-shaped lens in said end facet.

35. The optical coupling system of claim 30 wherein said waveguide is an optical fibre.

36. The optical coupling system of claim 35 wherein said optical fibre is a single mode fibre or a multimode fibre.

37. The optical coupling system of claim 30 wherein said laser beam is from a semiconductor laser diode.

38. The optical coupling system of claim 37 wherein said laser diode is a single mode or multimode laser diode.

39. A method of increasing the coupling efficiency between a semiconductor laser source emitting a laser beam and an optical waveguide both positioned along a common optical path so that the waveguide receives the laser beam as an input, comprising the steps of:

forming a lens on an end facet of the waveguide;

providing a lens system between the laser source and the waveguide end facet for collimating and focusing the laser beam; and

positioning the facet lens in the optical path where the orthogonal transverse axes of the laser beam are substantially the same.

40. The method of claim 39 wherein the lens is concaved and its radius of curvature is determined by an aspect ratio of the laser beam.

41. The method of claim 39 comprising the further step of:

determining the radius of curvature by the formula of:

$$R_L = (n-1)z\left[1+\left(\frac{\pi\omega_\perp'^2}{\lambda z}\right)^2\right]$$

where λ is the wavelength of the laser beam, n is the refractive index of the lens system, $\omega_\perp'$ is the beam radius in a first orthogonal transverse direction at the focal plane of said lens system and z is a position along the optical axis where beam diameter of said laser beam in both the first and in a second orthogonal transverse direction are substantially equal to one another.

42. The method of claim 39 wherein said facet lens is a cylindrical lens.

43. The method of claim 42 wherein said cylindrical lens is a concave-shaped lens in said end facet.

44. The method of claim 39 wherein said waveguide is an optical fibre.

45. The method of claim 44 wherein said optical fibre is a single mode fibre or a multimode fibre.

46. The method of claim 39 wherein said laser beam is from a semiconductor laser diode.

47. The method of claim 46 wherein said laser diode is a single mode or multimode laser diode.

48. A method of fabricating a concave cylindrical microlens on an end facet of an optical waveguide comprising the step of moving a cylindrical medium in contact with and across the end facet so that frictional engagement between the wire and the end facet creates a concave, cylindrical groove in said end facet.

49. The method of fabrication of claim 49 comprising the step of moving the motion of said cylindrical medium bidirectionally and parallel with the longitudinal axis of the wire.

50. The method of fabrication of claim 49 wherein the formed groove is a concave cylindrical microlens with a focal length given by

$$f = \frac{R_L}{n-1}$$

where R_L is the radius of curvature of the microlens and n is the refractive index of the waveguide.

51. The method of claim 48 wherein the waveguide is a single mode fibre and the cylindrical medium is a fine wire.

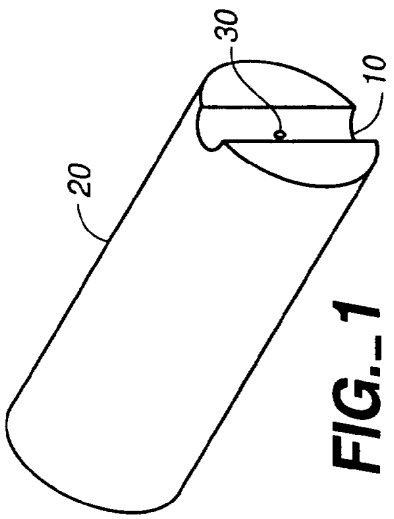


FIG._1

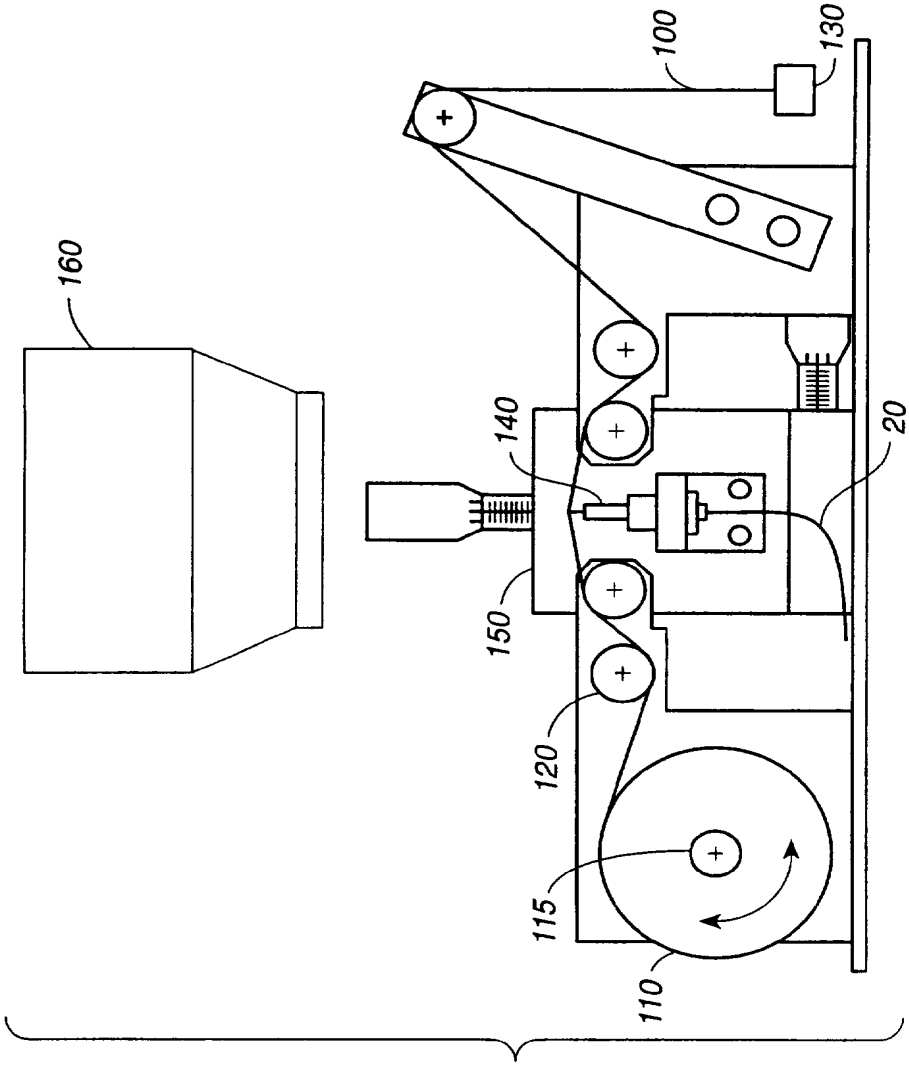


FIG._3

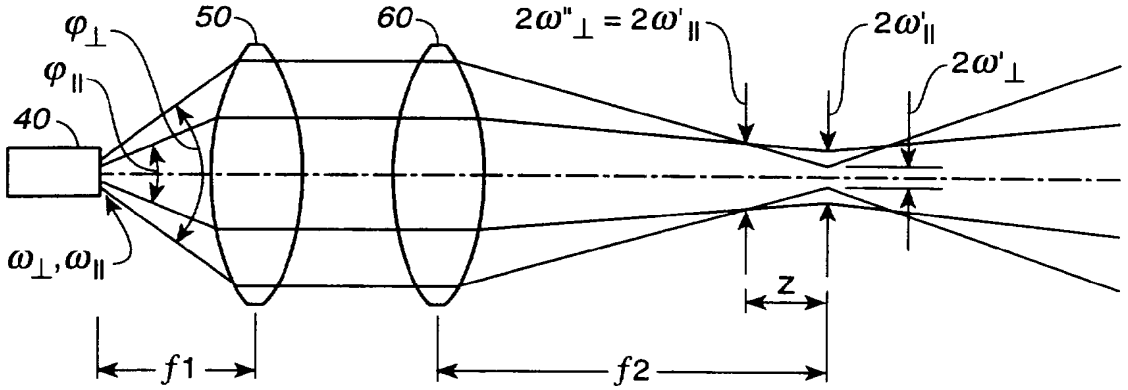


FIG._2A

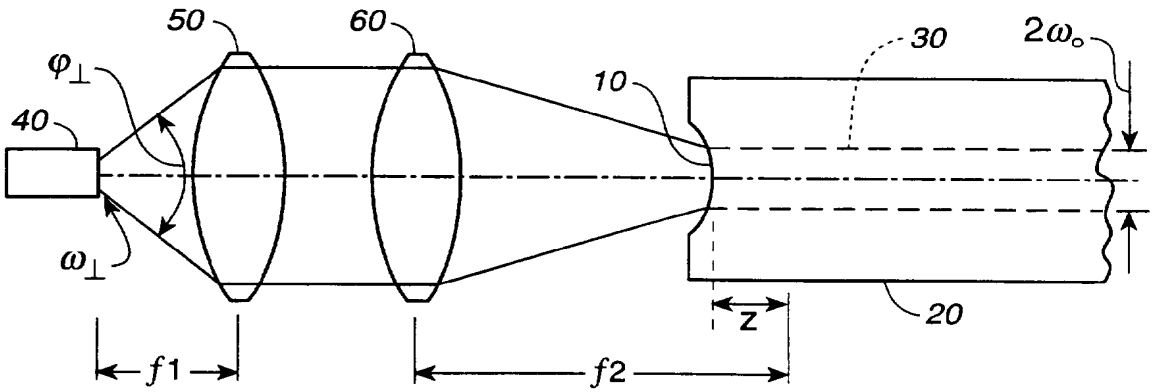


FIG._2B

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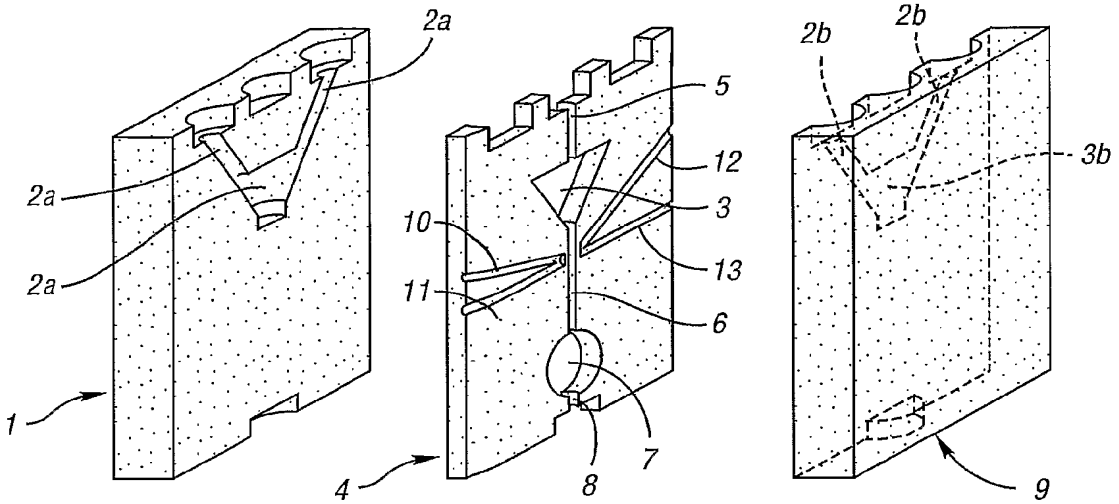
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[Continued on next page]

(54) Title: MICRO FLOW CYTOMETER WITH MULTIANGULAR WAVEGUIDE DETECTORS HAVING SPECTRAL CAPABILITIES



(57) Abstract: A micro flow cytometer having the ability to distinguish light emanating from a particle in the interrogation zone of the cytometer contains a plurality of optical waveguides for receiving emanating light, the optical waveguides positioned at different angles from the axis of the interrogation zone and in the same plane. Photodetectors measuring emanated light provide an electrical signal which is processable to reveal an angular dependence of wavelength with respect to the interrogation channel.

WO 2005/033654 A2

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

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MICRO FLOW CYTOMETER WITH MULTIANGULAR WAVEGUIDE DETECTORS HAVING SPECTRAL CAPABILITIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional application Serial No. 60/498,453 filed August 28, 2003., herein incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention pertains to micro flow cytometers having spectral capabilities.

2. Background Art

Micro flow cytometers are known, for example as described in PCT/US02/22903, incorporated herein by reference. Such flow cytometers are distinguished from conventional cytometers by their small size and inexpensive construction. The small channel sizes of such devices encourage laminar fluid flow, and their small volume requires correspondingly small amounts of analyte and focusing fluids, when the latter are used. In PCT/US02/22903, a unique detection system employing PIN diodes as detectors has been described. By modulating the incident light and amplifying the detector signal synchronously with the frequency of modulation, a detector output having lower noise than conventional photomultiplier tubes is achieved. PCT/US02/22903 also describes the use of multiple wavelengths of light with suitably filtered detectors, so as to detect fluorescence from more than one species.

In PCT published application WO 85/05680 is disclosed a conventional flow cytometer of macroscopic size in which the interrogation channel is interrupted by an orifice plate transverse to the axis of the interrogation channel. The orifice plate contains a plurality of optical waveguides which are perpendicular to the channel. However, WO 85/05680 does not disclose any variation of spectral response at the different waveguides, which rather are for the purpose of gathering a plurality of differing kinds of information based on interaction of particles in the channel with an exciting light source which flows through one of the waveguides.

It would be desirable to provide new modes of detection in micro flow cytometers, particularly enhanced ability to gather wavelength-dependent spectral data. Gathering such data may provide means to further increase detection characteristics such as signal to noise ratio, and to provide information on particles being detected which is not currently available.

SUMMARY OF THE INVENTION

It has now been surprisingly discovered that interrogation waveguides having different angular relationships with the interrogation channel of a micro flow cytometer can provide increased signal to noise ratio in particle detection, as well as allowing for investigating spectral response characteristics. In such devices, a plurality of detectors for a single color, color band, etc. may be used, but at different angles to the flow channel. The detector response exhibits an angular dependence on wavelength.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 illustrates one embodiment of a micro flow cytometer having microgrooves adapted to receive optical waveguides.

FIGURE 2 illustrates a test setup used to investigate detector response in a micro flow cytometer employing red and blue light sources.

FIGURE 3 illustrates a block diagram for detection and signal amplification of fluorescence emitted by a particle in an interrogation zone of a MFC.

FIGURES 4a and 4b represent typical traces from a MFC using a PMT detector and PIN detector with lock-in, respectively.

FIGURE 5 illustrates one relationship between signal to noise ration (S/N) at various detector angles for various colored microspheres.

FIGURE 6 illustrates the dependency of S/N ratio on wavelength at various detector angles.

FIGURE 7 illustrates actual and theoretical S/N variation with different sample column widths.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The micro flow cytometers ("MFC") of the subject invention contain at least one optical waveguide which supplies incident light to an interrogation zone of a MFC, and a plurality of collecting optical waveguides to gather light from the interrogation zone, the collecting optical waveguides positioned at different included angles with respect to the axis of the interrogation zone, and in the plane of the axis of the interrogation zone. The incident and collecting optical waveguides are preferably optical fibers.

The flow cytometer itself may be made of any convenient material, including but not limited to micromachined glass, quartz, silicon, stainless steel, ceramic, etc., injection molded plastics, and the like. However, the material of construction is preferably a cast silicone elastomer, preferably a transparent elastomer. Such MFCs can be readily cast from a micromachined master. They may also be fabricated as multiple layer structures which are subsequently bonded together. One embodiment of such a device is shown in Figure 1, where a three

layer construction is used. In leftmost layer 1, the inner face contains gas channels 2a which communicate with leftmost portion of focusing zone 3a. A middle layer 4 contains the bulk of the focusing zone 3 and a sample fluid passage 5 in communication therewith. The focusing zone communicates with the interrogation zone or "channel" 6 which communicates with the outlet reservoir 7 and outlet passage 8. The rightmost layer 9 is, in this case, a mirror image of leftmost layer 1, and contains gas passages 2b and, rightmost focusing zone portion 3b.

The terminations of the various channels external to the device are advantageously adapted to be single plug-in connectors as are common for supply of fluids, or in the case of the connectors for the light supply and detector passageways, simple snap fittings which allow suitable coupling to external light emitters (lasers, laser diodes, etc.) and detectors (photomultiplier tubes, PIN diodes, etc). In preferred devices, these latter companies may be incorporated into the body of the cell, such that only electrical connections (electrical contacts, jacks, coaxial cable), etc., may be required. The central layer 4 is also configured to contain microgrooves for receiving minimally one incident waveguide and two collector waveguides. The device shown contains microgrooves for two incident waveguides 10, 11 and two collector waveguides, 12 and 13.

As indicated previously, the micro flow cytometer ('MFC") devices may be constructed of various materials. For example, the entire device may be constructed of a hydrophobic polymer such as polydimethylsiloxane (PDMS), or may be constructed of alternative materials, including conventional thermosetting polymers, thermoplastics, glass, ceramic, metal, and the like, provided that the portions of the internal passageways of the device preceding and including the interrogation zone where both liquid and optionally gas are simultaneously present are transparent to the wavelengths of interest in an area between the light source and the detector within the interrogation zone, so that light scattered by particles passing through the interrogation zone can be analyzed appropriately as in conventional flow cytometers. The walls of the internal passages are preferably of hydrophobic material, or are coated or otherwise treated to render them hydrophobic.

A convenient method of constructing the focusing chamber, interrogation zone, and further channels and reservoirs is the use of soft lithography as disclosed by Y.N. Xia et al., SCIENCE, V. 273 p.347 ff (1996). The use of this technique also allows a simple lens or even a grating to be fabricated in the device. Y.N. Xia et al., "SOFT LITHOGRAPHY" Annu. Rev. Matter SC1 V. 28, pp 153 - 184, (1998). The lens and grating may form part of the wall of the interrogation zone, for example, for light focusing and/or collimation or spectral dispersion.

The devices may be unfocused, *i.e.* configured such that the only fluid flowing through the interrogation zone of the device is the fluid being analyzed. This type of configuration is particularly useful at low flow rates in channels of very small dimension. However, in general, it is preferable that the devices be focused, either by means of a surrounding sheath of liquid, or of gas. As is well known, focused devices maintain the fluid being analyzed in a narrower and more well defined flow through the interrogation channel.

The optical waveguides used are preferably optical fibers of glass, and may be cast in place in the device or positioned by any other suitable means. Most preferably, MFC devices are constructed of layers of materials, with one or more layers, preferably a layer also containing the interrogation channel or a portion thereof, containing grooves or channels sized to receive and emplace the optical waveguides. Providing of such channels simplifies the construction process and renders the devices uniform from device to device. Since the MFCs are preferably designed to be low cost and disposable, uniformity is particularly desirable. Traditional large scale flow cytometers are both expensive as well as specifically designed for multiple use. The concepts expressed herein can be used with conventional flow cytometers as well.

The geometry of the focusing chamber, interrogation zone, etc., is such that the sample stream is focused, either two dimensionally or multiply dimensionally, without substantial breakup of the sample stream when used as a flow cytometer. Numerous geometries are possible, and the various channel chambers and zones may take numerous cross-sections. A suitable geometry is

provided in Figure 7 of PCT/US02/22903, which should not be construed as limiting. Dimensions are in mm. Focusing of the sample fluid is illustrated in Figure 8 of PCT/US02/22903, wherein sample fluid stream 41 enters focusing chamber 42 together with air sheaths or "focusing streams" 43a and 43b. As the focusing chamber narrows, the sample stream is altered in shape and constricted 44 to a narrow and coherent stream 45 within the interrogation zone. The geometry may be determined by standard commercially available fluid dynamics software.

In a typical device, the diameter of the liquid sample channel may be from 1 - 1000 μm, preferably 10-300 μm, and most preferably about 20 μm at the downstream end where it communicates with the focusing chamber. The gas channels at this point are preferably somewhat larger in dimension, for example 200-600 μm, preferably 250-500 μm, and more preferably 300-400 μm, and converge with the fluid sample channel at an acute included angle, preferably from 15-45°, more preferably 20-40°, and most preferably ca. 30°. The walls of the focusing chamber are defined by the outer walls of the gas inlet channels at its "upper" end, for example 1000 μm to 2000 μm, preferably about 1500 μm, and at the "lower" end, by the diameter of the interrogation zone into which the focusing chamber transitions, *i.e.* from 100 μm to 500 μm, preferably 200 μm to 400 μm, and most preferably about 300 μm. The transition of the upper end of the focusing chamber to the lower end, the beginning of the interrogation zone, is roughly V-shaped in plan, and the side walls may be slightly concave, as described in the figures, the actual geometry being straight, circular, elliptical, hyperbolic, parabolic, or other shape which suitably minimizes breakup of the sample stream.

The liquid sample and focusing gas flow rates (when used) are selected so as to form a focused but coherent liquid sample stream within the interrogation zone of the desired diameter. It should be noted that when gas focusing is used, the fluid sample is not necessarily completely enveloped by gas, although complete envelopment is preferred. The diameter of the sample stream in the interrogation zone will be a function of the gas flow rate, the liquid sample flow rate, and the vacuum (if any) applied at the outlet. Liquid focusing in the same manner may also be used. The lower end of the interrogation zone, for example the

outlet reservoir, is preferably maintained at less than atmospheric pressure, for example from 10 mm Hg to 700 mm Hg. A pressure of 45 mm Hg has been found quite suitable. Alternatively, the inlet reservoirs could be maintained at positive pressure without requiring application of vacuum at the outlet. The physical geometry of the device may also affect sample stream diameter.

For example, when gas is used for focusing, the gas flow rate may range from 40 mL/hr to 15,000 mL/hr, preferably ca. 800 mL/hr. At these gas flow rates, with a 45 mm Hg vacuum, and in a channel with a 100 μm height, the diameter of the focused liquid sample stream in the interrogation zone may be varied from as little as 15 μm at a liquid sample flow rate of 6 mL/hr to about 100 μm at a flow rate of 20 mL/hr. Calculations based on fluid dynamics indicate that in a device with a 300 μm wide interrogation zone, a gas flow rate of about 700-900 mL, and a vacuum at the outlet of 45 mm Hg, the maximum focused liquid stream flow rate is approximately 50 mL/hr, at which point the liquid will contact the walls of the interrogation chamber. These same calculations indicate a minimum sample flow rate of 6.6 mL/hr without losing stream coherency. A stable 6 mL/hr stream has been observed. However, lowering the flow rate to 5 mL/hr caused the stream to be discontinuous. The geometry and dimensions of the channel can be tailored to achieve stability at different flow rates. For further discussion of the necessary calculations, reference may be made to Huh, et al., "Use of Air-Liquid Two-Phase Flow in Hydrophobic Microfluidic Channels for Disposable Flow Cytometers," BIOMEDICAL MICRODEVICES, 4 (2), 141-49 (2002), based on the work of the present inventors.

The light source and light impingement system may comprise a conventional laser, numerous types of which are commercially available. With such lasers, optical systems are generally necessary to properly direct the laser beam through the interrogation zone. In this type of light supply, conventional auxiliary components can be used. "Auxiliary" components include conventional optics including but not limited to lenses, lens systems, filters, gratings, polarizers, quarter wave plates, beam splitters, etc. These auxiliary components comprise the light impingement system when standard light sources are used. The light source may

emit a range of light, may be monochromatic, or may be multichromatic. In one embodiment, a plurality of light sources of different spectral characteristics are employed. These, for example, may emit light in the UV, visible, or IR portions of the spectrum. The wavelengths are often chosen such that they maximize fluorescing modes of particles to be detected. For purposes of detection, it is also desirable that the light sources be of a wavelength other than that to be detected.

Similarly, conventional light collection systems and light detection systems can be used. These include, generally, a photomultiplier tube or equivalent device as part of the detector system, generally also associated with auxiliary components such as lenses or lens systems, beam splitters, filters, gratings, etc., which also comprise the light collection system. The output of the photomultiplier and of photodiodes, when used, are generally connected to standard data acquisition devices and processed using analog or digital circuitry to maximize the signal to noise ratio. These techniques are easily implemented by those skilled in the art based on the concepts presented herein. For example, a noise baseline may be established, and only peaks projecting above this baseline may be reported.

When lenses or lens systems are employed, these may be external to the flow cell, or may be cast or inserted into the flow cell to minimize the number of external auxiliary components. The impingement and collection pathways terminate internally such that light impinges upon the collection zone, and is detectable following passage through the collection zone by the detector. The pathways for conventional impingement and collection pathways generally terminate "proximate" to the interrogation zone. For transparent devices, for example of acrylic resin or polyorganosiloxane, the path termini may be at an outside wall of the device, or may be located in close proximity to the interrogation zone, with an optically transparent passage, for example a hole, transparent rod or window, etc., whose innermost closed end is close to the interrogation zone. The actual construction of the impingement and collection zones is not critical so long as their function is achieved. It should be noted that the material of construction, if optically transparent at the desired wavelengths, may be structured in the form of a lens element or grating. While light transmission from the interrogation zone will be

conveyed by optical waveguides, and while it is preferably that the impinging light also be so conveyed, the lenses, gratings, etc. heretofore described may precede or follow the optical waveguides *per se*. Most preferably, the optical waveguides communicate directly with the interrogation channel or an optically transparent wall thereof, as described more fully below.

To reduce size and cost, it is particularly preferred that the conventional light source be supplanted by one or more solid state "laser" diodes, which are preferably located within or on the flow cell or a housing thereof. Use of such devices is made practical by a light impingement system which employs optical waveguides, preferably fiber optics. In this embodiment, the light from a laser diode light source is transmitted to the proximity of the interrogation zone by fiber optics. The optical fiber(s) terminate as close as possible to the interrogation zone to minimize scattering effects, for example terminating from 10 to 100 μm, preferably 30-80 μm from the interrogation zone. Due to the nature of the signal carrying capabilities of the optical fibers, lens systems which are commonly used in conventional systems may be eliminated, thus providing significant savings in size and cost, to the degree that in a preferred embodiment, the laser diode light source(s) may be incorporated within the body of the flow cell itself or intimately associated therewith, for example in a compact case, such that the entire device is portable, handholdable, and/or disposable. In addition to focusing fluid and sample fluid connections, only standard electrical connections need be provided, which can constitute metal contacts, jacks, etc.

The light impingement system can also be used with a remotely located light source, either of the conventional laser type or a laser diode type. In such cases, the optical fibers embedded within the body of the flow cell are preferably terminated at the exterior of the cell body, for example by conventional fiber optics coupling devices.

In like manner, the light collection system preferably comprises fiber optics as well, and preferably does so for purposes of miniaturization and disposability. As in the case of the light impingement systems, the optical fibers

may be connected to an external detector, for example a photomultiplier tube and auxiliary components desired to maximize the signal to noise ratio. However, the fiber optics may also be connected to a detector system "on board," which allows for an integrated transportable and even handheld device. The waveguide configuration is also self-locating and fixtured, which minimizes the potential for interfering vibrations during measurements with a miniaturized device.

The detector system of the present invention is used in conjunction with an optical waveguide or fiber optic collection system, and preferably comprises a semiconductor detector, preferably a PIN diode. Such diodes are commercially available, are capable of being biased by relatively low voltages, and are of relatively small size and cost. The PIN diode preferably includes one or more filters grown directly on the PIN structure or attached thereto. Such a filter is useful for filtering out extraneous light. For example, the filter may allow light of a fluorescence wavelength to pass while filtering out the wavelength used to excite the fluorescent species, for example a dye marker. Alternatively, an optional filter may be contained in the collection pathway as a component of a collimating lens system.

The PDMS observation channel illustrated in Figure 1 was designed to have a cross-sectional are of $100x300\mu m^2$ and three fluid entry compartments. Design parameters for the channel such as lengths, angles, and volumes were determined by developing a microfluidic model within the computational fluid dynamics (CFD) software Fluent 5.5 (Fluent Inc., NH). A three-dimensional computational model was utilized to simulate the hydrodynamic focusing behavior of the sample at the interrogation point of the laser under different scenarios. Specifically, models which could predict the sample focusing effectiveness of sheath fluid injected at different impingement angles (20° - 60°) were constructed to find an optimal configuration for the impingement angle of the sheath fluid channels on the sample channel. Similarly, various ratios of sample flow rate to sheath fluid flow rate (1:1-1:10) were simulated to observe the effectiveness of sample fluid focusing. The final dimensions and operating parameters for the channel were selected such that the sample fluid is well focused with a constant velocity in the center of the channel well before the interrogation point. The design was also set

such that the sample fluid in entire thickness direction (100μm) would be uniformly illuminated as necessary to minimize device performance variation.

The optical sub-system of the micro flow cytometry system must serve 1) to deliver light, preferably laser light, to a well-defined observation zone, and 2) to collect fluorescent light from the observation zone and deliver it to an optical filter for separation from scattered laser light prior to electronic detection. In contrast to conventional flow cytometers, the present invention uses integrated optical fibers. The fiberoptic system has a more compact volume, yet is still capable of transmitting excitation and fluorescence light with high efficiency. In addition, by embossing groves that serve as fiber receptacles directly into the PDMS observation cell as shown in Figure 1, the optical fibers can be fit tightly in the system without separate alignment steps necessary during assembly. Figure 1 also illustrates that multiple light impingement and collection angles are also easily possible (45° and 90° shown), which is not the case in conventional flow cytometers. The detection system can therefore be configured to collect information from any or all angles simultaneously as desired in a given application.

In addition to the consideration of microfluidics and materials of construction, much effort has been directed to develop micro-optical components that could potentially be utilized within a flow cytometer microsystem. In a flow cytometer, the optical system usually consists of a laser, photodetectors, and optical components such as lenses, mirrors, and filters. In conventional flow cytometers photomultiple-tubes (PMTs), and occasionally avalanche photodiodes (APDs), are used for fluorescence detection in the wavelength range of 400 to 1000nm. These detectors are utilized in flow cytometry and other applications due to their high sensitivity, high internal gain ($>10^6$), and fast response (10^{-7}-10^{-9}s). However, PMTs are bulky, fragile, consume large amounts of power, and are generally sensitive to environmental conditions. APDs are also somewhat bulky and require high-power, resulting from their need for active thermal management to maintain high S/N. Although both PMT and APD photodetectors yield a large S/N due to their internal gain, optical systems incorporating these devices into flow cytometers are costly and difficult to miniaturize. For this reason, PIN-based photodetectors

which are 1) low-cost, 2) low power, and 3) tightly integratable with other optical components are preferably used.

Since the fluorescent signals generated from the detection of biological cells only generate a few nA of current in PIN photodiodes, a lock-in amplification technique is applied to increase the S/N of the micro flow cytometer. While common in other technical applications, the lock-in amplification approach, which is also described in PCT/US02/22903, has not been previously discussed in the literature for flow cytometry. The lock-in technique is effective because it acts as a narrow band-pass filter which removes much of the unwanted noise while allowing the signal which is to be measured to pass through. To achieve this, the laser excitation is modulated at a reference frequency which induces fluorescence emission from the interrogated sample at the same frequency. The modulated signals detected at the photodiode are then monitored with high sensitivity using the lock-in circuit. For reliable performance, the modulation frequency is set to be two orders of magnitude higher than the bandwidth required for detecting the fluorescence emission from moving sample particles. The lock-in circuit parameters such as the time constant and sensitivity are selected to maximize S/N at the given modulation frequency.

The spectral characteristics can best be understood by consideration of an MFC having two monochromatic light sources of different peak wavelengths, for example blue and red. The term "monochromatic" is used in its ordinary sense, and is meant to include light sources which emit the majority of their energy over a relatively narrow wavelength band. Examples of such monochromatic light sources include, but are not limited to, LEDs, lasers, etc. Spectral emission lamps such as mercury vapor lamps, which typically emit a line spectrum, may also be used, preferably with a filter to isolate one or more wavelengths of interest.

The two light sources of the present illustration may be applied to the interrogation channel through a single optical waveguide, for example through a combining prism, rotating mirror, etc, or may be applied through two different optical waveguides. When two or more optical waveguides are used for the incident

light, the waveguides are preferably positioned with their ends which are proximate the interrogation channel as close as possible to each other, and may be angled slightly with respect to each other to try to obtain a condition where a single particle is illuminated by both incident wavelengths in the same position in the channel, as shown in Figure 1. Otherwise, the output signals will be staggered from each other in time.

At least two output optical waveguides are employed in this illustration, one, for example, at an included angle of 90° to the axis of the interrogation channel, and one at an included angle of 45° to the axis of the interrogation channel. Typically, the two output optical waveguides will not receive the same light energy at any given wavelength. Rather, due to refractive, diffractive, and reflective effects, the signal strengths will differ. Moreover, the difference in signal strength will be wavelength dependent. Therefore, by measuring the difference in signal strengths at a first wavelength and the difference in signal strengths at a second wavelength, by comparing the relative differences of these "comparison" signals at the two different wavelengths, a plot of wavelength versus difference in comparison signal can be made. By measuring a difference in comparison signal strength during operation, each difference will correspond to a different wavelength. Thus, the wavelength emitted by a fluorescing particle may be measured by comparing the difference in output signal strength between two output waveguides at different included angles to the interrogation channel.

For example, once the output signal and wavelength relationship has been established, signals may be selected with regard to their comparison output to distinguish between multiple particles having different spectral signatures, *i.e.*, different fluorescent wavelengths. Thus, in a sample fluid containing two different microorganisms, each receiving a stain which will cause fluorescence at a different wavelength, the detectors, for example one or more photodiodes or PMTs may be filtered to remove the incident light, and the outputs sampled at two different angles with the interrogation channel. The signal strength at any given output waveguide will not yield any information which can distinguish between the different microorganisms. Rather, a signal merely indicates that a particle is pressing through

the interrogation zone. However, if at this instant, the difference between the two differently angled output waveguides is measured, the difference will indicate which type of particle is traversing the zone. Thus, a low difference in signal strength will correspond to one wavelength of fluorescence, attributable to one type of particle, while a higher difference will correspond to a second wavelength and hence a second type of particle. By synchronizing particle detection with output signal strength difference between the two output waveguides, a count of each type of particle can be made. In a like manner, the S/N ratio may be used in lieu of a difference or "comparison" signal. As indicated in the Example and as illustrated in Figure 6, the S/N ratio is also wavelength dependent when expressed as a ratio between variously angled detectors.

The means for measuring signal strength, amplifying signals, measuring signal strength differences, etc., are all accomplished by means well known in the electronic arts. For example, signal strengths may be amplified by conventional operational amplifiers which are readily available, or amplifiers constructed of discrete components. Differences in signal strength may be made by means of comparators or differential amplifiers. The signals may be exclusively analog, or may be digitized by analog-to-digital converters. Once digitized, the signals, their difference (comparison signals), etc., may be processed by suitable software. An output may be presented as a trace on a CRT as shown in Figures 4a, 4b, or in tabular form, etc.

The micro flow cytometers must have an inlet for supplying test fluid, and an outlet. However, these need not be made in such a fashion that fluid necessarily needs to enter or exit the device during measurement. For example, the inlet may comprise a well or reservoir holding fluid until fluid flow is desired to be established. In like fashion, the outlet may continually remove fluid from the device, or again may comprise a well or reservoir to receive fluid from the interrogation channel. In disposable MFCs, outlet wells or reservoirs may be highly desirable, to avoid the necessity of disposing of microorganism containing fluids.

In preferred devices, photodiodes are employed as the detector, for reasons advanced earlier. Due to the lack of internal amplification and noise associated with such devices, it is preferable also as earlier indicated, to modulate the incident light and to amplify detected output light synchronously. For example, a function generator may be used to modulate the incident light source such as an LED, and also to provide a signal to the amplifier for synchronous amplification. The function generator may produce any type of output, for example sinusoidal, square wave, sawtooth waves, etc. When two or more incident wavelengths are desired to be used, they are most preferably driven substantially out-of-phase with respect to each other. For two sources, for example, they may be 180° out-of-phase. Some overlap of the light intensities at multiple wavelengths may be tolerated. However, the greater the overlap, the less will be the signal-to-noise ratio. A function generator may be easily arranged to provide signals which would produce no overlap. The term "substantially out-of-phase" implies that the peak intensities of the various wavelengths can be distinguished from each other with respect to time.

Figure 3 illustrates a schematic of a synchronous ("lock in") detection system which can be assembled with conventional components. A 1 MHZ oscillator 20 is chosen to provide signal to both the laser driver 21 and synchronous detector 22. The laser driver provides a suitable driving signal for laser diode or LED 23, causing it to emit pulses of light a the 1 MHZ signal rate. The laser light, upon contacting a particle 24 in the interrogation zone causes a fluorescent output which is detected by PIN photodiode 25. The resulting electrical signal is amplified by a low noise differential amplifier 26, and optionally further amplified by additional amplifier(s) 27. The amplified signal is routed to a high Q (e.g. Q≈100) bandpass filter 28, which may, for example, limit the output signal to the range of 995 KHz to 1.005 MHZ, only a 10 KHz bandwidth. This output signal from the bandpass filter is routed to the synchronous detector 22, the output of which may be digitized by analog to digital (A/D) converter 29, and the data subsequently processed and/or collected by microcontroller or microcomputer 30.

The term "emanating" is used to describe light from a particle having been illuminated by incident light. Light emanating from such a particle may have the same wavelength as the incident light, for example by the incident light having been reflected from or scattered by the particle. In most cases, however, the particles will fluoresce, *i.e.*, emit one or more wavelengths different from the incident light wavelength. Fluorescence at a different wavelength provides the opportunity to eliminate the rather strong incident light from the output, resulting in a significant increase in the signal-to-noise (S/N) ratio which may be obtained. The term "comparator" means a device or plurality of devices which are able to compare relative signal strengths. A comparator need not be a discrete device, but may, for example, include detectors, amplifiers, analog to digital converters, and a microprocessor. The output of the comparator may be processed further to provide any desired type of signal.

Thus, in one aspect, the subject invention pertains to a spectral micro flow cytometer device with a micro flow cytometer having an inlet, an outlet, and an interrogation channel in fluid communication with the inlet and outlet, the interrogation channel defining an interrogation plane in which the channel is positioned; minimally three optical waveguides terminating proximate to the channel and in the interrogation plane, each optical waveguide directed toward the channel at a different angle with respect to the axis of the channel, the inlet direction of the channel being 0°; minimally one incident light source coupled to an optical waveguide and illuminating the channel; and a comparator which compares a light intensity or a signal to noise ratio at one optical waveguide positioned at a first included angle, with a light intensity or a signal to noise ratio at a further optical waveguide positioned at another included angle, the difference in intensity or in signal to noise ratio being proportional to the wavelength of light emanating from a particle passing through the channel.

In another aspect, the micro flow cytometer contains a modulated, first monochromatic light source which directs light into an incident optical waveguide such that modulated monochromatic light impinges upon particles within an interrogation channel; optionally, a modulated, second monochromatic light

source of a different color whose modulation is substantially out-of-phase with the modulation of the first monochromatic light source, and which directs light to the interrogation channel through a second incident light optical waveguide, which may be the same as or different from the first incident light waveguide; a first photodiode detector sensitive to light from the first monochromatic light source, the second monochromatic light source, or both, or sensitive to a fluorescent wavelength emitted by a particle in the channel as a result of the particle being illuminated by one or more light sources, the photodiode detector communicating with the channel through a first output waveguide in a plane with the first incident waveguide and the channel, and positioned at a first included angle with respect to the channel; a second photodiode detector communicating with the channel through a second output waveguide in a plane with the interrogation channel and the first incident waveguide, but at a different included angle to the channel than the first output optical waveguide; at least one amplifier which amplifies an output from the photodiode detector(s); a means for selectively sampling an amplified output from the amplifier synchronously with a respective modulation frequency of the first or second monochromatic light sources to provide an output proportional to incident light fluorescent light impinging on the detector, and a second output proportional to light of a different incident color or fluorescent color; a means for comparing the signal strength obtained from the first detector with the second detector at at least two different wavelengths to obtain an angularly dependent comparison signal or signal to noise ratio for each wavelength; and a means for comparing the angularly dependent comparison signals or signal to noise ratios to each other to provide spectral differentiation of light collected by the respective output waveguides.

A yet further aspect of the invention pertains to a method for providing spectral information for particles in an interrogation channel of a micro flow cytometer, by illuminating the particles with incident light directed to the channel through an incident optical waveguide; transmitting reflected, scattered, or fluorescent light from a particle in the channel through separate output optical waveguides positioned in a plane which includes the interrogation channel, the output optical waveguides positioned at different included angles with respect to the axis of the channel; measuring light intensity or signal to noise ratio from each

output optical waveguide at two different wavelengths, and providing comparison output signals proportional to the difference between the intensity of each respective wavelength from the output optical waveguides, thus providing a comparison output signal for a first wavelength and a second comparison output signal for a second wavelength; and comparing the comparison output signals or signal to noise ratios, by means of which spectral information relating to the particles being observed can be collected, and if desired, further processed.

Example

The PDMS observation cell with a microfluidic channel and optical fiber microgrooves is fabricated using replica molding. The process begins with the patterning of a negative tone SU-8 photoresist (SU-8 2050, Microchem Co., MA) mold on a silicon wafer using lithography. This is followed by silanization of the wafer surface using 3-(trimethoxysilyl)propyl methacrylate (Sigma-Aldrich Co., MO), which facilitates the parting of a PDMS layer during the next process step. A PDMS precursor liquid is then well mixed with a curing agent (Sylgard 184 Silicone Elastomer Base, Dow Corning Co., MI) and poured onto the SU-8 micromold on the silicon wafer. After curing for 24 hours at room temperature, the PDMS slab with embossed observation channel features is peeled off. The final size of the fabricated flow cell is $4x2cm^2$. The microfluidic channel has a length of 1 cm and cross-sectional area of $100 \times 300\mu m^2$.

The flow profile of the hydrodynamically-focused sample liquid is observed using optical and confocal microscopy. These methods permit characterization of the hydrodynamic focusing performance of the PDMS microfluidic channel and provide cross-sectional images of the sample fluid. For the confocal microscopy study, sample water containing fluorescein (Sigma-Aldrich, MO) is utilized as the characterized fluid. The sample fluid is excited by a blue laser contained within the confocal microscope (LSM-510, Zeiss, Germany).

Figure 2 illustrates the experimental testbed used to evaluate the performance of the microfluidic and optical design of the PDMS micro flow

cytometer previously described. FluoSpheres® polystyrene microspheres (Molecular Probes, OR) are used as a reference sample to illustrate the performance of the micro flow cytometer, using scarlet fluorescent (F-8843) microspheres with 15.5μm average diameter, and green fluorescent (F-21010) microspheres with 15 μm average diameter, as listed in Table 1. Significant variation in the absorption and emission wavelengths and intensities is observed experimentally.

TABLE 1

Sample	Maximum Absorption Wavelength (nm)	*Maximum Fluorescence Emission Wavelength (nm)*
FluoSpheres® polystyrene microspheres, F-21010 (green fluorescent, 15.0 μm)	645	680
FluoSpheres® polystyrene microspheres, F-8843 (scarlet fluorescent, 15.5 μm)	450	480

The semiconductor laser diodes used to excite the reference fluorescent microspheres include 1) a 6mW red iode laser (SCFC2-0635-0006-T-25, Optical Fiber System Inc., MA) with a peak wavelength of approximately 635nm, and 2) a 5mW blue diode laser (NDHB500APAE1 Engineering Sample, Nichia Co., Japan) with a peak wavelength of approximately 440nm. The blue and red laser excitations are modulated at 100 kHz with differing phase angles (*e.g.*, signals 180 degrees apart in phase). Multimode optical fibers (F-MCB-T, Newport Co., CA) with 100μm core diameter and 110μm cladding diameter are utilized to transmit energy to and from the interrogation zone as shown in Figure 2. The optical filters are selected according to the transmission spectral characteristics listed in Table 2 in order to separate the high-intensity excitation light from the relatively weak fluorescent light emitted by the above-described microspheres. The photocurrent generated at the PIN photodiode (S7329-01, Hamamatsu Photonics Co., NJ) is amplified using an OP-amplifier-based circuit (10^4X, OPA655, Texas Instruments Inc., TX). The circuit converts the photocurrent signal to an amplified voltage signal. The voltage signal is processed using a lock-in amplifier (SR-840, Stanford

Research, Inc., CA) and extracted from the background noise. A block diagram is illustrated in Figure 3.

TABLE 2

Sample	*Optical Filter*
645/680 Polystyrene Bead	695 Band Pass (FWHM: 11.5 nm)
450/480 Polystyrene Bead	480 Long Pass (50% transmission at 480 nm)

Two independent experiments are conducted to fully validate the performance of the PIN-based signal detection process. First, fluorescence detection with and without the lock-in amplifier was performed. To achieve this, the signal detected at the PIN photodiode is guided to a pre-amplifier circuit and then identically divided into two cables. One of the divided signals is directly monitored and the other was processed by the lock-in amplifier prior to monitoring. The signals are then compared with each other.

Second, signals simultaneously obtained from a PMT (P30CWAD5-01, Electron Tubes Inc., NJ) and PIN photodiode are compared with each other to validate the performance of the signal detection system using the PIN photodiode and the lock-in amplifier. The PMT was selected as a reference detector, as it is widely used in cytometry and known to perform well in this application. The PMT is powered by a 900-1200V voltage source to obtain maximum S/N. For this experiment, one excitation and two detection optical fibers are embedded into the observation cell at the same angle. The detection fibers, one of which is connected to the PIN photodiode and the other connected to the PMT, simultaneously capture the fluorescent signals and send them to the photodetectors. The signals from the PIN photodiode with lock-in amplifier and the PMT (without signal processing) are then compared.

To better understand the influence of experimental conditions on the PDMS micro flow cytometer, scarlet/green inorganic and biological particles are

tested under variable sample/sheath flow rate ratios, and multiple optical detection angles. Three major parameters are investigated: 1) S/N, 2) S/N variation, and 3) recovery rate. The recovery rate is estimated by injecting a sample fluid with polystyrene microspheres of known concentration (10^6/30 beads/ml) into the microchannel. The final microsphere concentration is achieved by a 1:30 dilution in buffer solution (Tris Buffered Saline with bovine serum albumin (BSA), T6789, Sigma-Aldrich Co., MO).

To demonstrate two-color excitation and detection using the developed micro flow cytometer, two pairs of optical fibers are arranged at an angle of 135° between the excitation and detection fibers as shown in Figure 2. A mixed sample consisting of scarlet and green microspheres (each diluted 1:30 with buffer solution) was injected into the microfluid channel and hydrodynamically focused. The sample was excited at a single interrogation point using two lasers with 440nm and 635nm wavelengths. In order to prevent the coupling of the two excitation light sources, the red and blue laser diodes were driven 180° out-of-phase (100kHz).

To demonstrate the feasibility of using the micro flow cytometer to detect fluorescent labeled biological samples, an experiment with the fungus *Saccharomyces cerevisiae* is performed. To prepare the fungus sample, a powder sample of dried cells is mixed into warm water and maintained at 38°C for about 20 minutes to revive the culture. After full activation of the cells, a 2μl yeast solution is stained using Syto® 62 (or Syto® 44) (Molecular Probes, OR) and incubated for 20 minutes at room temperature. The yeast cells, with fluorescently labeled nucleic acid, are injected into the flow cell at a sample flow rate of 5ml/hr with total flow rate of 15ml/hr and excited using the 635nm red (or 440nm blue) laser diode. The optical signals are separated from the excitation radiation using the 695nm band-pass (or 480 long-pass) filter.

When examined by optical and confocal microscopy, the sample fluid is well confined in the center of the channel in the horizontal direction. It is found, as predicted, that the width of the focused sample fluid can be controlled by changing the ratio of sample/sheath flow rate. It is observed that by tuning the ratio

of flow rates between the sample fluid and the sheath fluid (maintaining fixed total flow rate of 15ml/hr), experimental results conferred with CFD simulation results to within 10% error.

Figure 4a demonstrates the simultaneous detection by the PMT (without advanced signal processing) and the PIN photodiode detection (4b) with lock-in technique. It is observed that all particles detected with PMT are observed with the PIN photodiode with equal or higher S/N.

The micro flow cytometer performance was evaluated for the case of fluorescent microsphere detection. Three parameters were used as performance metrics: S/N, S/N variation, and recovery rate. These performance parameters are discussed in the following paragraphs.

S/N: Figure 5 shows the average S/N corresponding to various angles between the excitation and detection fibers. The S/N values obtained from the experiments show that the device can successfully detect the fluorescence emission in different angles for 15μm polystyrene microspheres. The detection angle resulting in maximum S/N (~9.5) is 180°. For the scarlet polystyrene microspheres, the S/N is slightly reduced when measuring at 45° and 135° off the excitation direction. In contrast, the S/N at 45° and 135° for green polystyrene microspheres measurement is reduced to about 35% of that in 180°. These results have useful and significant ramifications for estimating fluorescence color that will be discussed later.

The S/N variation (expressed as the coefficient of variation percentage) varies at different angles for numerous ratios of sample/sheath flow rates. The minimum variation occurs at 180° detection and at the lowest ratio of sample/sheath flow rate. The S/N variation for the 180° detection is observed to be approximately 25% and 35% for the scarlet and green polystyrene microspheres respectively over most of the flow rate ratios investigated. The variation over this range is believed to be in part due to the variation in the dimensions and intensity of the microspheres, and in part due to the variation in the vertical position of the

microspheres in the microfluidic channel. Furthermore, the S/N variation is observed to increase as the ratio of sample/sheath flow rate became higher. This variation increase is more obvious at 45° and 135° detection than it was at 180°. As will be discussed later, this is likely due to the wider column of sample fluid at higher at higher sample/sheath flow rate ratios, and the increased possibility of multiple particles in the interrogation zone at the same time under this condition.

The recovery rate also varies for different angles at different ratios of sample/sheath flow rate. The maximum recovery rate (75%) is obtained at 180° detection. Higher recovery rates generally are observed with increasing sample/sheath flow rate ratio. Deviations from 100% recovery are discussed later.

Experiments with simultaneous two-color excitation/detection demonstrates the feasibility of the compact optical arrangement as shown in Figure 2. The high S/N ratio (6.4 and 2.1 for scarlet and green microspheres, respectively) of the detected signal shows that the system is capable of simultaneous two-color excitation/detection with a single interrogation zone. Moreover, the recovery rates of the two-color excitation/detection system are observed to be only slightly lower than those of the single-color results. The differences are approximately 10-20%, which may be due to experimental variation, or correctable interference between the detection systems of the two colors.

In observing the electronic signal corresponding to the detection of *Saccharomyces cerevisiae* labeled with Syto 62®, the concentration of the yeast cells calculated from the experimental results is about 1.90×10^4 cells/ml, with a S/N of approximately 2.8. The results show that the micro flow cytometer is effective in detecting yeast cells, with high potential for application to other biological cells.

S/N: The data in Figure 5 indicate that the average S/N is almost invariant regardless of the detection angles for the scarlet fluorescent microspheres, whereas it is highly influenced by angle for the green fluorescent microspheres. Moreover, it is observed that the signal intensity is reduced at the 45° and 135°

angles compared to that at the 180° angle. The reduced off-angle signal intensities are likely due to numerous opportunities for scattering losses at the off angles. For instance, when the detection optical fiber is not aligned normal to the channel, the optical path can be significantly bent due to the difference in index of refraction at the interfaces between the PDMS channel wall and the fluid, and between the PDMS flow cell and the air gap in front of the optical fiber. In addition to optical path bending, the distance between the end of the detector fiber and the center of the interrogation zone, d_c, highly influences the magnitude of the detected light intensity. In the current flow cell design, the 180° detection arrangement has a shorter distance between these two points ($d_c \sim 200$ μm) than the 45° and 135° detection arrangements ($d_c \sim 330$μm). The longer distance leads to weaker detected signals at the 45° and 135° angles, as would be predicted if the sample particles were assumed to be point light sources positioned at the center of the interrogation zone. In this case, the intensity of the light collected by the optical fiber is proportional to d_c^{-2}, and it would be predicted that the signal intensity at the 45° and 135° angles should be about 37% as that at 180°. From the experimental results, the average signal intensities at 45° and 135° were 36-42% of that at 180°, which is in reasonable agreement with the prediction.

Moreover, experimental results show that the relative S/N between various detection angles varies for sample particles with different spectra. Consequently, by taking advantage of simultaneous multiple-angle detection, a micro flow cytometer with spectrometer characteristics is possible. To test this hypothesis, both scarlet and green fluorescent microspheres (F-2101 and F-8843) and *Saccharomyces cerevisiae* labeled by Syto® 62 and Syto® 44 are examined, and the ratio of average S/N at 180° and 45° detection is calculated for both green and scarlet particle/cell detection. As shown in Figure 6, both microsphere and biological detection yield a distinct ratio of 180° to 45° S/N depending on the fluorescent color of the interrogation target. The difference of the ratio is consistent with color. Thus, spectral flow cytometers for specialized biological assays targeted at simultaneous multi-species detection are possible with the devices of the subject invention.

To determine whether particles smaller than 15 μm can be detected using this micro flow cytometer design, scarlet fluorescent polystyrene microspheres with 6 μm average diameter are also tested in the micro flow cytometer using the 180° optical fiber arrangement. The S/N (~ 1.5) is low but detectable, suggesting that the signal will be prohibitively weak if the particle size becomes smaller than 6 μm. Ideally, the intensity of fluorescence emission is proportional to the surface area of the fluorescence particles, and therefore the fluorescence intensity of the 15 μm microspheres is expected to be about 6.25 times larger than that of the 6 μm microspheres. The experimentally observed results are in close agreement with this prediction, showing a S/N difference of 6.3 (9.5/1.5).

S/N Variation: The variation of the S/N at a given detection angle can be correlated with the shape and area of the interrogation zone, Ω_i, that is defined by the intersection of the sample fluid column, the optical excitation zone, and the optical detection zone as shown in Figure 12. To account for the S/N variation observed experimentally, it is assumed that:

- The positional variation of the particle passing through Ω_i is negligible in the vertical direction.
- The particle can pass through any point in Ω_i at the same velocity, with the same probability.
- The optical excitation in Ω_i is spatially uniform.
- The detected particle is a point light source.
- The optical path bending can be ignored at any interfaces.
- The optical attenuation loss in the optical fiber is negligible.

Based on these assumptions, the intensity of the fluorescence signal I detected from the sample particle at an arbitrary point $r = (x,y)$ in Ω_i is given by.

$$I(r) = \frac{I_0}{|r - r_d|^2} \tag{1}$$

where I_0 is the original total intensity of the excited fluorescent microsphere, and r_d represents the position of the end of the detection optical fiber in the x-y coordinate system in Figure 12. The maximum and minimum signal intensities are given by the maximum and minimum of the maximum intensity calculated at each x position within the interrogation zone, Ω_i, which can be expressed as

$$\Omega_i = \left\{ (x, y) \; \middle| \; |x| \le \frac{w_c}{2}, |y| \le \frac{d_f}{2}, f_1(x) \le y \le f_2(x) \right\} \tag{2}$$

$$f_1(x) = \frac{d_f}{\cos\theta} + \frac{x}{\cos\theta} \tag{3}$$

$$f_2(x) = \frac{d_f}{\cos\theta} + \frac{x}{\cos\theta} \tag{4}$$

where d_f is the excitation and detection optical fiber diameter, w_c is the width of sample fluid column, and θ is the angle between excitation and detection direction. In this case, the S/N variation ΔS/N is estimated as

$$\Delta S / N = \frac{I_{\max} - I_{\min}) / 2}{\langle I \rangle} \tag{5}$$

where $\langle I \rangle$ is the averaged signal intensity given by

$$\langle I \rangle = \frac{I_{max} + I_{min}}{2} \quad (6)$$

Figure 7 shows the calculated S/N variations at varying sample column width and detection angle. Due to the geometrical symmetry, the calculations for 45° and 135° detection angles yield the same predictions.

As can be seen from Figure 7, the estimated S/N variation of 180° detection is close to the observed experimental result obtained from green fluorescence detection, with the difference within 10%. In contrast, the predicted S/N variation of 45° or 135° detection is much lower than that observed experimentally. This contradiction is a likely result of ignoring optical path bending in the model, which makes 180° detection less sensitive to the position of the sample particles in the microfluidic channel when compared with 45° and 135° detection.

Recovery Rate: At a low sample flow rate relative to the sheath flow rate (~3:12), the sample stream becomes unstable due to the large pressure difference between the sample and sheath flows near the channel inlets, resulting in discontinuous fluid motion. The discontinuous sample flow causes particles to become trapped in the inlet reservoir, which is one of the major reasons for the low detection recovery rate at a low sample flow rate (10-30%). As the sample flow rate increases to nearly 50% of the sheath flow rate, the sample stream can establish a stable liquid column by overcoming the pressure difference at the channel inlets. The stable liquid column is more effective at centering the sample particles in the center of the interrogation zone in a consistent manner. The stable flow of the sample particles leads to a higher recovery rate (55-75%) at the higher sample/sheath flow rate ratio.

On the other hand, larger liquid column width resulting from a higher sample flow rate is likely to prohibit a single-file sample flow, allowing multiple particles to simultaneously enter into the interrogation zone. Fluorescence signal intensities detected from the scarlet polystyrene microspheres at sample/sheath flow

rate ratio of 6:9 and 7:8, respectively (total flow rate of 15ml/hr) show that the signal intensity distribution is approximately divided into two subgroups at a flow rate ratio increases to 7:8. The middle intensity of each subgroup relates to the others in a whole number ratio. For instance, the middle intensities in Figure 14(b) are approximately in the ratio of 1:2:3 (0.45:0.90:1.35). Assuming that the signal intensity is proportional to the number of the simultaneously detected beads, these results appear to indicate that multiple fluorescent particles are detected in the interrogation zone at the same time. To test this hypothesis, the detection algorithm is modified to account for the possibility of multiple beads passing through the interrogation zone. With this modification to the detection algorithm, the recovery rate increases by about 25-30% (for flow rate ratio 6:9 and 7:8), leading to about 95% recovery for the fluorescence detection in the micro flow cytometer.

Even with the intensity correction, 100% recovery is not observed due to some loss of microspheres in the microfluidic interconnects. To minimize such losses, a buffer fluid containing BSA is utilized. BSA is found to be effective in coating the PDMS channel wall surface to prevent particle adhesion. The BSA buffer doubles recovery rates relative to experiments with other buffers such as PBS (phosphate buffered saline) or de-ionized water, both of which have little effect on changing the surface chemistry of the channel wall.

Lower recovery rates are also observed at the 45° and 135° detection angles. This suggests that the fluorescence signal may become weak enough to be indistinguishable from the background noise when the sample particles take a fluidic path far away from the detection optical fiber. The observation is likely due to the fact that optical path bending can shift the detection zone from the center of excitation zone defined by the laser diodes. This shifting can generate a partial or total blind spot for the 45° or 135° detector fibers, resulting in a low recovery rate. This is readily correctable by modifying the optical pathways in the system through the use of micro optical components in a next generation design of the system.

Single-Color vs. Two-Color Excitation/Detection: For two-color excitation/detection setup, the S/N ratios are about 16% (for λ=635nm) and 50%

(for λ-440nm) lower than that observed for the single-color excitation/detection setup. For the most part, this is because the background noise level in the two-color system is approximately 30% and 100% larger for scarlet and green fluorescence detection respectively. The background noise increase for the 2-color system is likely caused by the excitation light of one color leaking into the detection optical fiber. The leakage of excitation light and imperfect filtering can be solved by use of customized optical filters based upon the specific wavelength spectra of the excitation and detection targets. This will yield a two-color excitation/detection microcytometer possessing nearly the same S/N as the single-color system discussed previously.

While the inventive microsystem is highly successful to demonstrate the feasibility of producing a two-color micro-flow cytometer with PIN photodiodes and color differentiation ability, a number of design modifications would be achieved by designing the flow cell to have a channel width more comparable to the sample particle diameter. This channel design would result in a larger ratio of the sample flow rate to the sheath flow rate at a fixed combined total flow rate. In this case, a stable sample liquid column could be established while keeping its width narrow enough to confine the particle in the center of the interrogation zone with high precision. The modified sample liquid column width would improve the recovery rate and reduce the S/N variation, and promote a single-file flow in the microfluidic channel.

With respect to the optics design, micro optical components such as micro lenses and apertures could be integrated into the flow cell to prevent the optical bending and reduce background noise. This would also help increase the amount of collected light and better define the shape of the excitation area, leading to a higher S/N. These optical components would be designed to allow optical excitation to be more spatially uniform within a smaller area in the channel, which would reduce the S/N variation. Moreover, the blind spot that appears for the off-angle detection can be eliminated with a new optics design, which will naturally lead to an improved recovery rate at the off-angles. In addition, a new optics system could incorporate a larger number of detection optical fibers embedded into the

observation cell. These optical fibers can simultaneously collect the fluorescence emission from a single particle within the interrogation zone. The signals collected at each optical fiber can be combined to amplify the signal and to cancel the random components of the background noise. It appears that practical and affordable particle detection below 6 microns will be readily achievable with this design approach.

While embodiments of the invention have been illustrated and described, it is not intended that these embodiments illustrate and describe all possible forms of the invention. Rather, the words used in the specification are words of description rather than limitation, and it is understood that various changes may be made without departing from the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A spectral micro flow cytometer device, comprising:

a) a micro flow cytometer having an inlet, an outlet, and an interrogation channel in fluid communication with said inlet and said outlet, said interrogation channel defining an interrogation plane in which said interrogation channel is positioned;

b) a plurality of minimally three optical waveguides terminating proximate said interrogation channel and positioned in said interrogation plane, each optical waveguide of said plurality of waveguides directed toward said interrogation channel at a different angle with respect to the direction of said interrogation channel, with the inlet direction of said interrogation channel being 0°;

c) minimally one incident light source coupled to one of said optical waveguides and illuminating said interrogation channel;

d) a comparator which compares a light intensity or a signal to noise ratio at one optical waveguide at a first included angle, with a light intensity or a signal to noise ratio at a further optical waveguide at a further included angle, the difference in intensity or in signal to noise ratio being proportional to the wavelength of light emanating from a particle passing through said interrogation channel.

2. The device of claim 1, wherein a first included angle of a first optical waveguide with the interrogation channel and a further included angle of a further optical waveguide with the interrogation channel are such that the change in intensity of a first wavelength of light measured at said first optical waveguide and said further optical waveguide is different from the change in relative intensity of a second wavelength of light measured at said first optical waveguide and said further optical waveguide.

3. The device of claim 1, wherein said optical waveguides comprise optical fibers.

4. The device of claim 1, wherein two or more incident light sources of differing spectral characteristics are employed.

5. The device of claim 4, wherein at least two incident light sources are supplied to two different optical waveguides.

6. The device of claim 1, wherein at least one incident light source is monochromatic.

7. The device of claim 1, wherein at least two monochromatic incident light sources are employed.

8. The device of claim 7, wherein said two incident monochromatic light sources are pulsed light sources out-of-phase with respect to each other.

9. The device of claim 1, comprising first and second output optical waveguides positioned at different included angles with respect to the axis of the interrogation zone, and means for calculating the signal to noise ratio of the electrical signal from photodetectors receiving light from the respective output optical waveguides.

10. The micro flow cytometer of claim 1, comprising:

a modulated, first monochromatic light source having a first peak wavelength directing light into a first incident optical waveguide such that modulated monochromatic light impinges upon particles within said interrogation channel;

optionally, a modulated, second monochromatic light source having a second peak wavelength different from said first peak wavelength, whose modulation is substantially out-of-phase with the modulation of said first monochromatic light source, said second monochromatic light source directing light

to said interrogation channel through a second incident light optical waveguide, said first and second incident waveguides being the same or different;

a first photodiode detector sensitive to light from said first monochromatic light source, said second monochromatic light source, or both said first and said second monochromatic light sources or to a wavelength corresponding to a fluorescence emitted by a particle in said interrogation channel as a result of said particle being illuminated by one or more of said first and second light sources, said at least one photodiode detector communicating with said interrogation channel through a first output optical waveguide in a plane with said first incident waveguide and said interrogation channel, and having a first included angle with said interrogation channel;

at least a second photodiode detector communicating with said interrogation channel through a second output optical waveguide in a plane with said interrogation channel and said first incident waveguide, but at a different included angle to said interrogation channel than said first output optical waveguide;

at least one amplifier which amplifies an output from said photodiode detector(s);

means for selectively sampling an amplified output from said at least one amplifier synchronously with a respective modulation frequency of said first and said second monochromatic light sources to provide a first output proportional to light of an incident wavelength or a fluorescent wavelength impinging on said detector, and a second output proportional to light of a different incident wavelength or a different fluorescent wavelength;

means for comparing the signal strength obtained from said first photodiode detector with said second photodiode detector at at least two different wavelengths to obtain an angularly dependent comparison signal for each wavelength, and/or means for calculating a signal to noise ratio from said first photodiode detector and said second photodiode detector;

means for comparing the angularly dependent comparison signals or the signal to noise ratios to each other to provide spectral differentiation of light collected by said first output waveguide and said second output waveguide.

11. A method for providing spectral information for particles in an interrogation channel of a micro flow cytometer of claim 1, comprising:

illuminating said particles with incident light from at least one incident optical waveguide;

transmitting reflected, scattered, or fluorescent light from a particle in said interrogation channel through at least first and second output optical waveguides, all of said optical waveguides in a plane including said interrogation channel, said first and second output optical waveguides at different included angles with respect to the axis of said interrogation channel;

measuring light intensity or signal to noise ratio from each output optical waveguide at each of at least two different wavelengths and providing comparison output signals proportional to the difference between the intensity of each respective wavelength from the first and second output optical waveguides, thus providing a first comparison output signal for a first wavelength and a second comparison output signal for a second wavelength;

comparing said first and said second comparison output signals or signal to noise ratios, a difference being spectrally dependent.

12. The process of claim 11, wherein an output signal is amplified by a photomultiplier tube.

13. The process of claim 11, wherein incident light is modulated, and output signals are amplified in phase with modulated incident light.

14. The process of claim 11, wherein said incident light comprises two or more modulated, monochromatic light sources of different peak wavelengths, each of said monochromatic light sources modulated out-of-phase with all other monochromatic light sources.

15. A method for spectrally distinguishing output signals from a device of claim 1, comprising

illuminating particles in said interrogation channel with an incident light wavelength which causes said particles to fluoresce with a fluorescent wavelength different from the incident light wavelength;

measuring the signal to noise ratio of a photodetector signal from a first output optical waveguide and a photodetector signal from a second output optical waveguide, said first and second output optical waveguides positioned at different angles with respect to said interrogation channel;

identifying the fluorescent wavelength emitted by a particle by the ratio of signal to noise ratio at one photodetector to the signal to noise ratio at the other photodetector.

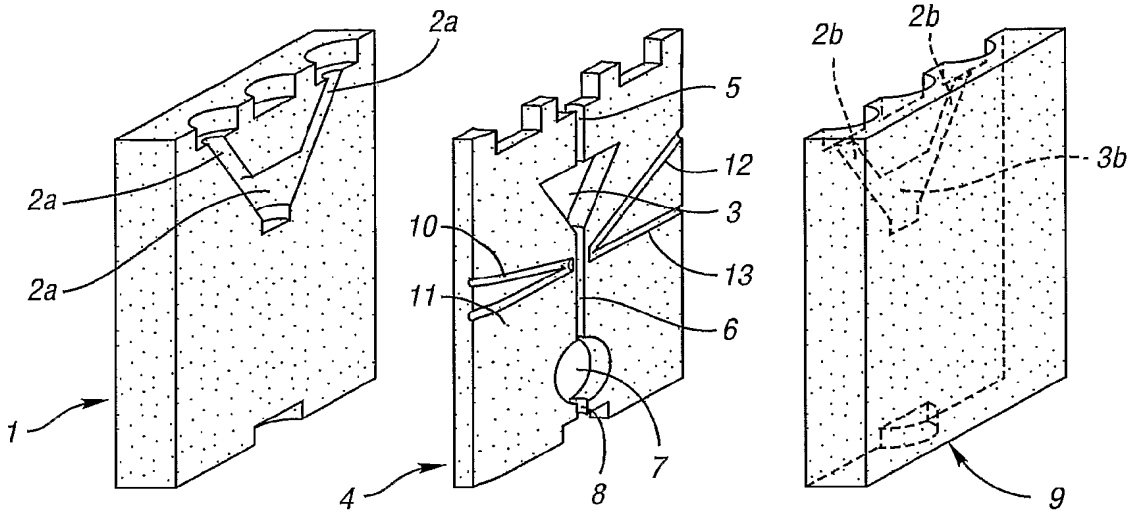

2a
2a
2a
1
4
10
11
5
12
3
13
6
7
8
2b
2b
3b
9

Fig. 1

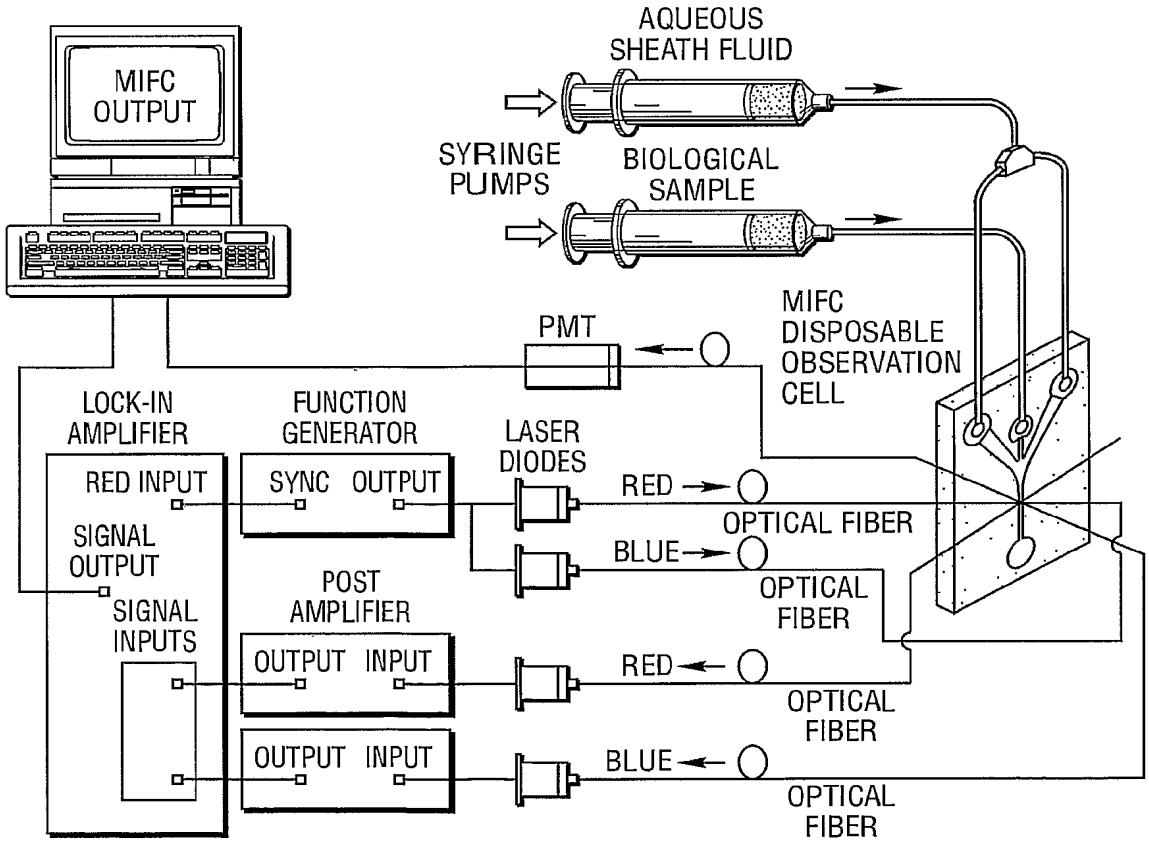

MIFC
OUTPUT
AQUEOUS
SHEATH FLUID
SYRINGE
PUMPS
BIOLOGICAL
SAMPLE
PMT
MIFC
DISPOSABLE
OBSERVATION
CELL
LOCK-IN
AMPLIFIER
FUNCTION
GENERATOR
LASER
DIODES
RED INPUT
SYNC OUTPUT
RED
OPTICAL FIBER
SIGNAL
OUTPUT
BLUE
OPTICAL
FIBER
POST
AMPLIFIER
SIGNAL
INPUTS
OUTPUT INPUT
RED
OPTICAL
FIBER
OUTPUT INPUT
BLUE
OPTICAL
FIBER

Fig. 2

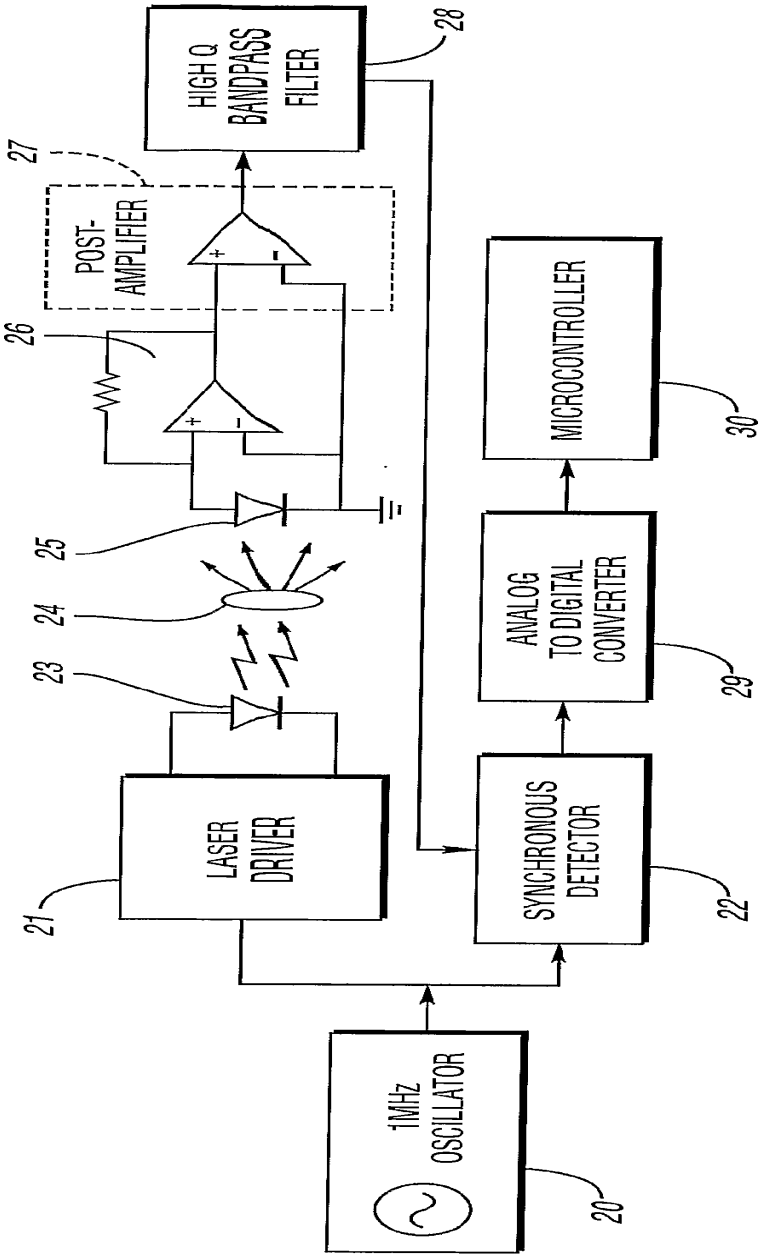

1MHz OSCILLATOR
20
LASER DRIVER
21
SYNCHRONOUS DETECTOR
22
ANALOG TO DIGITAL CONVERTER
29
MICROCONTROLLER
30
23
24
25
26
POST-AMPLIFIER
27
HIGH Q BANDPASS FILTER
28

Fig. 3

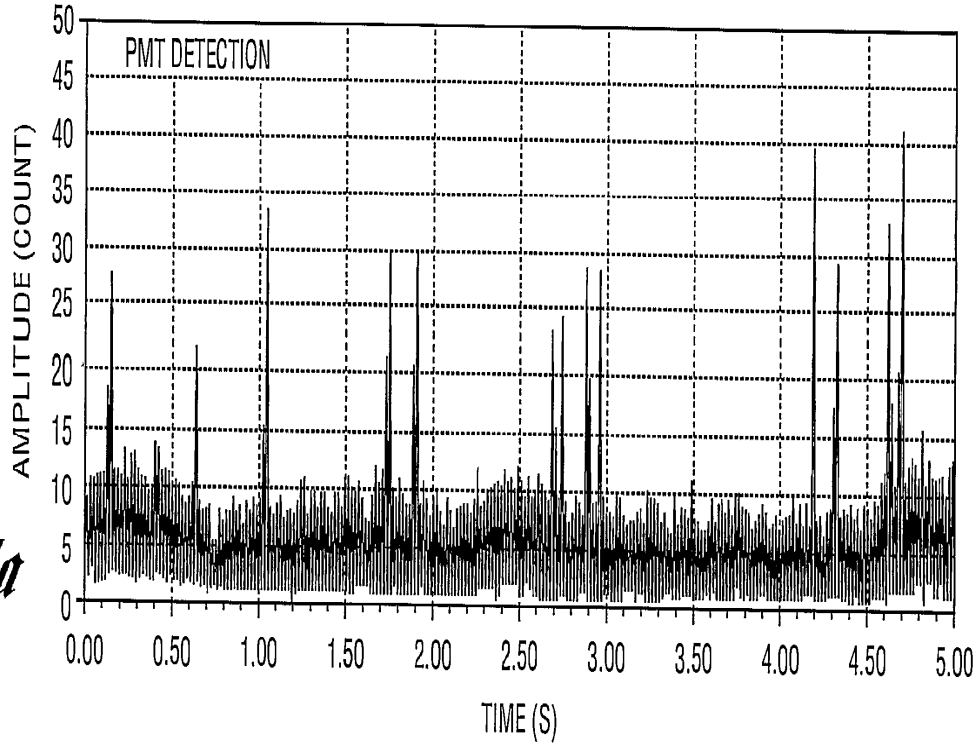

PMT DETECTION
AMPLITUDE (COUNT)
0
5
10
15
20
25
30
35
40
45
50
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
TIME (S)

Fig. 4a

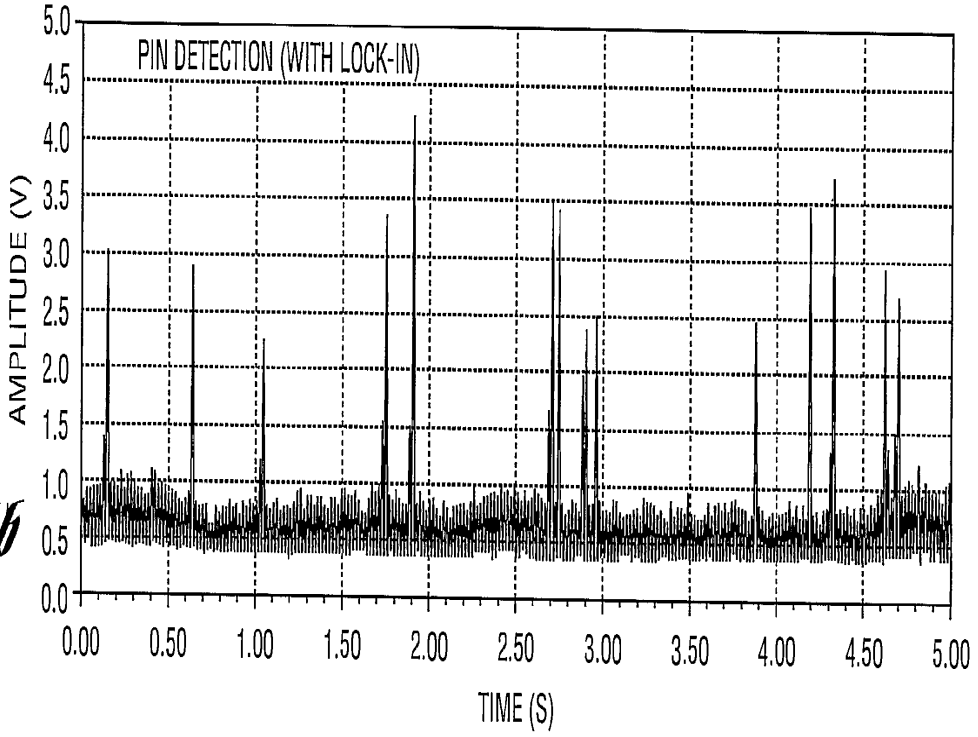

PIN DETECTION (WITH LOCK-IN)
AMPLITUDE (V)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
TIME (S)

Fig. 4b

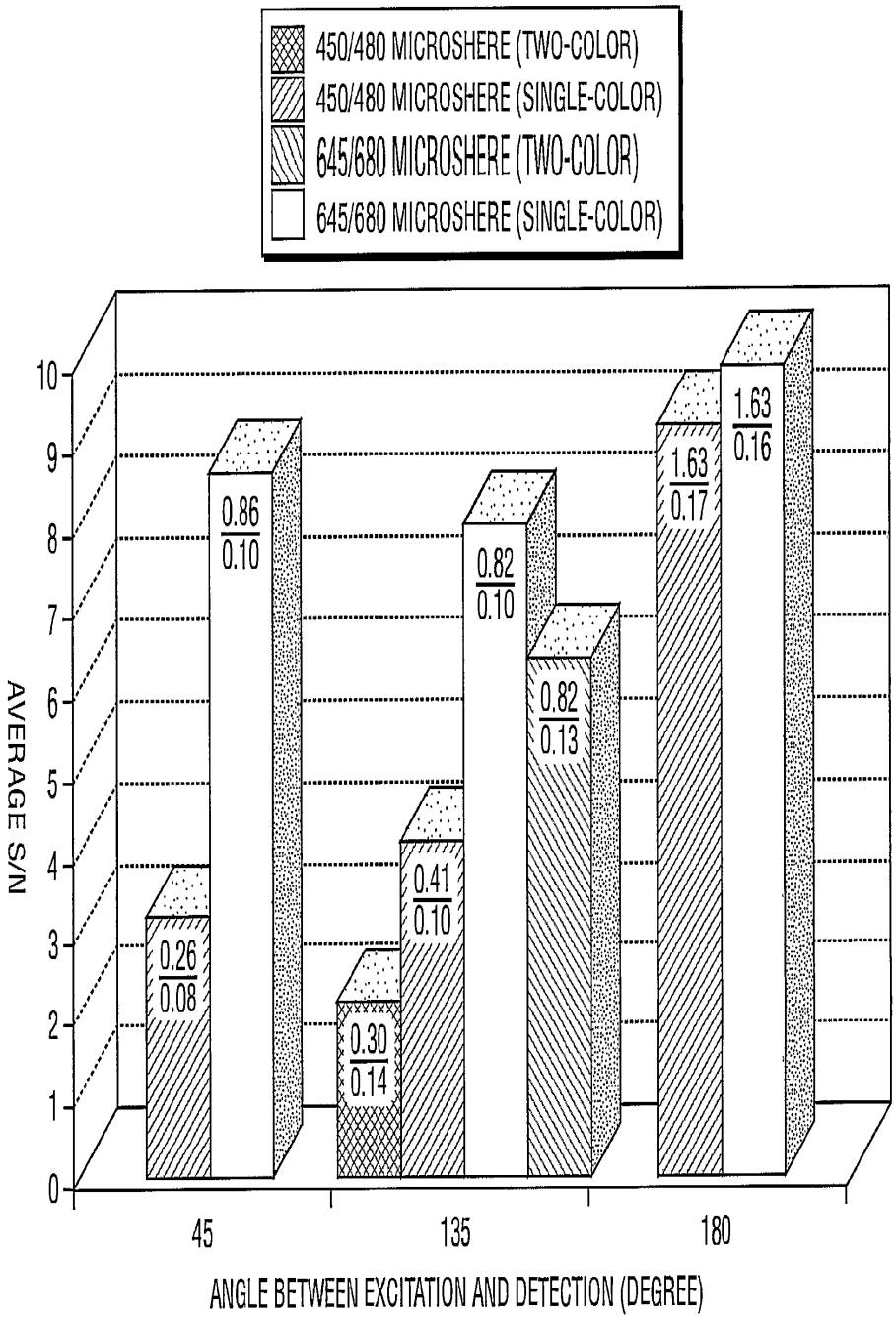
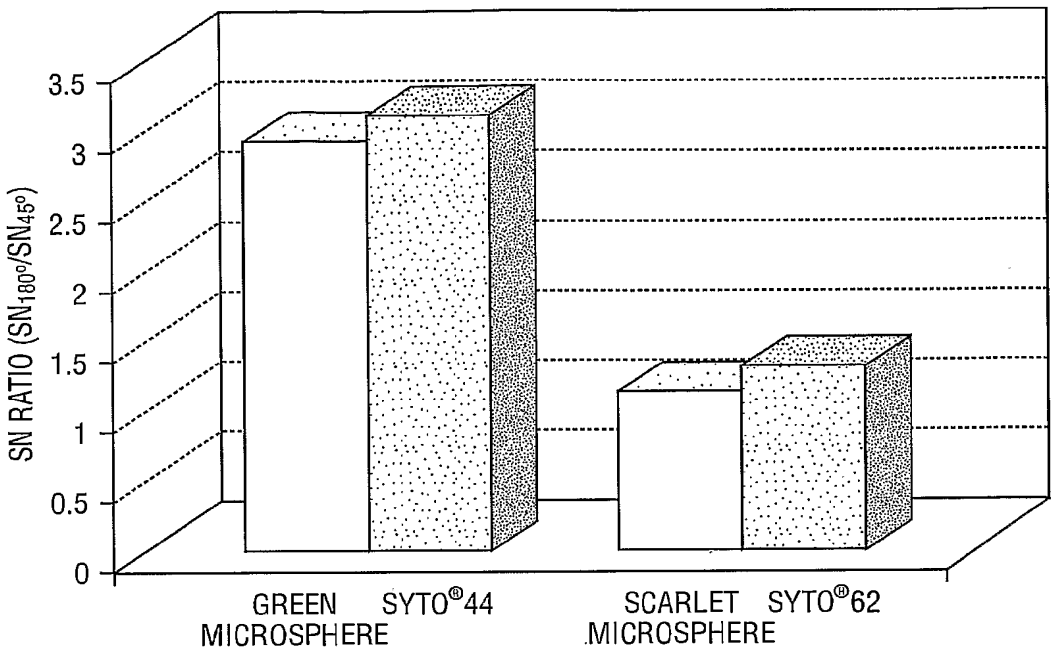

450/480 MICROSHERE (TWO-COLOR)
450/480 MICROSHERE (SINGLE-COLOR)
645/680 MICROSHERE (TWO-COLOR)
645/680 MICROSHERE (SINGLE-COLOR)
AVERAGE S/N
0
1
2
3
4
5
6
7
8
9
10
0.26/0.08
0.86/0.10
0.30/0.14
0.41/0.10
0.82/0.10
0.82/0.13
1.63/0.17
1.63/0.16
45
135
180
ANGLE BETWEEN EXCITATION AND DETECTION (DEGREE)

Fig. 5



AVERAGE SN RATIO ($SN_{180°}/SN_{45°}$)
FOR RED AND BLUE EXCITATION SAMPLE PARTICLES

Fig. 6

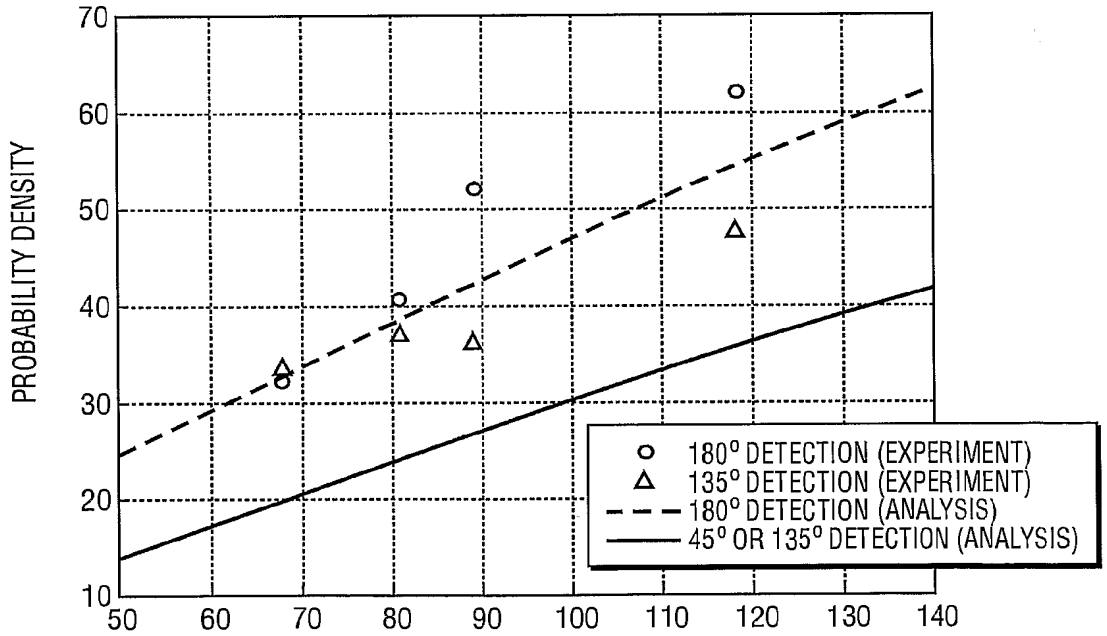


Fig. 7

THE ESTIMATED S/N VARIATION FOR
DIFFERENT SAMPLE COLUMN WIDTH

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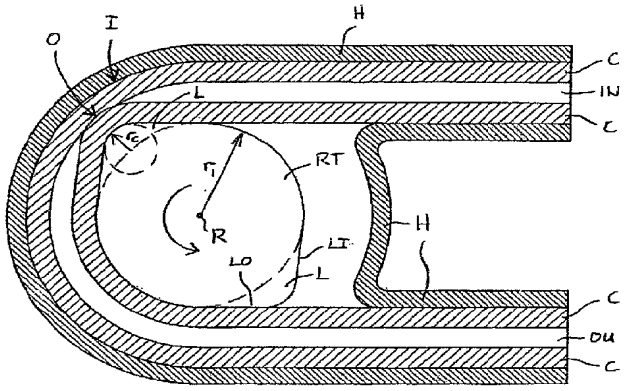
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(54) Title: PERISTALTIC PUMP AND ROTOR



(57) Abstract: A peristaltic pump is described and claimed, the pump comprising: a compressible conduit; an arcuate support surface arranged to support the compressible conduit, the arcuate support surface following a substantially circular path around an axis and the conduit being arranged to extend around the arcuate support surface; and a rotor arranged to rotate about the axis. The rotor comprises at least one lobe arranged to periodically compress the conduit against the support surface to form a constriction and to sweep the constriction along the conduit as the rotor rotates. The rotor has a profile in a plane perpendicular to the axis of rotation, that profile being described by a function r(θ) in polar coordinates where r is the radial distance of a point on the profile from the axis of rotation and θ is the angular position of the point relative to a reference position. The rotor profile comprises a lobe portion corresponding to the lobe and determining the compression of the conduit as the rotor rotates, the lobe portion comprising a curved portion of the rotor profile in which r increases smoothly, with no discontinuity in dr/dθ, from a value r1 at an angle θ1 to a second value r2 at an angle θ2, and dr/dθ = 0 at θ1 and at θ2. A rotor for such a pump is also described and claimed.

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PERISTALTIC PUMP AND ROTOR

Field of the Invention

The present invention relates to peristaltic pumps.

Background to the Invention

Peristaltic pumps are well known, and one general type comprises a mechanical rotor arranged to rotate inside a housing so as to periodically compress a hose or tube against an inner wall of the housing, thereby occluding the hose and sweeping the occlusion axially along the hose as the rotor rotates. If the hose contains a fluid, this action pushes fluid ahead of the occlusion, and the natural resilience of the hose draws in fluid behind the occlusion. Peristaltic pumps offer advantages and find use in a number of applications, such as the pumping of abrasive media, corrosive media, sterile media, and live or shear-sensitive media.

A known peristaltic pump is illustrated schematically in figure 1. The pump comprises a housing H inside which a compressible hose C is arranged to follow a generally U-shaped path from an inlet IN to an outlet OU. The housing includes an inner surface I which follows a circular path, centred on an axis R, against which the hose C is periodically compressed by lobes L of a rotor RT as the rotor rotates about the axis R. Compression of the hose forms an occlusion O which is axially swept along the hose as the rotor rotates. In this known design, the rotor is a single, solid component, having a base radius r1. The outer tip of each lobe has a relatively small radius of curvature rc which is approximately (1/4).r1. Each lobe also comprises straight lead-in and lead-out surfaces LI, LO which are tangential to the curved tips and the portion of the rotor having the base radius r1. A problem with the pump shown in figure 1, however, is that when the rotor is driven at constant speed the pump produces a pulsating discharge. Pulsation can be regarded as the repeated acceleration and deceleration of the pumped media, and is undesirable in some circumstances. For example, uneven flow throughout each cycle makes metering difficult.

Peristaltic pumps with other forms of rotors are also known. For example, rotors are known in which the hose-compressing members are rollers, of relatively small diameter compared with the overall rotor diameter, mounted on suitable supports or arms. Also known are rotors in which curved "shoes" are attached to a rotor body having a basic radius, the shoes extending radially outwards from the body to provide hose-compressing structures. The curved hose-compressing surfaces of these shoes typically have a constant radius of curvature (the surfaces have profiles which follow circular paths), that radius of curvature being substantially smaller than the basic radius of the rotor body. However, a problem with pumps employing these roller- and shoe-type rotors is again that they produce pulsations.

GB 2 290 582 A describes an attempted solution to the pulsation problem. That document discloses a peristaltic pump having a rotor comprising two diametrically opposed rollers arranged to periodically compress a flexible pipe against internal support walls. These support walls comprise a substantially semi-circular wall of constant radius, centred on the rotor axis, joined to walls of larger radius having centres offset from the rotor axis. One of these walls of larger radius supports the flexible pipe on the lead in to the semi-circular section, providing gradual closure of the pipe, and the other supports the flexible pipe on the lead out from the semi-circular section, providing gradual opening of the pipe. Although reducing pulsations, a disadvantage of the disclosed device is that it requires a particular support wall configuration, and so is not applicable to pumps in which the housing has already been manufactured to provide a typical semi-circular, part-circular, or U-shaped support surface.

An important factor associated with the use of peristaltic pumps is hose life. Hose life has previously been regarded as a quantity which could simply be expressed in terms of a number of compressions to which the hose could be subjected. Thus, doubling rotor speed or changing from a two-lobe rotor to a four-lobe rotor, keeping rotor speed constant, would result in the hose life expiring in half the time.

However, the present inventor has determined that this "rule of thumb" for hose life represents an oversimplification; rather than the lifetime of a hose simply being determined by the number of times it can be compressed, the inventor has determined that the shape (i.e. profile) of the peristaltic pump rotor, and in particular

the shape of its lobes, has a major effect on hose life, pumping performance, and other pumping characteristics. Observations and analysis of known rotor profiles have led to the inventor determining that on contact with, and on decompression of the hose, prior art rotors have created acceleration spikes, resulting in large cyclic pressure changes in the hose. The fluid in the hose transmits these pressure fluctuations, which are absorbed by the hose at the point where the rotor leaves the hose after compression. This is typically the only area where the hose is both under discharge pressure and unconstrained by the housing and rotor. Hence the hose is free to stretch like a balloon, which it does, causing fatigue and eventually resulting in failure.

With regard to the pump shown in figure 1, the inventor has determined that the curved tips of the lobes, with small radii, in particular result in the hose wall experiencing relatively high accelerations as the rotor lobes strike and then leave the hose. The high transient pressures generated in the pumped fluid can make hose life very short, and may cause pipe work to rupture

With regard to peristaltic pumps comprising the roller- and shoe-type rotors described above, the inventor has determined that when one considers the dynamics of how the rotor compresses the hose, there is a sharp increase in velocity of the hose wall, and hence it experiences a large acceleration, as the rotor both strikes and leaves the hose. Thus, the hose is subjected to a large force, and there is also a rapid change in fluid velocity inside the hose (which is manifested as a pressure pulsation). Both of these components contribute to hose fatigue, and can ultimately lead to premature failure.

These problems will be better appreciated from a consideration of figures 2-5. Figure 2 is a highly schematic representation of a known rotor, in which two shoes S are attached to a rotor body B of basic radius r1. The shoes extend the rotor to a peak radius of r2 (i.e. the shoes provide a lift h, where $h = r2 - r1$). Each shoe has an outer surface which has a generally semi-circular profile, of radius rc. Looking at just one quadrant of the overall profile of the rotor, which in polar coordinates can be expressed as a function $r(\theta)$, we see that r is constant at $r = r1$ for $0 \leq \theta \leq x$, but then as we enter the portion of the profile corresponding to the shoe there is an abrupt transition to increasing r; there is a discontinuity in $dr/d\theta$ at x. As a result, the second

derivative of r with respect to θ, which is proportional to the acceleration experienced by a flexible hose being displaced by the rotating rotor, includes a large spike at x.

It is therefore an object of embodiments of the present invention to provide peristaltic pumps and peristaltic pump rotors which overcome, at least partially, one or more of the above-described problems associated with the prior art. Particular embodiments aim to reduce hose (i.e. conduit) fatigue and reduce pressure pulsations (previously caused by rapid hose compression). Embodiments also aim to provide increased hose life and/or enable the pump to be operated at higher rotor speeds.

Summary of the Invention

According to a first aspect of the present invention, there is provided a peristaltic pump comprising:

a compressible conduit;

an arcuate support surface arranged to support the compressible conduit, the arcuate support surface following a substantially circular path around an axis and the conduit being arranged to extend around the arcuate support surface; and

a rotor arranged to rotate about said axis and comprising at least one lobe arranged to periodically compress the conduit against the support surface to form a constriction and to sweep the constriction along the conduit as the rotor rotates, the rotor having a profile in a plane perpendicular to the axis of rotation, the profile being described by a function $r(\theta)$ in polar coordinates where r is the radial distance of a point on the profile from the axis of rotation and θ is the angular position of the point relative to a reference position,

wherein the rotor profile comprises a lobe portion corresponding to the lobe and determining the compression of the conduit as the rotor rotates, the lobe portion comprising a curved portion of the rotor profile in which r increases smoothly, with no discontinuity in $dr/d\theta$, from a value r1 at an angle $\theta 1$ to a second value r2 at an angle $\theta 2$, and $dr/d\theta = 0$ at $\theta 1$ and at $\theta 2$.

Employing a rotor of the defined profile provides the advantage that pressure pulsations in pump output are reduced compared with the prior art, and the accelerations experienced by the conduit material are reduced, thereby increasing conduit life for a given rotor speed, or enabling increased rotor speed to be used for the same conduit life.

In certain preferred embodiments the arcuate support surface is concave, although the invention also finds application in peristaltic pumps where the rotor compresses a conduit radially inwards against a convex support surface.

It will be appreciated that the description of the support surface following a circular path is not intended to require that the surface extends around a complete circle. Instead, it merely indicates that the surface follows a circular path for an arc of at least a few degrees. In certain embodiments, the extent of the support surface may be approximately 180 degrees, such that the surface has semicircular profile, but in other embodiments the extent may be less or more than 180 degrees.

It will also be appreciated that the profile of the rotor is to be understood as an overall shape or outline of the rotor on a macroscopic scale related to the size and flexibility of the conduit. Features on the rotor surface which are small enough to have no substantial influence on the deformation of the conduit by the rotor are not to be regarded as part of the rotor profile. Similarly, the support surface follows a generally circular path, and small deviations from a perfect circular path, having no substantial effect on the pump operation, are to be disregarded.

Preferably, the lobe portion further comprises a curved portion of the rotor profile in which r decreases smoothly, with no discontinuity in $dr/d\theta$, from value r2 at angle $\theta 3$ to value r1 at an angle $\theta 4$, where $\theta 4 > \theta 3 \geq \theta 2 > \theta 1$ and $dr/d\theta = 0$ at $\theta 3$ and at $\theta 4$. Thus, in addition to providing reduced conduit acceleration on compression, embodiments may also provide reduced acceleration as the conduit is released.

In certain embodiments $(\theta 4 - \theta 3) = (\theta 2 - \theta 1)$, such that the rise and fall intervals are the same. The rise and fall portions may furthermore be symmetrical.

In certain embodiments, $\theta 3 = \theta 2$, such that there is no dwell at maximum radius (i.e. at maximum lift).

The lobe portion may have a radius of curvature rc2 at θ2, where rc2>(1/3)r1, and even more preferably rc2 may be equal to r2.

In alternative embodiments, θ3>θ2 and the lobe portion comprises a curved portion of the rotor profile in which r is substantially constant at value r2 from θ2 to θ3. In other words, the profile may include a dwell at maximum lift, which provides the advantage that a higher pressure seal may be achieved. A low dwell will increase the slug size of the pump, and a high dwell will improve the sealing of the hose and its ability to develop high pressure without over compression.

In addition, or alternatively, the rotor profile may comprise a portion of constant radius r1 immediately adjacent the lobe portion on one side of the lobe portion (e.g. preceding the lobe portion, relative to the direction of rotation of the rotor in normal use). The rotor profile may also comprise a second portion of constant radius r1 immediately adjacent the lobe portion and on the opposite side of the lobe portion (e.g. following the lobe portion). Thus, the rotor may comprise dwells at base radius r1 before and/or after the or each lobe.

In certain preferred embodiments, the curved portion of the profile in which r increases smoothly is described by the relation $r = r1 + ((r2 - r1)/2).(1 - \cos(\pi(\theta - \theta1)/(\theta2 - \theta1)))$ for $\theta1 \leq \theta \leq \theta2$. Similarly, the curved portion of the profile in which r decreases smoothly may be described by the relation $r = r1 + ((r2 - r1)/2).(1 - \cos(\pi(\theta4 - \theta)/(\theta4 - \theta3)))$ for $\theta3 \leq \theta \leq \theta4$.

Preferably, the pump rotor comprises two lobes, the rotor profile comprising two lobe portions each corresponding to a respective one of the two lobes. The two lobes may be diametrically opposed on the rotor, and/or may be separated by portions of the rotor profile having constant radius r1 (i.e. dwells).

The pump rotor may comprise a plurality of lobes, the rotor profile comprising a plurality of lobe portions each corresponding to a respective one of the plurality of lobes, and the plurality of lobes may be evenly spaced around the rotor. Each adjacent pair of lobe portions may be separated by a respective dwell.

In certain preferred embodiments the rotor profile is elliptical.

In alternative preferred embodiments the rotor profile is given by:

$r = r1$ for $(-\alpha) \leq \theta \leq \alpha$

and $(\pi - \alpha) \leq \theta \leq (\pi + \alpha)$;

$r = r1 + (h/2).[1 - \cos(\pi(\theta - \alpha) / \beta)]$

for $\alpha \leq \theta \leq (\alpha + \beta)$

and $(\pi + \alpha) \leq \theta \leq (\pi + \alpha + \beta)$;

$r = r1 + h$ for $(\alpha + \beta) \leq \theta \leq \pi - (\alpha + \beta)$

and $(\pi + \alpha + \beta) \leq \theta \leq 2\pi - (\alpha + \beta)$; and

$r = r1 + (h/2).[1 - \cos(\pi(\theta + \alpha - \pi) / \beta)]$

for $\pi - (\alpha + \beta) \leq \theta \leq \pi - \alpha$

and $2\pi - (\alpha + \beta) \leq \theta \leq 2\pi - \alpha$

where

r1 = a base circle radius,

h = a radial lift provided by each lobe = r2 –r1,

α = an angle, per quadrant (i.e. per 90 degree, or $\pi/2$ radian section), over which the rotor profile has a radius equal to the base circle radius,

β = a rise angle, over which the radius of each lobe portion smoothly increases from r1 to r2, and a fall angle over which the radius of each lobe portion smoothly decreases from r2 to r1.

Although embodiments of the invention may utilise flexible conduits of various forms, in certain preferred embodiments the conduit is a flexible hose.

The circular path followed by the arcuate support surface may have a radius r3, and r3 – r1 may correspond to a radial thickness of the conduit in an uncompressed

state. The quantity r3 – r2 may then correspond to a radial thickness of the conduit in a compressed state in which the conduit is fully occluded.

A second aspect of the invention provides a pump rotor for a peristaltic pump of the type comprising a compressible conduit and an arcuate support surface arranged to support the compressible conduit, the arcuate support surface following a substantially circular path around an axis and the conduit being arranged to extend around the arcuate support surface,

the rotor having a rotational axis about which the rotor, in use, is arranged to rotate, and which, in use, is arranged so as to coincide with the axis of the circular path followed by the support surface, the rotor comprising at least one lobe for periodically compressing the conduit against the support surface to form a constriction and sweeping the constriction along the conduit as the rotor rotates, the rotor having a profile in a plane perpendicular to the axis of rotation, the profile being described by a function $r(\theta)$ in polar coordinates where r is the radial distance of a point on the profile from the axis of rotation and theta is the angular position of the point relative to a reference position,

wherein the rotor profile comprises a lobe portion corresponding to the lobe and determining the compression of the conduit as the rotor rotates, the lobe portion comprising a curved portion of the rotor profile in which r increases smoothly, with no discontinuity in $dr/d\theta$, from a value r1 at an angle $\theta 1$ to a second value r2 at an angle $\theta 2$, and $dr/d\theta = 0$ at $\theta 1$ and at $\theta 2$.

It will be appreciated that a pump rotor embodying the second aspect of the invention may comprise any of the rotor features described above in relation to the first aspect, with corresponding advantage.

Brief description of the Figures

Embodiments of the invention will now be described with reference to the accompanying drawings of which:

Figure 1 is a schematic representation of a peristaltic pump according to the prior art;

Figure 2 is a schematic representation of a peristaltic pump rotor according to the prior art;

Figures 3, 4 & 5 illustrate the radius of the rotor of figure 2 as a function of angular position around the axis R, and its first and second derivates respectively;

Figure 6 illustrates the profile of a rotor embodying the invention, together with the profile of a rotor in accordance with the prior art, for comparison;

Figure 7 is a plot of the radius of the rotor of figure 6 as a function of an angular position, together with the equivalent plot for a rotor according to the prior art;

Figure 8 is a plot indicative of the radial velocity of the surface of the rotor of figure 6 as a function of angular position on the rotor profile, again including a corresponding plot for a rotor in accordance with the prior art;

Figure 9 is a plot indicative of the radial acceleration at the surface of the rotor of figure 6 as a function of angular position on the rotor profile, again with a corresponding plot for a rotor in accordance with the prior art;

Figure 10 illustrates the profile of another peristaltic pump rotor embodying the invention;

Figure 11 illustrates the variation of radius as a function of angular position for the rotor profile shown in figure 10;

Figure 12 is a plot illustrating the variation of radial velocity with angular position for the rotor of figure 10;

Figure 13 is a plot illustrating the variation of radial acceleration as a function of angular position for the rotor of figure 10;

Figure 14 is a plot indicative of the jerk experienced by a hose or other conduit in contact with the surface of the rotor shown in figure 10, as a function of angular position with respect to the rotor, as the rotor rotates;

Figures 15, 16 &17 are front, rear and isometric views respectively of a peristaltic pump rotor embodying the invention;

Figure 18 is a schematic representation of a peristaltic pump embodying the invention;

Figure 19 is a schematic representation of yet another peristaltic pump rotor embodying the invention;

Figure 20 is a schematic representation of a further pump rotor embodying the invention; and

Figure 21 is a schematic representation of part of another peristaltic pump embodying the invention.

Detailed description of Preferred Embodiments

Referring now to figure 6, a first peristaltic pump rotor 1 embodying the invention comprises a core portion 10 of base radius b, and two diametrically opposed lobes 2a and 2b. Each lobe provides a lift, h. The profile of the rotor comprises a first lobe portion 3a and a second lobe portion 3b. Lobe portion 3a comprises a curved portion of the rotor profile P in which the radius r increases smoothly, with no discontinuity in $dr/d\theta$, from value b at 0° to (b + h) at 90°. Furthermore, $dr/d\theta$ equals zero at 0° and at 90°. Similarly the first lobe portion comprises another curved portion of the rotor profile from 90° to 180°, in which r decreases smoothly from (h + b) to b. The second lobe has the same shape as the first. Also shown in figure 6 is a broken line indicating the profile of a rotor in accordance with the prior art. In this prior art rotor, rather than comprising only curved portions of the rotor profile, the lobes have lead in and lead out surfaces which are straight in profile. These straight sections connect the portion of the prior art rotor having the base radius to the lobe tips, which are curved sections with relatively small radii of curvature. Figure 7 shows the variation of the rotor profile radius as a function of angle for the rotor

embodying the invention in figure 6 in the first quadrant (i.e. from 0° to 90°) and also shows the corresponding plot, as a broken line, for the prior art rotor depicted in figure 6. Figure 8 is a corresponding velocity plot, which corresponds to the first derivative of the relationships shown in figure 7. Figure 9 is a corresponding plot of radial acceleration, which corresponds to the second derivative of the relationship shown in figure 7. It will be seen, from figure 9 in particular, that the prior art rotor, in use, would result in the adjacent compressible hose or other conduit experiencing a high acceleration spike, and hence a high force. In contrast, when using the pump rotor embodying the invention, illustrated in figure 6, the acceleration experienced by the pump conduit is considerably reduced.

Referring now to figure 10, a further peristaltic pump rotor embodying the invention comprises two lobes 2a, 2b diametrically opposed about the rotor axis of rotation R. The rotor profile is symmetrical. The profile may be divided into segments, of which just five s1-s5 are labelled on the figure. The first lobe portion of the profile, which corresponds to the first lobe 2a, consists of segments s2, s3, and s4. In segments s1 and s5 the rotor profile has constant radius, equal to the base radius b. Segment s2, a rise segment, is a curved portion of the profile in which r increases smoothly, with no discontinuity in $dr/d\theta$, from b at an angle α to $(b + h)$ at an angle $(\alpha + \beta)$, and $dr/d\theta = 0$ at $\theta = \alpha$ and at $\theta = (\alpha + \beta)$. The first lobe portion then comprises segment s3, from $(\alpha + \beta)$ to $(180 - (\alpha + \beta))$, over which radius r remains constant at $(b + h)$. Finally, the first lobe portion comprises segment s4, a fall segment, which is a curved portion of the profile in which r decreases smoothly, with no discontinuity in $dr/d\theta$, from $(b + h)$ at an angle $(180 - (\alpha + \beta))$ to b at an angle $(180 - \alpha)$ and $dr/d\theta = 0$ at $\theta = (180 - (\alpha + \beta))$ and at $\theta = (180 - \alpha)$. The second lobe 2b has the same shape as the first lobe 2a. Segments s1 and s5, in which radius is constant may be described as base radius dwells. Similarly, segment s3, and the corresponding unlabelled segment of the second lobe profile, may be described as maximum lift dwells. The incorporation of maximum lift dwells provides the advantage that the rotor can produce an extended seal (occlusion) along the hose, which is able to withstand higher pressures without necessitating overcompression of the hose material. By avoiding overcompression, hose life can be greatly extended. Thus, by incorporating a rotor as shown in figure 10, one can provide a pump with high pressure capabilities and with long hose life.

The profile of the rotor of figure 10 can be described as follows:

Segments 1 and 5 – constant base radius dwell

$s = b$ where $(180 - \alpha) \leq \theta \leq \alpha$

Segment 2 – rise

$s = b + (h/2)[1 - \cos(\pi(\theta - \alpha)/\beta)]$

where $\alpha \leq \theta \leq (\alpha + \beta)$

Segment 3 – constant max lift dwell

$s = b + h$ where $(\alpha + \beta) \leq \theta \leq (180 - (\alpha + \beta))$

Segment 4 – fall

$s = b + (h/2)[1 - \cos(\pi(\theta + \alpha - 180)/\beta)]$

where $(180 - (\alpha + \beta)) \leq \theta \leq (180 - \alpha)$

where:

s = radius of profile (or, equivalently, displacement)

b = base circle radius

h = radial lift

θ = angle

α = angle of constant base circle radius per 90 degree section

β = rise / fall angle.

The rise segment may be referred to as a sinusoidal rise, from b to (b + h), and the fall segment may similarly be referred to as a sinusoidal fall. The overall algorithm may be referred to as a sinusoidal displacement algorithm, with dwells.

In embodiments of the invention these parameters may be selected in the following ways:

Base circle radius b is the amount of displacement required to produce zero compression on the hose (or other form of conduit).

Radial lift h is the extra amount required when added to the base circle radius to go from zero hose compression to a fully sealed state. This figure will usually be a little larger than the hose bore.

The angle of constant base circle radius, α, is used to increase the volume per slug. However, increasing this value also increases the peak velocity and acceleration on the hose, so α should be selected to obtain a suitable compromise.

The rise and fall angles β should ideally be as long as possible in order to minimise peak velocity and accelerations and to help prevent inversions (i.e. negative curvatures in cartesian space).

The angle of constant lift (providing the dwells at maximum radius) is used to provide an extended contact and extended seal along the hose, rather than a point contact. This angle is represented by the quantity $(90 - (\alpha + \beta))$ in figure 10. This enables high pressure to be developed without the need for shims and over compression which would have a detrimental effect on hose life. However, it should be noted that increasing the extent of the dwells at maximum radius decreases the volume per slug of pump output.

It should be noted that the above algorithm is valid only for $(\alpha + \beta) \leq 90$ degrees. The rise algorithms are designed to work in the range 0 – 90 degrees and the fall algorithms in the range 90 – 180 degrees, and produce a two-lobe rotor.

Figure 11 is a plot of lift versus rotor angle over the five segments s1-s5 for the rotor of figure 10 (and hence corresponds to a plot of rotor profile radius, r, as a function of θ, the angle from the nominal zero degree reference position on the profile). Figure 12 is a corresponding "velocity" plot, which may also be regarded as

a plot of $dr/d\theta$ as a function of theta. Figure 13 is the corresponding "acceleration" plot, which may also be regarded as a plot of $d^2r/d\theta^2$ as a function of θ. Figure 14 illustrates the corresponding "jerk" , which corresponds to $d^3r/d\theta^3$.

From figures 11 to 14 it will be appreciated that the above-mentioned sinusoidal displacement algorithm, with dwells, produces non-zero acceleration at the ends of each section. However, the peak accelerations are very small compared with those produced by prior art rotor profiles, and the rotor enables substantially reduced pulsation and increased hose life to be achieved.

Thus, the rotor of figure 10 is designed to smooth the pressure peaks in a pumped fluid by controlling velocity and acceleration of the hose walls. It does, however, reduce flow per revolution (and hence flow rate for a given rpm) to approximately 75% of that achievable with an equivalent rotor having the shape illustrated in figure 1, and so requires an increase of approximately 33% to achieve an equivalent flow rate. However, even with this speed increase, the peak acceleration is much reduced using the rotor of figure 10.

Referring now to figures 15-17, another peristaltic pump rotor 1 embodying the invention is a one-piece component comprising a central hub, with an array of attachment holes, and two lobes 2a, 2b. In this embodiment the hub and lobes have been machined from a single piece of suitable material, although in alternative embodiments the rotor may be an assembly of separate components. The rotor profile P consists of a first lobe portion 3a and a second lobe portion 3b, and in this embodiment has no dwells. The lobes provide a smooth, continuously curving, hose-engaging surface 6 for engaging (directly or indirectly) a pump hose so as to produce periodic compression. The rotor profile is given by the relation:

$$r = r1 + (r2 - r1)/2.[1 - \cos(2.\theta)] \qquad \text{for } 0 \leq \theta \leq 360 \text{ degrees}$$

Moving on to figure 18, this shows a peristaltic pump embodying the invention. The peristaltic pump comprises a compressible conduit in the form of a hose 8, having an inlet and an outlet, and arranged to follow a substantially U-shaped path within a pump housing 7. The housing 7 provides a concave arcuate support surface 71 which is arranged to support the compressible conduit 8. The arcuate support surface follows

a substantially circular path of radius r3 around an axis R (in this example the support surface is part-cylindrical, with a semicircular profile) and the conduit is arranged to extend axially around the arcuate support surface. The housing 7 also provides straight sections of support surface 72 adjacent the straight portions of the U-shaped conduit. A rotor 1 is arranged inside the housing 7 to rotate about the axis R and has an elliptical profile, having a minor radius r1 and a major radius r2. The elliptical rotor thus provides two lobes which periodically compress the conduit 8 against the support surface 71 to form a constriction 82 and sweep the constriction along the conduit as the rotor rotates. It will be appreciated that the elliptical profile may be described by a function $r(\theta)$ in polar coordinates where r is the radial distance of a point on the profile from the axis of rotation R and theta is the angular position of the point relative to a reference position. The rotor profile comprises two lobe portions, which determine the compression of the conduit as the rotor rotates, each lobe portion comprising a respective curved portion of the rotor profile in which r increases smoothly, with no discontinuity in $dr/d\theta$, from a value r1 at a respective first angle to a second value r2 at a respective second angle, and $dr/d\theta = 0$ at the respective first and second angles. In figure 18, the quantity r3 –r1 corresponds to the thickness t of the conduit 8 in an uncompressed state, and r3 – r2 corresponds to the radial thickness of the conduit in a compressed state in which the conduit is fully occluded.

Moving on to figure 19, another pump rotor embodying the invention comprises three lobes 2a, 2b, 2c providing a radial lift (r2 –r1) from a body portion 10. The lobes are evenly spaced around the rotational axis R, and between each pair of adjacent lobes there is a respective dwell portion of constant radius r1. All three lobes have the same shape. Considering just the first lobe 2a, between angles a1 and a2 its profile is curved and r increases smoothly, with no discontinuity in $dr/d\theta$, from a value r1 at angle a1 to r2 at angle a2, and $dr/d\theta = 0$ at a1 and at a2. Similarly, between angles a2 and a3 its profile is curved and r decreases smoothly, with no discontinuity in $dr/d\theta$, from r2 to r1, and $dr/d\theta = 0$ at a2 and at a3. The curved rise portion between a1 and a2 may be a sinusoidal rise as described above, or may have a different curved shape. Similarly, the fall portion between a2 and a3 may be sinusoidal or a different smooth, curved shape. The rotor of figure 19 with long base radius dwells can produce long slugs and reduced hose accelerations, to prolong hose life.

Figure 20 is a highly schematic representation of a fabricated rotor, having a body portion 10 with a rectangular profile, and two lobes 2a, 2b attached to the body to provide lift displacement from r1 to r2. Although the overall rotor profile includes flats, the lobe portions of the profile are curved, and satisfy the relation that $dr/d\theta$ equals zero at the beginning and end of each rise and fall portion.

Figure 21 is a highly schematic representation of part of another pump embodying the invention. Here, the support surface 71 is convex, and the rotor 1 is arranged to rotate around the outside of the support surface. Thus, rather than the rotor having lobes projecting radially outwards, in this example the two lobes 2a and 2b project radially inwards, so as to compress a hose arranged between the rotor and the convex support surface. The lobes have the same shape, and considering just the first lobe 2a, it has a curved profile between angle $\theta 1$ and angle $\theta 2$ in which r smoothly increases, and $dr/d\theta$ is zero at both $\theta 1$ and $\theta 2$.

It will be appreciated that pumps embodying the present invention are able to offer reduced pulsation compared with the prior art. Rotor profiles in embodiments of the invention are shaped so as to reduce, and even minimise, the rate of change of hose internal volume, and hence reduce, or minimise, the rate of change of flow of the pumped medium (i.e. accelerations and decelerations are reduced). Thus, embodiments of the invention are able to increase hose life (by a factor of ten or more, increase pumphead reliability, increase overall system reliability (as there is a lower risk of pipework rupture with reduced pressure pulses), increase pump efficiency , provide a pumping mechanism suitable for materials with even greater shear sensitivity, and provide an improved metering capability (metering is facilitated as pulsations are reduced).

By selecting a rotor profile to control the dynamics of the rotor on the hose, embodiments of the invention are able to reduce peak velocity and acceleration, offering a substantial improvement over prior art pumps in which the rotors created large acceleration spikes in their actions on the hose.

Embodiments of the invention are able to reduce fatigue on the pump hose by controlling the fluctuations of pressure in the hose, and hence pump flow may be

increased by increasing rotor speed, whilst retaining the same, or similar, hose life as in prior art pumps operating at reduced speed.

CLAIMS:

1. A peristaltic pump comprising:

a compressible conduit;

an arcuate support surface arranged to support the compressible conduit, the arcuate support surface following a substantially circular path around an axis and the conduit being arranged to extend around the arcuate support surface; and

a rotor arranged to rotate about said axis and comprising at least one lobe arranged to periodically compress the conduit against the support surface to form a constriction and to sweep the constriction along the conduit as the rotor rotates, the rotor having a profile in a plane perpendicular to the axis of rotation, the profile being described by a function $r(\theta)$ in polar coordinates where r is the radial distance of a point on the profile from the axis of rotation and θ is the angular position of the point relative to a reference position,

wherein the rotor profile comprises a lobe portion corresponding to the lobe and determining the compression of the conduit as the rotor rotates, the lobe portion comprising a curved portion of the rotor profile in which r increases smoothly, with no discontinuity in $dr/d\theta$, from a value r1 at an angle $\theta 1$ to a second value r2 at an angle $\theta 2$, and $dr/d\theta = 0$ at $\theta 1$ and at $\theta 2$.

2. A pump in accordance with claim 1, wherein the arcuate support surface is concave.

3. A pump in accordance with any preceding claim, wherein the lobe portion further comprises a curved portion of the rotor profile in which r decreases smoothly, with no discontinuity in $dr/d\theta$, from value r2 at angle $\theta 3$ to value r1 at an angle $\theta 4$, where $\theta 4 > \theta 3 \geq \theta 2 > \theta 1$ and $dr/d\theta = 0$ at $\theta 3$ and at $\theta 4$.

4. A pump in accordance with claim 3, wherein $(\theta 4 - \theta 3) = (\theta 2 - \theta 1)$.

5. A pump in accordance with claim 3 or claim 4, wherein $\theta 3 = \theta 2$.

6. A pump in accordance with any preceding claim, wherein the lobe portion has a radius of curvature rc2 at $\theta 2$, where $rc2 > (1/3)r1$.

7. A pump in accordance with claim 6, wherein $rc2 = r2$.

8. A pump in accordance with claim 3 or claim 4, wherein $\theta 3 > \theta 2$ and the lobe portion comprises a curved portion of the rotor profile in which r is substantially constant at value r2 from $\theta 2$ to $\theta 3$.

9. A pump in accordance with any preceding claim, wherein the rotor profile comprises a portion of constant radius r1 immediately adjacent the lobe portion on one side of the lobe portion.

10. A pump in accordance with claim 9, wherein the rotor profile comprises a second portion of constant radius r1 immediately adjacent the lobe portion and on the opposite side of the lobe portion.

11. A pump in accordance with any preceding claim wherein said curved portion of the profile in which r increases smoothly is described by the relation $r = r1 + ((r2 - r1)/2).(1 - \cos(\pi(\theta - \theta 1)/(\theta 2 - \theta)))$ for $\theta 1 \leq \theta \leq \theta 2$.

12. A pump in accordance with claim 3, or any one of claims 4 to 11 as dependent on claim 3, wherein said curved portion of the profile in which r decreases smoothly is described by the relation $r = r1 + ((r2 - r1)/2).(1 - \cos(\pi(\theta 4 - \theta)/(\theta 4 - \theta 3)))$ for $\theta 3 \leq \theta \leq \theta 4$.

13. A pump in accordance with any preceding claim, comprising two said lobes, the rotor profile comprising two said lobe portions each corresponding to a respective one of the two lobes.

14. A pump in accordance with claim 13, wherein the two said lobes are diametrically opposed on the rotor.

15. A pump in accordance with claim 13 or claim 14 wherein said two lobe portion are separated by portions of the rotor profile having constant radius r1.

16. A pump in accordance with any one of claims 1 to 12, comprising a plurality of said lobes, the rotor profile comprising a plurality of said lobe portions each corresponding to a respective one of the plurality of lobes.

17. A pump in accordance with claim 16, wherein the plurality of lobes are evenly spaced around the rotor.

18. A pump in accordance with claim 16 or claim 17 wherein each adjacent pair of lobe portions are separated by a respective portions of the rotor profile having constant radius r1.

19. A pump in accordance with any one of claims 1 to 6, wherein the rotor profile is elliptical.

20. A pump in accordance with any one of claims 1 to 4, wherein the rotor profile is given by:

$r = r1$ for $(-\alpha) \leq \theta \leq \alpha$

and $(\pi - \alpha) \leq \theta \leq (\pi + \alpha)$;

$r = r1 + (h/2).[1 - \cos(\pi(\theta - \alpha)/\beta)]$

for $\alpha \leq \theta \leq (\alpha + \beta)$

and $(\pi + \alpha) \leq \theta \leq (\pi + \alpha + \beta)$;

$r = r1 + h$ for $(\alpha + \beta) \leq \theta \leq \pi - (\alpha + \beta)$

and $(\pi + \alpha + \beta) \leq \theta \leq 2\pi - (\alpha + \beta)$; and

$r = r1 + (h/2).[1 - \cos(\pi(\theta + \alpha - \pi)/\beta)]$

for $\pi - (\alpha+\beta) \leq \theta \leq \pi - \alpha$

and $2\pi - (\alpha+\beta) \leq \theta \leq 2\pi - \alpha$

where

r1 = a base circle radius,

h = a radial lift provided by each lobe = r2 –r1,

α = an angle, per quadrant, over which the rotor profile has a radius equal to the base circle radius,

β = a rise angle, over which the radius of each lobe portion smoothly increases from r1 to r2, and a fall angle over which the radius of each lobe portion smoothly decreases from r2 to r1.

21. A pump in accordance with any preceding claim, wherein the conduit is a flexible hose.

22. A pump in accordance with any preceding claim, wherein the circular path followed by the arcuate support surface has a radius r3, and r3 – r1 corresponds to a radial thickness of the conduit in an uncompressed state.

23. A pump in accordance with claim 22, wherein r3 – r2 corresponds to a radial thickness of the conduit in a compressed state in which the conduit is fully occluded.

24. A pump rotor for a peristaltic pump of the type comprising a compressible conduit and an arcuate support surface arranged to support the compressible conduit, the arcuate support surface following a substantially circular path around an axis and the conduit being arranged to extend around the arcuate support surface,

the rotor having a rotational axis about which the rotor, in use, is arranged to rotate, and which, in use, is arranged so as to coincide with the axis of the circular path followed by the support surface, the rotor comprising at least one lobe for periodically compressing the conduit against the support surface to form a constriction and sweeping the constriction along the conduit as the rotor rotates, the rotor having a profile in a plane perpendicular to the axis of rotation, the profile being described by a function $r(\theta)$ in polar coordinates where r is the radial distance of a point on the profile from the axis of rotation and θ is the angular position of the point relative to a reference position,

wherein the rotor profile comprises a lobe portion corresponding to the lobe and determining the compression of the conduit as the rotor rotates, the lobe portion comprising a curved portion of the rotor profile in which r increases smoothly, with

no discontinuity in $dr/d\theta$, from a value r1 at an angle $\theta 1$ to a second value r2 at an angle $\theta 2$, and $dr/d\theta = 0$ at $\theta 1$ and at $\theta 2$.

25. A rotor in accordance with claim 24, wherein the lobe extends radially outwards from the rotor.

26. A rotor in accordance with any one of claims 24 or 25, wherein the lobe portion further comprises a curved portion of the rotor profile in which r decreases smoothly, with no discontinuity in $dr/d\theta$, from value r2 at angle $\theta 3$ to value r1 at an angle $\theta 4$, where $\theta 4 > \theta 3 \geq \theta 2 > \theta 1$ and $dr/d\theta = 0$ at $\theta 3$ and at $\theta 4$.

27. A rotor in accordance with claim 26, wherein $(\theta 4 - \theta 3) = (\theta 2 - \theta 1)$.

28. A rotor in accordance with claim 26 or claim 27, wherein $\theta 3 = \theta 2$.

29. A rotor in accordance with any one of claims 24 to 28, wherein the lobe portion has a radius of curvature rc2 at $\theta 2$, where $rc2 > (1/3)r1$.

30. A rotor in accordance with claim 29, wherein $rc2 = r2$.

31. A rotor in accordance with claim 26 or claim 27, wherein $\theta 3 > \theta 2$ and the lobe portion comprises a curved portion of the rotor profile in which r is substantially constant at value r2 from $\theta 2$ to $\theta 3$.

32. A rotor in accordance with any one of claims 24 to 31, wherein the rotor profile comprises a portion of constant radius r1 immediately adjacent the lobe portion on one side of the lobe portion.

33. A rotor in accordance with claim 32, wherein the rotor profile comprises a second portion of constant radius r1 immediately adjacent the lobe portion and on the opposite side of the lobe portion.

34. A rotor in accordance with any one of claims 24 to 33, wherein said curved portion of the profile in which r increases smoothly is described by the relation $r = r1 + ((r2 - r1)/2).(1 - \cos(\pi(\theta - \theta 1)/(\theta 2 - \theta 1)))$ for $\theta 1 \leq \theta \leq \theta 2$.

35. A rotor in accordance with claim 26, or any one of claims 27 to 34 as dependent on claim 26, wherein said curved portion of the profile in which r

decreases smoothly is described by the relation $r = r1 + ((r2 - r1)/2).(1 - \cos(\pi(\theta4 - \theta)/(\theta4 - \theta3)))$ for $\theta3 \leq \theta \leq \theta4$.

36. A rotor in accordance with any one of claims 24 to 35, comprising two said lobes, the rotor profile comprising two said lobe portions each corresponding to a respective one of the two lobes.

37. A rotor in accordance with claim 36, wherein the two said lobes are diametrically opposed on the rotor.

38. A rotor in accordance with claim 36 or claim 37 wherein said two lobe portion are separated by portions of the rotor profile having constant radius r1.

39. A rotor in accordance with any one of claims 24 to 35, comprising a plurality of said lobes, the rotor profile comprising a plurality of said lobe portions each corresponding to a respective one of the plurality of lobes.

40. A rotor in accordance with claim 39, wherein the plurality of lobes are evenly spaced around the rotor.

41. A rotor in accordance with claim 39 or claim 40 wherein each adjacent pair of lobe portions are separated by a respective portions of the rotor profile having constant radius r1.

42. A rotor in accordance with any one of claims 24 to 29, wherein the rotor profile is elliptical.

43. A rotor in accordance with any one of claims 24 to 27, wherein the rotor profile is given by:

$r = r1$ for $(-\alpha) \leq \theta \leq \alpha$

and $(\pi - \alpha) \leq \theta \leq (\pi + \alpha)$;

$r = r1 + (h/2).[1 - \cos(\pi(\theta - \alpha)/\beta)]$

for $\alpha \leq \theta \leq (\alpha + \beta)$

and $(\pi + \alpha) \leq \theta \leq (\pi + \alpha + \beta)$;

$r = r1 + h$ for $(\alpha + \beta) \leq \theta \leq \pi - (\alpha + \beta)$

and $(\pi + \alpha + \beta) \leq \theta \leq 2\pi - (\alpha + \beta)$; and

$r = r1 + (h/2).[1 - \cos(\pi(\theta + \alpha - \pi) / \beta)]$

for $\pi - (\alpha+\beta) \leq \theta \leq \pi - \alpha$

and $2\pi - (\alpha+\beta) \leq \theta \leq 2\pi - \alpha$

where

r1 = a base circle radius,

h = a radial lift provided by each lobe = r2 –r1,

α = an angle, per quadrant, over which the rotor profile has a radius equal to the base circle radius,

β = a rise angle, over which the radius of each lobe portion smoothly increases from r1 to r2, and a fall angle over which the radius of each lobe portion smoothly decreases from r2 to r1.

44. A peristaltic pump substantially as hereinbefore described with reference to figures 6 to 21 of the accompanying drawings.

45. A peristaltic pump substantially as shown in figures 6 to 21 of the accompanying drawings.

46. A peristaltic pump rotor substantially as hereinbefore described with reference to figures 6 to 21 of the accompanying drawings.

45. A peristaltic pump rotor substantially as shown in figures 6 to 21 of the accompanying drawings.

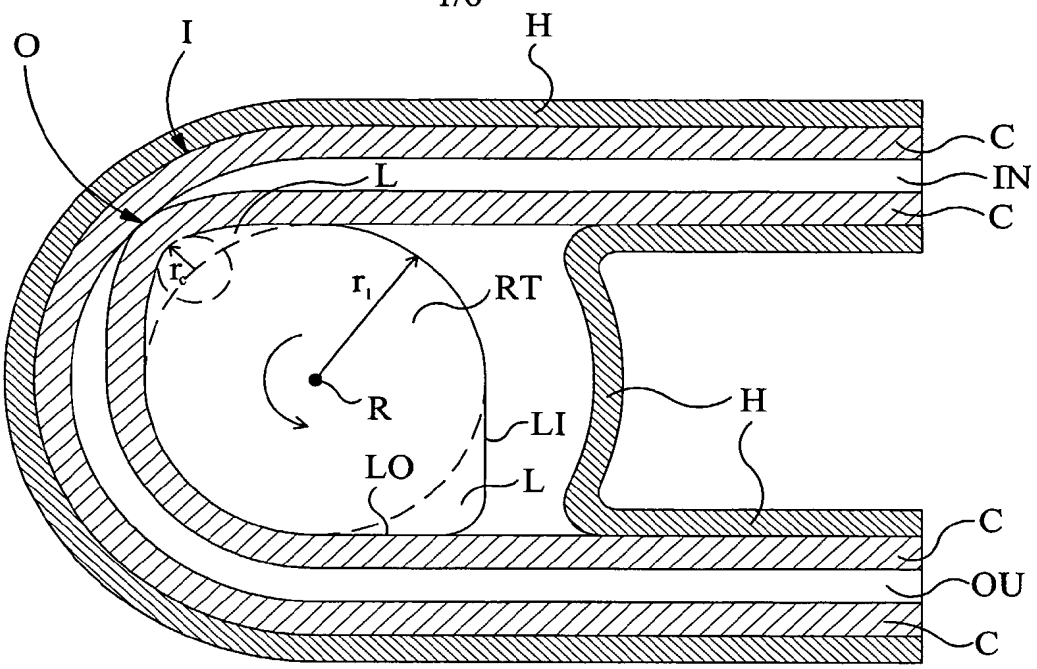
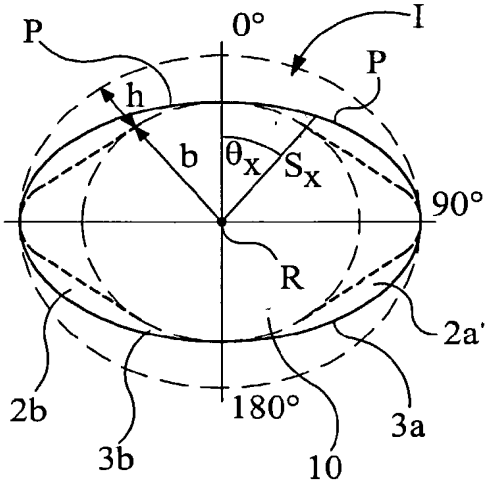


FIG 1

Current State of the Art Rotor ----

Present Invention Rotor ——

Rotor Profile Geometry



Result is controlled velocity and acceleration

FIG 6

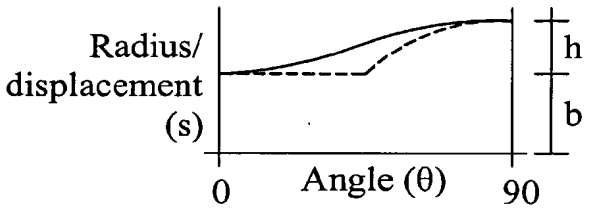


FIG 7

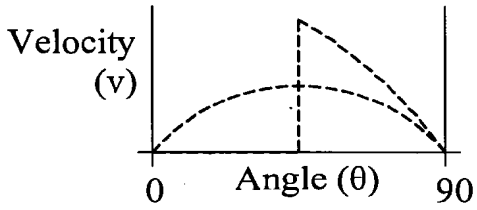


FIG 8

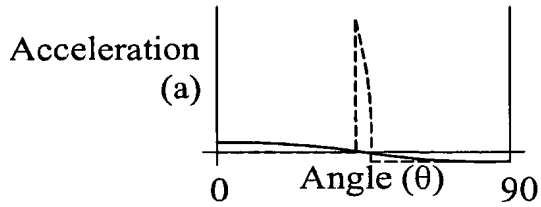


FIG 9

FIG 2

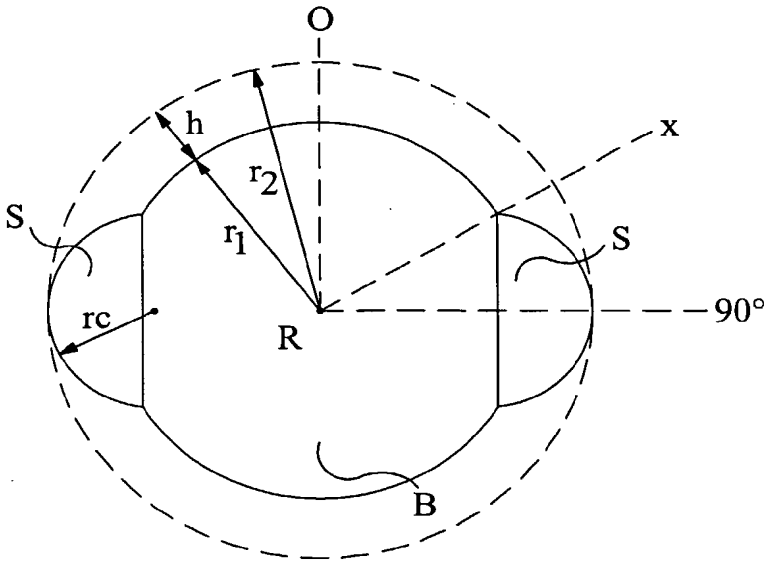


FIG 3

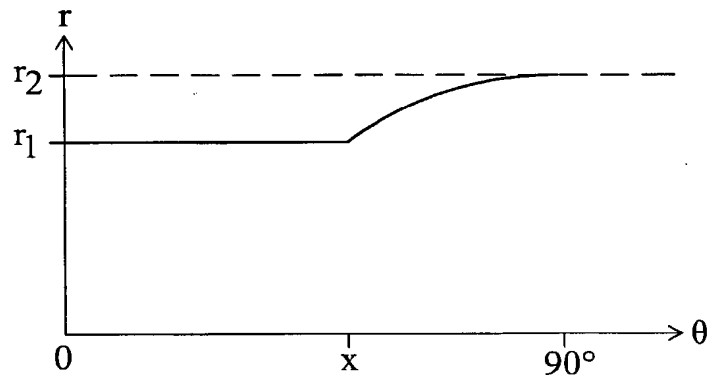


FIG 4

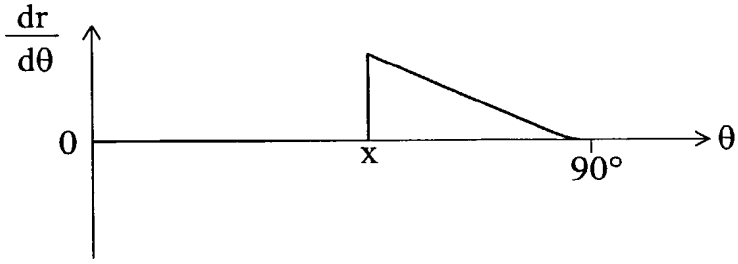
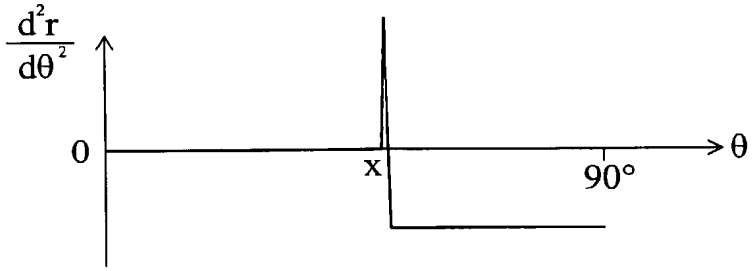


FIG 5



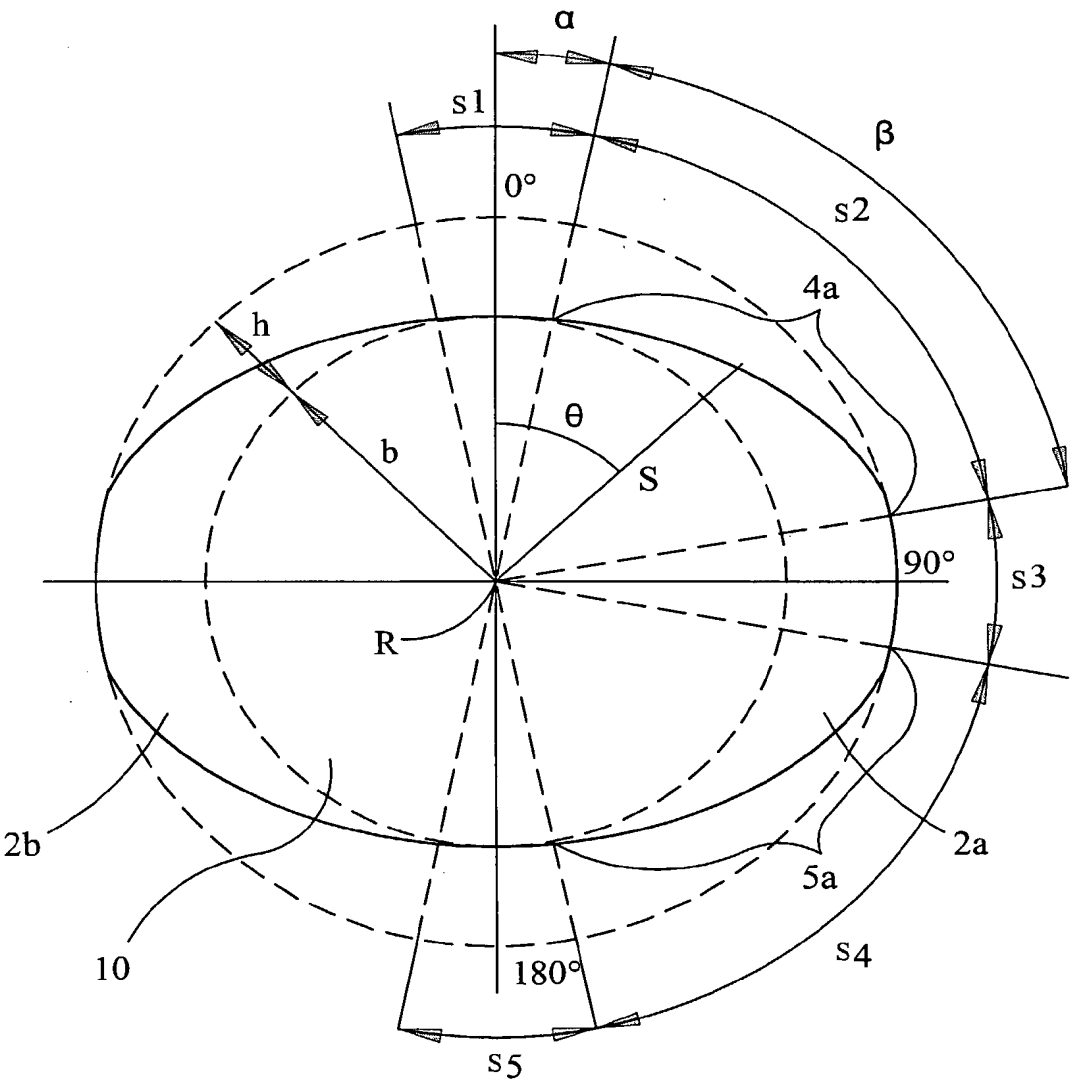
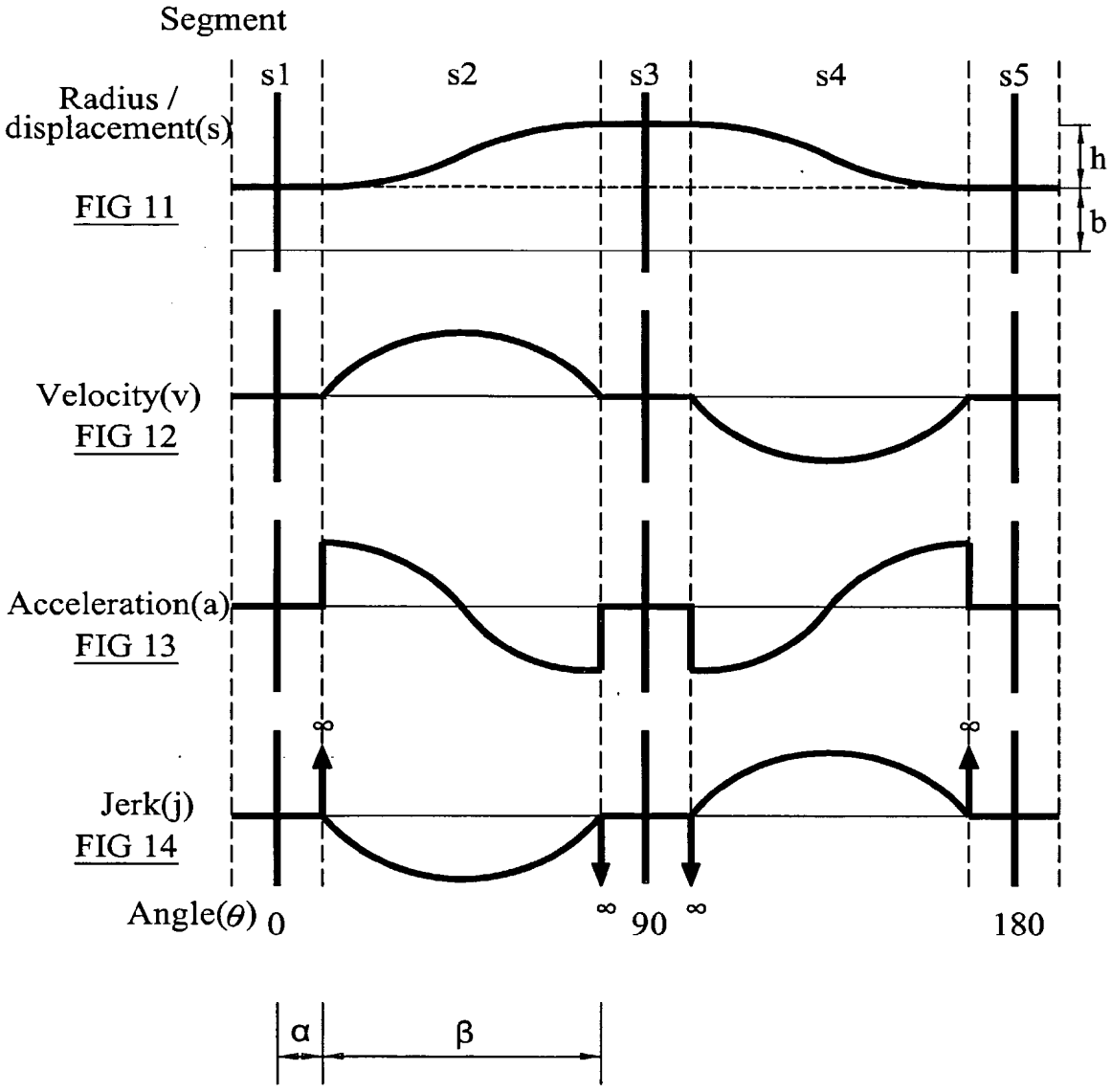


FIG 10


Segment
s1
s2
s3
s4
s5
Radius / displacement(s)
h
b
FIG 11
Velocity(v)
FIG 12
Acceleration(a)
FIG 13
∞
∞
∞
Jerk(j)
FIG 14
Angle(θ)
0
90
180
α
β

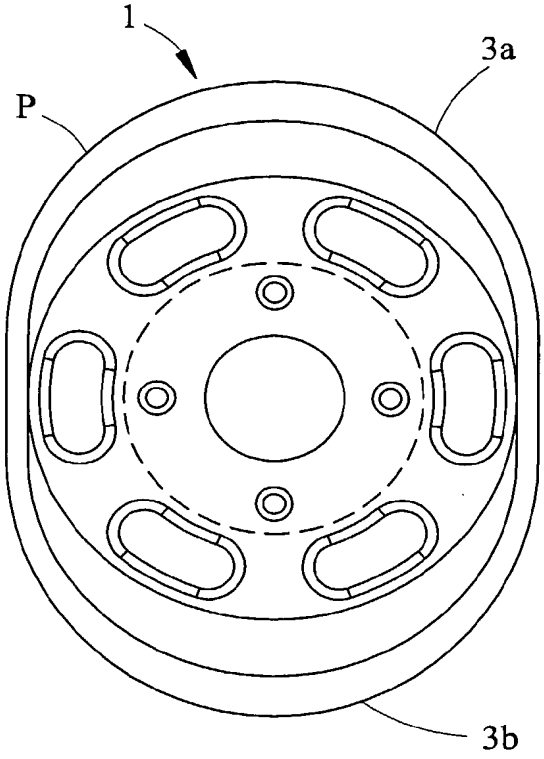


FIG 15

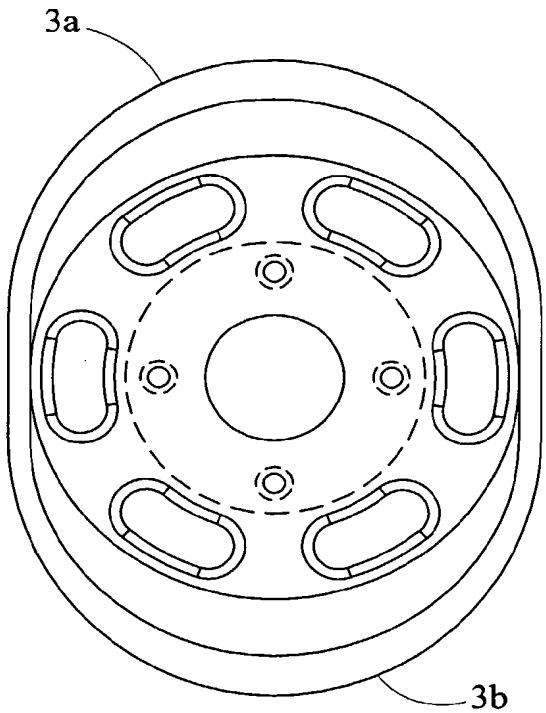


FIG 16

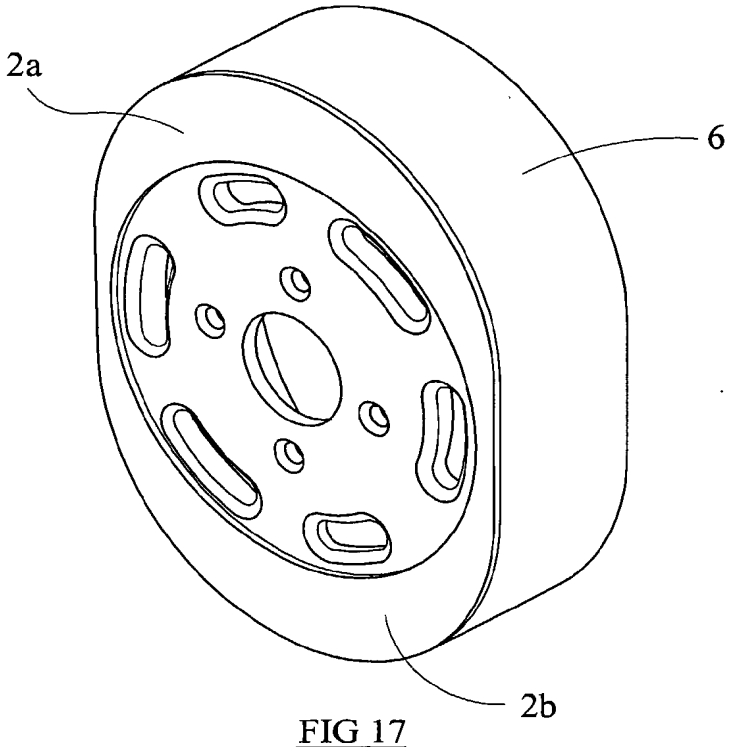


FIG 17

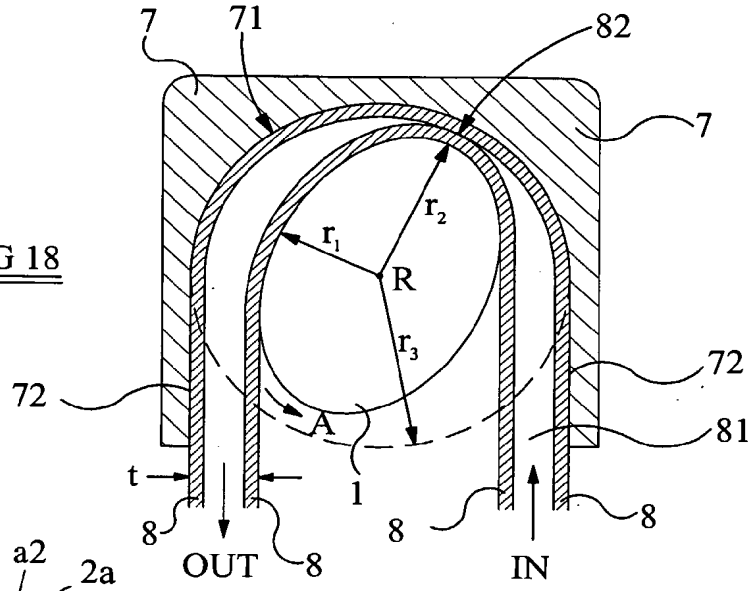


FIG 18

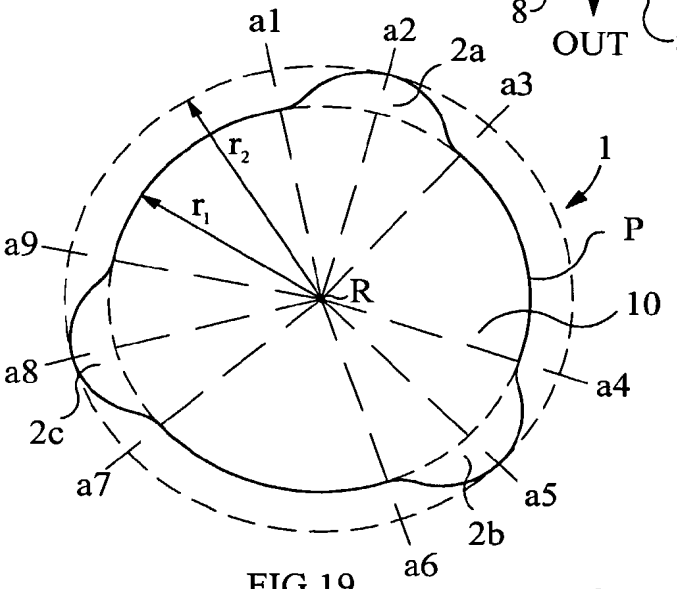


FIG 19

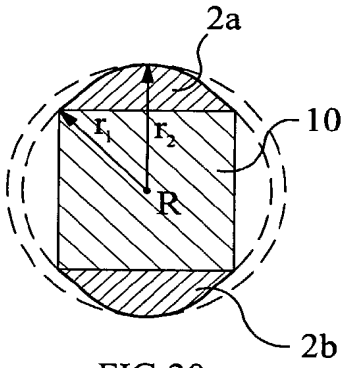


FIG 20

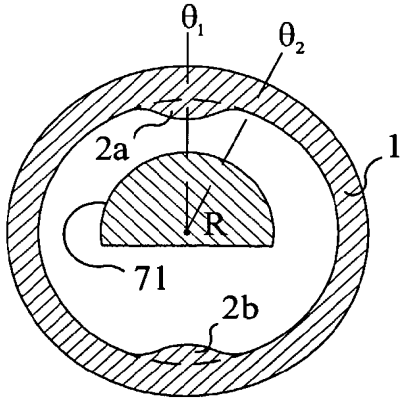


FIG 21

INTERNATIONAL SEARCH REPORT

In ial Application No
PCT/GB2005/002763

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 F04B43/12 F04B43/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 F04B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 1996, no. 11, 29 November 1996 (1996-11-29) & JP 08 177744 A (KUWABARA KAZUHIRO), 12 July 1996 (1996-07-12)	1-5,21, 24,25
Y	abstract; figures 1-3	6-20,22, 23,26-43
X	PATENT ABSTRACTS OF JAPAN vol. 1999, no. 08, 30 June 1999 (1999-06-30) & JP 11 062854 A (AQUA TEC:KK), 5 March 1999 (1999-03-05)	1-5,21, 24,25
Y	abstract; figure 1	6-20,22, 23,26-43

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 September 2005	06/10/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Avramidis, P

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Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PATENT ABSTRACTS OF JAPAN vol. 1999, no. 11, 30 September 1999 (1999-09-30) & JP 11 159445 A (NISSAN MOTOR CO LTD), 15 June 1999 (1999-06-15) abstract; figure 3	1-43
Y	US 5 488 769 A (NATWICK ET AL) 6 February 1996 (1996-02-06) abstract; figures 8-10	1-43
Y	PATENT ABSTRACTS OF JAPAN vol. 008, no. 182 (M-319), 22 August 1984 (1984-08-22) & JP 59 074387 A (ASAHI KOGYO KK), 26 April 1984 (1984-04-26) abstract; figure 1	1-43
A	GB 2 290 582 A (* RIVERLYNX LIMITED) 3 January 1996 (1996-01-03) cited in the application the whole document	1-43
A	US 3 798 982 A (LUNDQUIST I,US) 26 March 1974 (1974-03-26) the whole document	1-43

4

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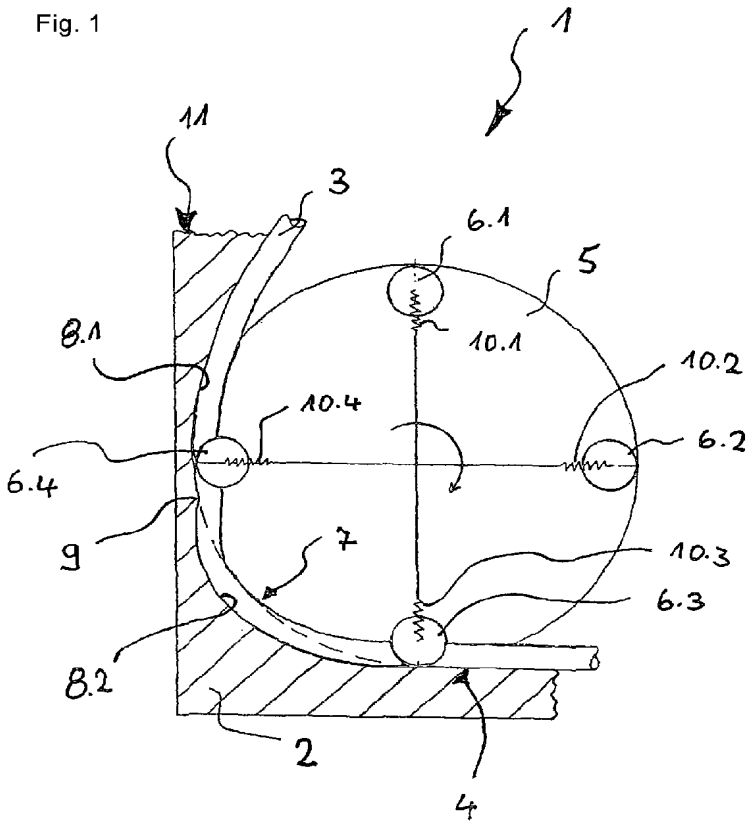
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(54) Title: HOSE PUMP FOR CONVEYING FLUIDS

(54) Bezeichnung: SCHLAUCHPUMPE ZUR FÖRDERUNG VON FLUIDEN

Fig. 1



(57) Abstract: The invention relates to a hose pump (1) for conveying fluids, comprising a stator (2) in which a flexible hose (3) is integrated into a track (4) that is substantially in the form of a pitch circle, and a rotor (5) is mounted in the stator (2), which rotor (5) has radially arranged, elastically mounted conveying elements (6) that press the hose (3), which is integrated in the track (4), onto said track (4) along a section (7) containing the fluid, and wherein the compressed fluid section (7) is advanced progressively in the hose (3) under the action of a motor which is connected to the rotor (5). The track (4) has, over the course of its extension up to the pressure-side outlet of the pump (1), at least two track regions (8.1) and (8.2) with different radii, wherein the first track region (8.1), which lies toward the pressure-side outlet, has a radius that increases along its extension, which increasing radius influences the circumferential speed of each elastically mounted conveying element (6) which is passing through, in such a way that the conveyed volume flow of the fluid to the pressure-side outlet of the pump emerges without pulsation, and wherein the second, upstream track region (8.2) produces a increased volume flow that compensates the outflowing volume flow of the first track region (8.1).

[Fortsetzung auf der nächsten Seite]

MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Bestimmungsstaaten** *(soweit nicht anders angegeben, für jede verfügbare regionale Schutzrechtsart)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), eurasisches (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Erklärungen gemäß Regel 4.17:

— *hinsichtlich der Identität des Erfinders (Regel 4.17 Ziffer i)*

— *hinsichtlich der Berechtigung des Anmelders, ein Patent zu beantragen und zu erhalten (Regel 4.17 Ziffer ii)*

— *hinsichtlich der Berechtigung des Anmelders, die Priorität einer früheren Anmeldung zu beanspruchen (Regel 4.17 Ziffer iii)*

— *Erfindererklärung (Regel 4.17 Ziffer iv)*

Veröffentlicht:

— *mit internationalem Recherchenbericht*

(57) Zusammenfassung: Schlauchpumpe (1) zur Förderung von Fluiden umfassend einen Stator (2) in dem ein flexibler Schlauch (3) auf einer im wesentlichen teilkreisförmigen Bahn (4) eingebunden ist, und im Stator (2) ein Rotor (5) gelagert ist, welcher über radial angeordnete, elastisch gelagerte Förderelemente (6) verfügt, die den in der Bahn (4) eingebundenen Schlauch (3) über einen das Fluid enthaltenden Abschnitt (7) auf der Bahn (4) abdrücken, und wobei unter Einwirkung eines mit dem Rotor (5) verbundenen Motors der abgedrückte Fluidabschnitt (7) sich fortschreitend im Schlauch (3) bewegt. Die Bahn (4) weist über den Verlauf ihres Erstreckungsbereichs zum druckseitigen Ausgang der Pumpe (1) wenigsten zwei Bahnbereiche (8.1) und (8.2) mit unterschiedlichen Radien auf, wobei der zum druckseitigen Ausgang liegende erste Bahnbereich (8.1) über seinen Erstreckungsbereich einen zunehmenden Radius aufweist, der jeweils das durchlaufende elastisch gelagerte Förderelemente (6) in seiner Umfangsgeschwindigkeit derart beeinflusst, so dass der geförderte Volumenstrom des Fluids zum druckseitigen Ausgang der Pumpe pulsfrei austritt, und wobei der zweite vorgeschaltete Bahnbereich (8.2) eine kompensierende Volumenstromerhöhung zum abströmenden Volumenstrom des ersten Bahnbereichs (8.1) erzeugt.

Beschreibung

Schlauchpumpe zur Förderung von Fluiden.

Technisches Gebiet

[0001] Die Erfindung betrifft eine Schlauchpumpe zur Förderung von Fluiden umfassend einen Stator in dem ein flexibler Schlauch auf einer im wesentlichen teilkreisförmigen Bahn eingebunden ist, und im Stator ein Rotor gelagert ist, welcher über radial angeordnete, elastisch gelagerte Förderelemente verfügt, die den in der Bahn eingebundenen Schlauch über einen das Fluid enthaltenden Abschnitt auf der Bahn abdrücken bzw. abgreifen, und wobei unter Einwirkung eines mit dem Rotor verbundenen Motors der abgegriffene Fluidabschnitt sich fortschreitend im Schlauch bewegt.

Stand der Technik

[0002] Aus dem Stand der Technik sind unterschiedliche Ausführungen von Schlauchpumpen zur Förderung von Fluiden bekannt. So werden diese in der Medizin und hier bei der Dialyse beispielsweise zum Bluttransport verwendet. Üblich sind Rollenpumpen, bei denen ein rotierender Rollenträger vorgesehen ist, wobei die Rollen ein Stück des Schlauches über seine Länge fortschreitend zusammendrücken, und so die Flüssigkeit vorwärts befördern.

[0003] So ist beispielsweise aus der WO 2007/036931 A2 eine derartige Schlauchpumpe bekannt, bei der ein rotierender Rollenträger mit seinen Rollen einen Bereich des Schlauches über seine Länge abgreift und somit das Fortschreiten des Fluides in dem Schlauch bewirkt. Bei der vorbekannten Schlauchpumpe befindet sich in einem halbkreisförmigen Stator ein flexibler Schlauch, wobei im Stator ein Rotor gelagert ist, welcher über radial angeordnete Förderelemente in Form von Rollen verfügt. Im Bereich der halbkreisförmigen Bahn drücken die Rollen einen bestimmten Abschnitt des eingebundenen Schlauches ab, so dass infolge der Rotation der abgedrückte Fluidabschnitt sich fortschreitend im Schlauch bewegt. So ergibt sich eine Schlauchpumpe, die auf der Saugseite entsprechend wieder Fluid aufnimmt, wobei zur Druckseite hin der abgedrückte Fluidabschnitt freigegeben wird und aus dem System

befördert wird.

[0004] Bei dieser bekannten Schlauchpumpe ergibt sich jedoch das Problem, dass die Förderung des Volumenstromes des Fluids pulsartig austritt, wobei dies insbesondere der Fall ist, wenn Fluide mit geringem Druck gefördert werden sollen. Diese peristatischen Pumpen werden vornehmlich eingesetzt, wenn Fluide mit geringem Druck gefördert werden sollen. Diese sind hinsichtlich des Bauraums sehr klein, einfach im Aufbau und somit auch kostengünstig. Durch das Bau- und Funktionsprinzip dieser Pumpen entsteht aber zwangsläufig ein Puls im Förderstrom.

Darstellung der Erfindung

[0005] Der Erfindung stellt sich somit das Problem die zuvor beschriebene Schlauchpumpe dahingehend zu verbessern, dass ein zu fördernder Volumenstrom möglichst gleichmäßig austritt.

[0006] **Lösung**

[0007] Erfindungsgemäß wird das Problem durch den Hauptanspruch gelöst. Vorteilhafte Weiterbildungen der Erfindung ergeben sich aus den Unteransprüchen.

[0008] Die erfindungsgemäße Schlauchpumpe arbeitet, wie eine herkömmliche Schlauchpumpe, und zwar durch Verdrängung eines Fluidvolumens mit Hilfe von Förderelementen. Wie bei einer herkömmlichen Schlauchpumpe entsteht an der Förderaustrittsseite ein Puls durch das Anheben der Förderelemente vom Schlauch. Dieser Puls wird mit Hilfe eines weiteren Förderelementes dadurch ausgeglichen, weil das folgende Förderelement um eine definierte Geschwindigkeit schneller läuft, als das an der Förderseite befindliche. Das schneller laufen wird dadurch realisiert, dass das zweite Förderelement einen größeren Radius durchläuft und somit einen längeren Weg in der gleichen Zeit zurücklegt. Der Stator weist hierzu druckseitig zum Ausgang hin einen Bahnbereich auf, der mit zunehmendem Radius versehen ist. Durch diese Ausbil-dung überstreicht das nachfolgende Förderelement in dergleichen Zeit ein größeres Volumen als das vorauseilende Förderelement. Dadurch wird das beim Abheben des Förderelementes entstehende Fehlvolumen ausgeglichen, und die Pulsation der Pumpe weitgehend unterdrückt bzw. beseitigt.

[0009] Der Rotor weist an mehreren Stellen seines Umfangs gleichmäßig in festem Winkel zueinander verteilte Abquetschelemente in Form von Förderelementen auf, mit denen ein ortsfester Schlauch, der in einem Gehäuse befindlich ist, das einen Abstand zum Rotor hat in dem der Schlauch selbst Platz hat, Sequenzweise abgequetscht wird und somit ein entsprechendes Volumen zwischen den Abquetschstellen bewegt wird. Dabei hat das Gehäuse eine Ausbuchtung über einen Teilkreis, in dem der Schlauch zwischen Rotor und Gehäuse liegt, und die Abquetschele-mente dieser Ausbuchtung geometrisch folgen, so dass bei gleicher Rotationsgeschwindigkeit die Umfangsgeschwindigkeit sich vergrößert und somit das geförderte Volumen beschleunigt und wobei die Ausbuchtung geometrisch so bemessen ist, dass das fehlende Volumen bei der Überlagerung der Abquetschelemente mit der Schlauchaustrittsöffnung kompensiert wird.

[0010] Erfindungsgemäß weist hierzu die Bahn über den Verlauf eines Erstreckungsbereichs zum druckseitigen Ausgang der Pumpe wenigsten zwei Bahnbereiche mit unterschiedlichen Radien auf, wobei der zum druckseitigen Ausgang liegende erste Bahnbereich über seinen Erstreckungsbereich einen zunehmenden Radius aufweist, der jeweils das durchlaufende, elastisch gelagerte Förderelement in seiner Umfangsgeschwindigkeit derart beeinflusst, dass der geförderte Volumenstrom des Fluids zum druckseitigen Ausgang der Pumpe pulsfrei austritt.

[0011] In Weiterbildung ist druckseitig vor dem ersten Bahnbereich mit zunehmendem Radius, ein zweiter Bahnbereich mit konstantem Radius auf der Bahn angeordnet. Dabei nimmt der zweite Bahnbereich mit konstantem Radius auf der Bahn vorzugsweise ein Bogenmaß ein, welches geringfügig geringer ist als 90°. Mit den Bahnbereichen wirken wenigstens vier elastisch gelagerte Förderelemente zusammen, die jeweils um 90° versetzt an dem Rotor angeordnet sind. Somit greifen zumindest zwei elastisch gelagerte Förderelemente, die um 90° versetzt angeord-net sind, einen Fluidabschitt auf der Bahn derart ab, dass das voreilende Förderelement sich auf dem ersten Bahnbereich mit

zunehmenden Radius befindet, wohingegen das nachlaufende Förderelement sich am Anfang des zweiten Bahnbereichs befindet. Somit ergibt sich beim Überstreichen der Förderelemente über die Bahn mit unterschiedlichen Radienbereichen folgende Situation, dass, wenn das voreilende Förderelement den Schlauchquerschnitt wieder freigibt, bereits das nachlaufende einen entsprechenden Druck in dem Schlauchabschnitt aufbaut, so dass dadurch der Impuls kompensiert wird, und es somit zu einem pulsfreien Volumenstromaustritt kommt.

[0012] In vorteilhafter Weise bestehen die elastisch gelagerten Förderelemente aus Wälzkörpern, die im Rotor gegen die Wirkung von Federn gelagert sind.

[0013] Das den Stator bildende Pumpengehäuse ist aus einem Gussteil hergestellt, in dem die die Bahnbereiche bildende Kurvengeometrie eingeformt ist, in die entsprechend der flexible Schlauch eingebunden ist.

Kurze Beschreibung der Abbildungen der Zeichnungen

[0014] Ein Ausführungsbeispiel der Erfindung wird anhand der nachstehenden Figur näher erläutert, die einzige Figur zeigt eine schematische teilweise geschnittene Seitenansicht der erfindungsgemäßen Schlauchpumpe.

Bester Weg zur Ausführung der Erfindung

[0015] Die einzige Figur zeigt eine Schlauchpumpe 1 zur Förderung von Fluiden. Dabei umfasst die Schlauchpumpe 1 einen Stator 2, in dem ein flexibler Schlauch 3 auf einer im Wesentlichen, teilkreisförmigen Bahn 4 eingebunden ist. Wie in der Figur infolge des Pfeils darge-stellt, ist in dem Stator 2 ein Rotor 5 gelagert, welcher über radial angeordnete elastisch gelagerte Förderelemente 6.1, 6.2, 6.3, 6.4 verfügt. Wie aus der Figur deutlich wird, drücken dabei insbesondere die Förderelemente 6.3 und 6.4 in der Bahn 4 den eingebundenen Schlauch 3 über einen das Fluid enthaltenden Abschnitt 7 auf der Bahn 4 ab. Dabei wirkt mit dem Rotor 5 ein nicht näher dargestellter Motor zusammen, der insbesondere den abgedrückten Fluidabschnitt 7 fortschreitend im Schlauch 3 entsprechend der Pfeilrichtung zur Druckseite bewegt.

[0016] Erfindungsgemäß wird nun eine derartige Schlauchpumpe dadurch weiter gebildet, dass die Bahn 4 über den Verlauf ihres Erstreckungsbereichs

unterschiedliche Bahnbereiche 8.1 und 8.2 aufweist, die die elastisch gelagerten Förderelemente 6.3 und 6.4 in ihrer Umfangsgeschwindigkeit derart beeinflussen, dass der geförderte Volumenstrom des Fluids auf der Druckseite der Pumpe 1 pulsfrei austritt. Dabei wird erreicht, dass im Bahnbereich 8.2 das Förderelement 6.3 den Fluidstrom dadurch ausgleicht, das es um eine definierte Geschwindigkeit schneller läuft als das an der Förderseite befindliche Förderelement 6.4. Das schneller laufen wird hierbei, wie aus der Figur zu erkennen ist, dadurch realisiert, dass das Förderelement 6.3 einen konstanten Radius, hier den Bahnbereich 8.2, durchläuft, und somit einen kürzeren Weg in der gleichen Zeit zurücklegt. Es versteht sich von selbst, dass nun, wenn in der Rotation das vorlaufende Förderelement 6.4 den Schlauch 3 wieder freigibt, das nachlaufende Förderelement 6.3 sich noch in der Radienbahn 8.2 befindet, so dass sich dadurch bei Freigabe ein so genannter nachdrückender Volumenstrom ergibt, der insbesondere den vorauseilenden Volumenstrom kompensiert, und so die pulsfreie Volumenstromförderung gewährleistet wird.

[0017] Dabei sind druckseitig wenigstens zwei Bahnbereiche 8.1 und 8.2 auf der Bahn 4 vorgesehen, wobei der Bahnbereich 8.2 vor dem Bahnbereich 8.1 auf der Bahn 4 liegt bzw. angeordnet ist. Wie aus der Figur zu erkennen ist, nimmt dabei der Bahnbereich 8.2 auf der Bahn 4 vorzugs-weise ein Bogenmaß ein, welches geringfügig geringer ist als 90°. Dabei wirken mit den Bahnbereichen 8.1 und 8.2 wenigstens vier elastisch gelagerte Förderelemente 6.1, 6.2, 6.3 und 6.4 zusammen, die jeweils um 90° versetzt an dem Rotor 5 angeordnet sind. Somit ergibt sich insbesondere für die um 90° versetzt angeordneten Förderelemente 6. 4 und 6.3 ein übergreifender Bereich bei den beiden Bahnbereichen 8.1 und 8.2, so dass insbesondere in den Bereich der versetzt angeordneten Förderelemente 6.4 und 6.3 der Übergang von dem einen Bahnbereich 8.2 zum anderen Bahnbereich 8.1 fällt, der hier insbesondere mit dem Bezugszeichen 9 bezeichnet ist.

[0018] Wie bereits schon gesagt, sind zumindest zwei elastisch gelagerte Förderelemente 6.3 und 6.4 vorzugsweise um 90° versetzt angeordnet.

Der dabei abgegriffene Fluidabschnitt 7 auf der Bahn 4 übergreift dabei die Radienbereiche 8.1 und 8.2, so dass das voreilende Förderelement 6.4 sich noch auf dem Bahnbereich 8.1 mit zunehmendem Radius befindet, wohingegen das nachlaufende Förderelemente 6.3 sich am Anfang des Bahnbereichs 8.2 mit konstantem Radius befindet.

[0019] Somit ergibt sich folgendes Förderbild, dass, wenn das Förderelement 6.4 bereits schon wieder den Schlauchquerschnitt freigibt, also das Förderelement 6.4 vom Schlauch 3 infolge des zunehmenden Radius abhebt, nimmt das nacheilende Förderelement 6.3 die Fördertätigkeit schon wieder auf, weil es sich noch in dem Bereich des Bahnbereiches 8.2 befindet, was zu dem kompensierten pulsfreien Fluidstrom führt.

[0020] In vorteilhafter Weiterbildung der Erfindung sind hierbei die elastisch gelagerten Förderelemente 6.1, 6.2, 6.3 und 6.4 als Wälzkörper ausgebildet, die im Rotor 5 gegen die Wirkung von Federn 10.1, 10.2, 10.3 und 10.4 gelagert sind. Dabei ist das den Stator 2 bildende Pumpengehäuse 11 aus einem Gussteil gefertigt, in dem die die Radien-be-reiche 8.1 und 8.2 bildende Kurvengeometrie eingeformt ist.

[0021] **Bezugszeichenliste**

[0022] 1 Schlauchpumpe

[0023] 2 Stator

[0024] 3 Schlauch

[0025] 4 Bahn

[0026] 5 Rotor

[0027] 6 Förderelemente (6.1, 6.2, 6.3, 6.4)

[0028] 7 Abschnitt

[0029] 8 Radienbereiche (8.1, 8.2)

[0030] 9 Übergang

[0031] 10 Federn (10.1, 10.2, 10.3, 10.4)

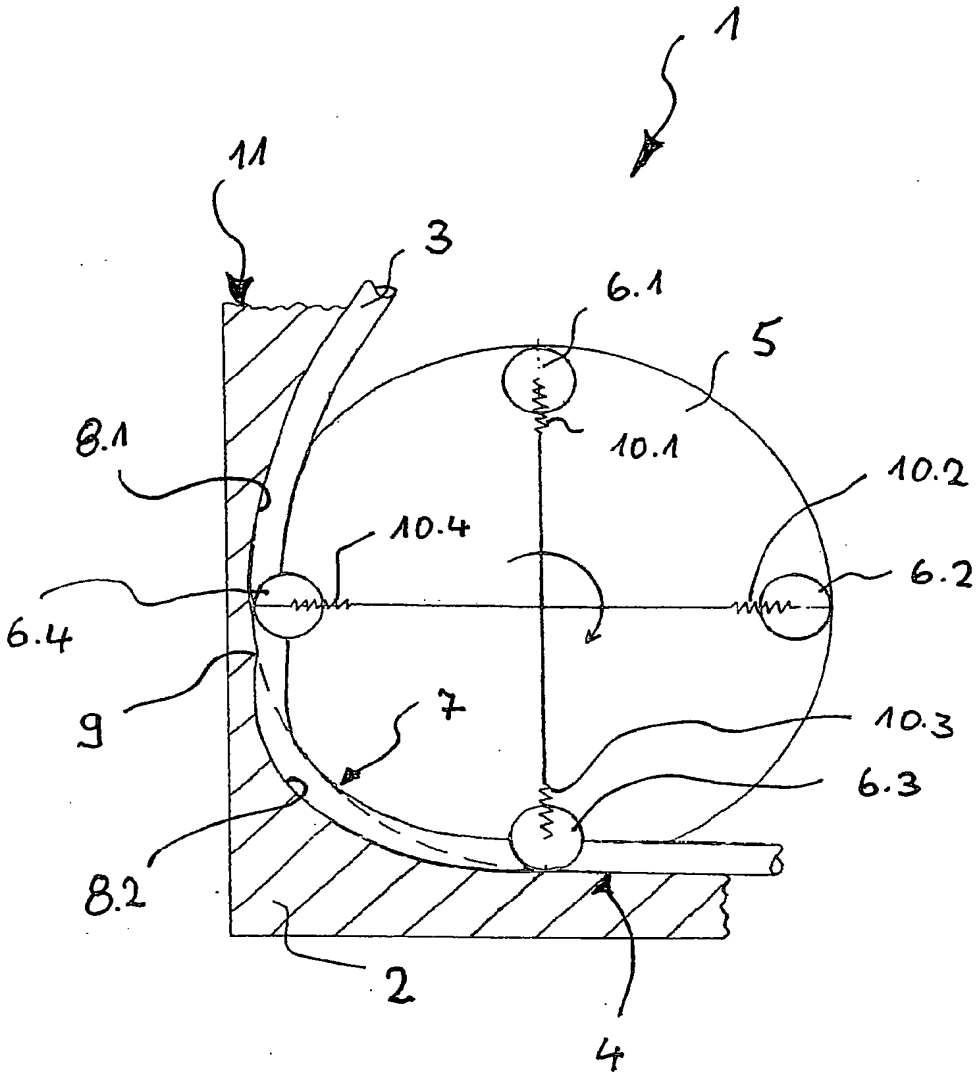
[0032] 11 Pumpengehäuse

Ansprüche

1. Schlauchpumpe (1) zur Förderung von Fluiden umfassend einen Stator (2) in dem ein flexibler Schlauch (3) auf einer im wesentlichen teilkreisförmigen Bahn (4) eingebunden ist, und im Stator (2) ein Rotor (5) gelagert ist, welcher über radial angeordnete, elastisch gelagerte Förderelemente (6) verfügt, die den in der Bahn (4) eingebundenen Schlauch (3) über einen das Fluid enthaltenden Abschnitt (7) auf der Bahn (4) abdrücken, und wobei unter Einwirkung eines mit dem Rotor (5) verbundenen Motors der abgedrückte Fluidabschnitt (7) sich fortschreitend im Schlauch (3) bewegt, **dadurch gekennzeichnet**, dass die Bahn (4) über den Verlauf ihres Erstreckungsbereichs zum druckseitigen Ausgang der Pumpe (1) wenigsten zwei Bahnbereiche (8.1) und (8.2) mit unterschiedlichen Radien aufweist, wobei der zum druckseitigen Ausgang liegende erste Bahnbereich (8.1) über seinen Erstreckungsbereich einen zunehmenden Radius aufweist, der jeweils das durchlaufende elastisch gelagerte Förderelemente (6) in seiner Umfangsgeschwindigkeit derart beeinflusst, dass der geförderte Volumenstrom des Fluids zum druckseitigen Ausgang der Pumpe pulsfrei austritt, und wobei der zweite vorgeschaltete Bahnbereich (8.2) eine kompensierende Volumenstromerhöhung zum abströmenden Volumenstrom des ersten Bahnbereichs (8.1) erzeugt.
2. Schlauchpumpe nach Anspruch 1, **dadurch gekennzeichnet,** dass druckseitig vor dem ersten Bahnbereich (8.1) mit zunehmendem Radius, ein zweiter Bahnbereich (8.2) mit konstantem Radius auf der Bahn (4) liegt bzw. angeordnet ist.
3. Schlauchpumpe nach Anspruch 1 und 2, **dadurch gekennzeichnet,** dass der zweite Bahnbereich (8.2) mit konstantem Radius auf der Bahn (4) vorzugsweise ein Bogenmaß einnimmt, welches geringfügig geringer ist als 90°.
4. Schlauchpumpe nach Anspruch 1 bis 3, **dadurch gekennzeichnet,** dass mit den Bahnbereichen (8.1) und (8.2) wenigstens vier elastisch gelagerte Förderelemente (6.1, 6.2, 6.3, 6.4) zusammenwirken, die jeweils um 90° versetzt an dem Rotor (5) angeordnet sind.
5. Schlauchpumpe nach Anspruch 4, **dadurch gekennzeichnet,** dass zumindest zwei elastisch gelagerte Förderelemente (6.3 und 6.4), die vorzugsweise um

90° versetzt angeordnet sind, einen Fluidabschitt (7) auf der Bahn (4) derart abgreifen, dass das voreilende Förderelement (6.4) sich auf dem ersten Bahnbereich (8.1) mit zunehmenden Radius befindet, wohingegen das nachlaufende Förderelement (6.3) sich am Anfang des zweiten Bahnbereichs (8.2) befindet.

6. Schlauchpumpe nach Anspruch 5, **dadurch gekennzeichnet,** dass die elastisch gelagerten Förderelemente (6.1, 6.2, 6.3, 6.4) aus Wälzkörpern bestehen, die im Rotor (5) gegen die Wirkung von Federn (10.1, 10.2, 10.3, 10.4) gelagert sind.
7. Schlauchpumpe nach Anspruch 1 bis 6, **dadurch gekennzeichnet,** dass das den Stator (2) bildende Pumpengehäuse (11) aus einem Gussteil hergestellt ist, in dem die die Bahnbereiche (8.1) und (8.2) bildende Kurvengeometrie eingeformt ist.


1
11
3
6.1
5
8.1
10.1
10.2
10.4
6.2
6.4
9
7
10.3
6.3
8.2
2
4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/050800

A. CLASSIFICATION OF SUBJECT MATTER
INV. F04B43/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
F04B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 22 00 595 A1 (VOGT WINHOLD DIPL BERGING) 12 July 1973 (1973-07-12) page 2, paragraphs 2,3; figure 3	1-7
X	DE 201 09 803 U1 (FRESENIUS HEMOCARE GMBH [DE]) 24 October 2002 (2002-10-24) abstract; figure 1	1-7
X	EP 1 647 712 A (JAPAN SERVO [JP]) 19 April 2006 (2006-04-19) abstract; figure 2	1-7
X	US 6 099 272 A (ARMSTRONG KEITH [US] ET AL) 8 August 2000 (2000-08-08) figures 1,2	1-7

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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/036931 A (LESOKHIN YURI [IL]) 5 April 2007 (2007-04-05) cited in the application the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2009/050800

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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DE 20109803 U1	24-10-2002	NONE	
EP 1647712 A	19-04-2006	NONE	
US 6099272 A	08-08-2000	WO 9914497 A1	25-03-1999
WO 2007036931 A	05-04-2007	NONE	

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C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	DE 22 00 595 A1 (VOGT WINHOLD DIPL BERGING) 12. Juli 1973 (1973-07-12) Seite 2, Absätze 2,3; Abbildung 3	1-7
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[X] Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen [X] Siehe Anhang Patentfamilie

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Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Olona Laglera, C

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C. (Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
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INTERNATIONALER RECHERCHENBERICHT

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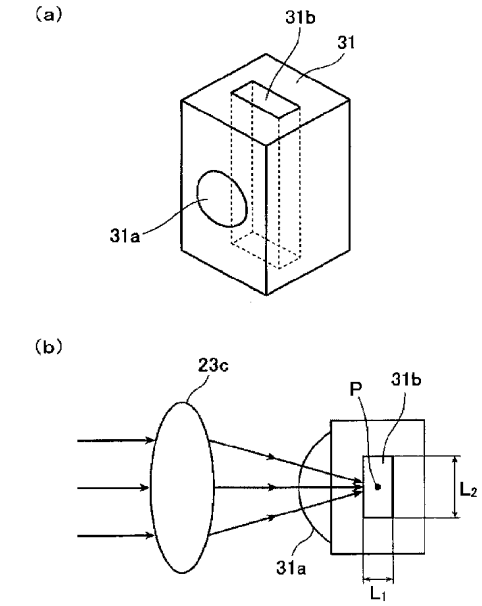
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[続葉有]

(54) **Title**: FLUORESCENT LIGHT DETECTION DEVICE AND FLUORESCENT LIGHT DETECTION METHOD

(54) 発明の名称: 蛍光検出装置および蛍光検出方法

[図4]



(57) **Abstract**: A fluorescent light detection device includes: a flow cell body having a flow channel in which a sample flows; a laser light source unit for applying a laser beam to an object to be measured and passing a measurement point in the flow channel; a light reception unit which receives the fluorescent light of the object to be measured to which the laser beam has been applied and outputs a reception light signal; and a processing unit which outputs an output value of the fluorescent light intensity from the reception light signal outputted from the reception light unit. A lens is arranged on the surface of the flow cell body. When the lens is virtually cut by a plane passing through a measurement point of the object to be measured and vertical to a movement direction of the object to be measured, the cross section of the lens forms a part of a circular shape around the measurement point.

(57) 要約: 蛍光検出装置は、試料が流れる流路が形成されるフローセル体と、流路中の測定点を通過する測定対象物に対してレーザ光を照射するレーザ光源部と、レーザ光の照射された測定対象物の蛍光を受光して受光信号を出力する受光部と、受光部から出力した受光信号から、蛍光強度の出力値を出力する処理部と、を有する。フローセル体の表面には、レンズが設けられ、このレンズは、測定対象物の測定点を通り、測定対象物の移動方向と垂直な面で仮想的に切断したとき、レンズの断面が測定点を中心とする円形形状の一部を成している。

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添付公開書類:

— 国際調査報告（条約第 21 条(3)）

明　細　書

蛍光検出装置および蛍光検出方法

技術分野

[0001] 本発明は、測定対象物にレーザ光を照射し、そのとき発する蛍光を測定する蛍光検出装置および蛍光検出方法に関する。

背景技術

[0002] 医療、生物分野で用いられるフローサイトメータには、レーザ光を照射することにより測定対象物の蛍光色素から発する蛍光を受光して、測定対象物の種類を識別する蛍光検出装置が組み込まれている。特に、近年、タンパク質等の細胞内の局所情報を調べるために、蛍光色素を用いて蛍光測定を行うことが試みられている。

細胞内の局所情報を調べるためには、計測分解能を従来に比べて向上させることが必要である。

[0003] 今日用いられるフローサイトメータでは、下記非特許文献１に示されるように、フローセル（フローセル体）が用いられる。このフローセルとは断面が四方形の細長いクォーツ製の中空チャンバで、レーザ光を透過し、サンプル中の細胞にレーザ光を照射するための部材である。このフローセルを通過したレーザ光は、フローセル中の測定点を通過する測定対象物に照射され、別途設けられた検出系で蛍光が検出される。

[0004] 当該文献では、以下の内容が記載されている。レーザ光のビームの光強度の分布はガウス分布である。このビームを楕円形に集光・収束させて、強度を高めるとともに、一度に複数の細胞が照射されないような光学系が構成されている。レーザ光は細胞が流れるサンプル流に到達する前に２つの円筒型集光レンズを通過し、楕円形のビームになる。１番目のレンズでビームの幅を調整し、２番目のレンズでビームの高さを調整する。２つのレンズを通過した後、楕円形のビームは、断面が四方形の細長いフローセル内を流れる細胞に照射される。サンプル圧を低くするとサンプル流は細くなり、レーザ光

の中央の光強度の変動のより少ない部分を通過するため、測定の分解能が向上する、と記載されている。

[0005] 非特許文献1：ＦＣＭの原理入門講座－Ｖ、http://www.bc-cytometry.com/FCM/fcmprinciple_5.html#5-1http://www.bc-cytometry.com/FCM/fcmprinciple_6-1.html （２００７年１１月２８日検索）

[0006] しかし上記非特許文献１に記載される装置では、タンパク質等の細胞内の局所情報を調べる程度に測定分解能を向上することはできないといった問題があった。

発明の開示

発明が解決しようとする課題

[0007] そこで、本発明は、上記問題点を解決するために、タンパク質等の細胞内の局所情報を調べる程度に測定分解能を向上することができる蛍光検出装置および蛍光検出方法を提供することを目的とする。

課題を解決するための手段

[0008] 上記目的を達成するために、本発明は、測定対象物にレーザ光を照射し、そのとき発する蛍光を測定する蛍光検出装置であって、測定対象物が流れる流路が形成されたフローセル体と、流路中の測定点を通過する測定対象物に対してレーザ光を照射するレーザ光源部と、レーザ光の照射された測定対象物の蛍光を受光して受光信号を出力する受光部と、前記受光部から出力した受光信号から、蛍光強度の出力値を出力する処理部と、を有し、

前記フローセル体の表面には、レーザ光の光路を横切るようにレンズが設けられレンズが設けられ、このレンズは、測定対象物の前記測定点を通る、測定対象物の移動方向と垂直な面で仮想的に切断したとき、前記レンズの断面が前記測定点を中心とした円形形状の一部分を成していることを特徴とする蛍光検出装置を提供する。

[0009] ここで、前記レンズは、前記測定点を焦点位置とする球体形状の一部分を成す球面レンズであることが好ましい。

[0010] 又、前記フローセル体に形成される前記流路の断面形状は、矩形形状であ

り、前記レーザ光は、光軸がこの矩形形状の一辺に平行になるように照射され、前記矩形形状の、前記レーザ光の光軸に平行な一辺の長さに対する、この一辺に対して直交する他辺の長さの比率が、１～２．５であることが好ましい。

[0011] その際、前記レーザ光の光軸に平行な一辺の長さは、測定対象物の平均直径に対して３０倍以上２００倍以下の長さであることが好ましい。

また、前記フローセル体と前記レンズは同じ材料で構成されていることが好ましい。

さらに、前記測定点と前記受光部との間の蛍光の光路中の前記フローセル体の表面に、レンズの断面が前記測定点を中心とした円形形状の一部分を成した別のレンズが設けられていることも好ましい。

[0012] なお、測定対象物は例えば細胞であり、前記レーザ光は、前記測定点において細胞内の一部分を照射し、前記受光部は、細胞内のタンパク質が発する蛍光を受光する。

[0013] さらに、上記目的を達成するために、本発明は、測定対象物にレーザ光を照射し、そのとき発する蛍光を測定する蛍光検出方法であって、

測定対象物を、フローセル体に設けられた流路に流すステップと、

測定対象物の測定点を通り、測定対象物の移動方向と垂直な面で仮想的に切断したとき、断面が前記測定点を中心とした円形形状の一部分を成している、前記フローセル体の表面に設けられたレンズを用いて収束させたレーザ光を、前記流路中の測定点を通過する測定対象物に対して照射させるステップと、

レーザ光の照射された測定対象物の蛍光を受光して受光信号を出力するステップと、

出力した前記受光信号から、蛍光強度の出力値を出力するステップと、を有することを特徴とする蛍光検出方法を提供する。

[0014] その際、前記レンズは、前記測定点を焦点位置とする球体形状の一部分を成す球面レンズであることが好ましい。

発明の効果

[0015] 本発明の蛍光検出装置は、測定対象物が流れる流路が形成されたフローセル体の表面に、レンズが設けられ、このレンズは、測定対象物の測定点を通り、測定対象物の移動方向と垂直な面で仮想的に切断したとき、前記レンズの断面が前記測定点を中心とする円形形状の一部分を成している。このため、従来に比べてＮＡ（開口数）を高めることができ、レーザ光の集光ビームの直径を小さくすることができる。特に、測定点を焦点位置とする球体形状の一部分を成す球面レンズを用いることで、レーザ光の集光ビームの直径を効率よく小さくすることができる。

また、上記レンズを用いて蛍光を検出する方法においても、従来に比べてＮＡ（開口数）を高めることができ、レーザ光の集光ビームの直径を小さくすることができる。特に、測定点を焦点位置とする球体形状の一部分を成す球面レンズを用いることで、レーザ光の集光ビームの直径を効率よく小さくすることができる。

したがって、タンパク質等の細胞内の局所情報を調べる程度に測定分解能を向上することができる。

図面の簡単な説明

[0016] [図1]本発明の蛍光検出装置を用いたフローサイトメータの概略構成図である。

[図2]本発明の蛍光検出装置に用いられるレーザ光源部の一例を示す概略構成図である。

[図3]本発明の蛍光検出装置に用いられる受光部の一例を示す概略構成図である。

[図4]（ａ）及び（ｂ）は、本発明の蛍光検出装置に用いられるフローセル体を説明する図である。

[図5]従来のフローセル体をレーザ光が通過する状態を説明する図である。

符号の説明

[0017] １０　フローサイトメータ

１２　試料

２０　信号処理装置

２２　レーザ光源部

２２ｒ　Ｒ光源

２２ｇ　Ｇ光源

２２ｂ　Ｂ光源

$２３ａ_1$，$２３ａ_2$，$２６ｂ_1$，$２６ｂ_2$　ダイクロイックミラー

２３ｃ．２６ａ　レンズ系

２４，２６　受光部

$２６ｃ_1$，$２６ｃ_2$，$２６ｃ_3$　バンドパスフィルタ

２７ａ～２７ｃ　光電変換器

２８　処理部

２９　制御部

３０　管路

３１　フローセル体

３１ａ　球面レンズ

３１ｂ　流路

３２　回収容器

３４ｒ，３４ｇ，３４ｂ　レーザドライバ

８０　分析装置

発明を実施するための最良の形態

[0018]　以下、本発明の蛍光検出装置および蛍光検出方法を詳細に説明する。

図１は、本発明の蛍光検出装置を用いたフローサイトメータ１０の概略構成図である。

フローサイトメータ１０は、レーザ光を測定対象とする細胞等の試料１２に照射し、試料１２中のタンパク質等の一部分から発する蛍光を検出して信号処理する信号処理装置（蛍光検出装置）２０と、信号処理装置２０で得られた処理結果をから試料１２中の測定対象物の分析を行なう分析装置８０と

を有する。

[0019] 信号処理装置２０は、レーザ光源部２２と、受光部２４、２６と、試料１２の蛍光強度の出力値を出力する処理部２８と、所定の強度でレーザ光を照射させ、各処理の動作の制御管理を行う制御部２９と、高速流を形成するシース液に含ませて試料１２を流す管路３０と、管路３０の端に接続され、試料１２のフローを形成するフローセル体３１と、を有する。フローセル体３１には、試料１２の流路中にレーザ光の照射点（測定点）が形成される。フローセル体３１の出口側には、回収容器３２が設けられている。フローサイトメータ１０には、レーザ光の照射により短時間内に試料１２中の特定の細胞等を分離するためのセル・ソータを配置して別々の回収容器に分離するように構成することもできる。

[0020] レーザ光源部２２は、波長の異なる３つのレーザ光、例えばλ_1＝４０５ｎｍ、λ_2＝５３３ｎｍおよびλ_3＝６５０ｎｍ等のレーザ光を出射する部分である。レーザ光は、フローセル体３１の流路中の所定の位置に集束するようにレンズ系が設けられ、この集束位置が試料１２の測定点となっている。

[0021] 図２は、レーザ光源部２２の構成の一例を示す図である。

レーザ光源部２２は、Ｒ光源２２ｒ、Ｇ光源２２ｇ、Ｂ光源２２ｂと、ダイクロイックミラー２３a_1、２３a_2と、レンズ系２３ｃと、レーザドライバ３４ｒ，３４ｇおよび３４ｂと、パワースプリッタ３５と、を有して構成される。

Ｒ光源２２ｒ、Ｇ光源２２ｇ、Ｂ光源２２ｂは、３５０ｎｍ～８００ｎｍの可視光の、レーザ光を出射する部分で、Ｒ光源２２ｒは、主に赤色のレーザ光Ｒを所定の強度で出射する。Ｇ光源２２ｇは、緑色のレーザ光Ｇを所定の強度で出射する。Ｂ光源２２ｂは、青色のレーザ光Ｂを所定の強度で出射する。

ダイクロイックミラー２３a_1、２３a_2は、特定の波長帯域のレーザ光を透過し、他の波長帯域のレーザ光を反射する。

レンズ系２３ｃは、レーザ光Ｒ，ＧおよびＢからなるレーザ光を管路３０

中の測定点に集束させる。レーザドライバ３４ｒ，３４ｇおよび３４ｂは、Ｒ光源２２ｒ、Ｇ光源２２ｇおよびＢ光源２２ｂのぞれぞれを駆動する。

パワースプリッタ３５は、供給された信号をレーザドライバ３４ｒ，３４ｇおよび３４ｂにそれぞれ分配する。

これらのレーザ光を出射する光源として例えば半導体レーザが用いられる。

[0022] ダイクロイックミラー２３ａ$_1$は、レーザ光Ｒを透過し、レーザ光Ｇを反射するミラーであり、ダイクロイックミラー２３ａ$_2$は、レーザ光ＲおよびＧを透過し、レーザ光Ｂを反射するミラーである。

この構成によりレーザ光Ｒ，ＧおよびＢが合成されて、測定点を通過する試料１２を照射する照射光となる。

[0023] レーザドライバ３４ｒ，３４ｇおよび３４ｂは、処理部２８及び制御部２９に接続されて、レーザ光Ｒ，Ｇ，Ｂの出射の強度が調整されるように構成される。

[0024] Ｒ光源２２ｒ、Ｇ光源２２ｇおよびＢ光源２２ｂは、レーザ光Ｒ、ＧおよびＢが蛍光色素を励起して特定の波長帯域の蛍光を発するように、予め定められた波長帯域で発振する。レーザ光Ｒ、ＧおよびＢによって励起される蛍光色素は測定しようとする生体物質等の試料１２に付着されており、測定対象物としてフローセル体３１の測定点を通過する際、測定点でレーザ光Ｒ、ＧおよびＢの照射を受けて特定の波長で蛍光を発する。

[0025] 受光部２４は、フローセル体３１を挟んでレーザ光源部２２と対向するように配置されており、測定点を通過する試料１２によってレーザ光が前方散乱することにより試料１２が測定点を通過する旨の検出信号を出力する光電変換器を備える。この受光部２４から出力される信号は、処理部２８に供給され、処理部２８において試料１２が管路３０中の測定点を通過するタイミングを知らせるトリガ信号として用いられる。

[0026] 一方、受光部２６は、レーザ光源部２２から出射されるレーザ光の出射方向に対して垂直方向であって、かつフローセル体３１の流路中の試料１２の

移動方向に対して垂直方向に配置されており、測定点にて照射された試料１２が発する蛍光を受光する光電変換器を備える。

図３は、受光部２６の一例の概略の構成を示す概略構成図である。

[0027] 図３に示す受光部２６は、試料１２からの蛍光信号を集束させるレンズ系２６ａと、ダイクロイックミラー２６ｂ$_1$，２６ｂ$_2$と、バンドパスフィルタ２６ｃ$_1$～２６ｃ$_3$と、光電子倍増管等の光電変換器２７ａ～２７ｃと、を有する。

レンズ系２６ａは、受光部２６に入射した蛍光を光電変換器２７ａ～２７ｃの受光面に集束させるように構成されている。

ダイクロイックミラー２６ｂ$_1$，２６ｂ$_2$は、所定の範囲の波長帯域の蛍光を反射させて、それ以外は透過させるミラーである。バンドパスフィルタ２６ｃ$_1$～２６ｃ$_3$でフィルタリングして光電変換器２７ａ～２７ｃで所定の波長帯域の蛍光を取り込むように、ダイクロイックミラー２６ｂ$_1$，２６ｂ$_2$の反射波長帯域および透過波長帯域が設定されている。

[0028] バンドパスフィルタ２６ｃ$_1$～２６ｃ$_3$は、各光電変換器２７ａ～２７ｃの受光面の前面に設けられ、所定の波長帯域の蛍光のみが透過するフィルタである。透過する蛍光の波長帯域は、蛍光色素の発する蛍光の波長帯域に対応して設定されている。

[0029] 光電変換器２７ａ～２７ｃは、例えば光電子増倍管を備えたセンサであり、光電面で受光した光を電気信号に変換するセンサである。

[0030] 制御部２９は、所定の強度でレーザ光を照射させ、処理部２８における各処理の動作の制御管理を行う部分である。

処理部２８は、所定の信号処理を行って蛍光強度の出力値を分析装置８０に出力する部分である。

[0031] 分析装置８０は、処理部２８から供給される出力値を用いて、フローセル体３１の測定点を通過する試料１２中に含まれる生体物質の種類等を特定し、試料１２中に含まれる生体物質の分析を行う装置である。こうして、分析装置８０は、例えば、試料１２中に含まれる生体物質の種類のヒストグラム

や各種特性を短時間に求める。

[0032] 管路３０の下端はフローセル体３１が接続されている。フローセル体３１は、本発明の特徴とする部分である。図４（ａ）は、フローセル体３１の概略斜視図であり、図４（ｂ）は、フローセル体３１をレーザ光が透過する状態を説明する図である。

フローセル体３１は、長方体を成した透明部材であり、石英等によって作られている。フローセル体３１の側面からレーザ光が入射され、フローセル体３１の内部を通過し、フローセル体３０に設けられた、管路３０から縦方向に延びる流路３１ｂの中心で集束するようになっており、この集束位置が測定点Ｐとなっている。この測定点Ｐは、レーザ光源部２２のレンズ系２３ｃの焦点位置である。

[0033] ここで、レーザ光が入射するフローセル体３１の側面には、測定点Ｐを焦点位置とする球体の一部分の形状を成す球面レンズ３１ａが設けられている。すなわち、レーザ光の光路を横切るように球面レンズ３１ａが設けられている。球面レンズ３１ａの球形状の中心、すなわち、球面レンズ３１ａの曲率中心位置は、試料１２の測定点Ｐと一致している。図４（ｂ）中の２３ｃは、図２に示すレーザ光源部２２のレンズ系（集束レンズ）である。球面レンズ３１ａは、フローセル体３１と同じ材料、例えば石英等によって作られている。

このように、フローセル体３１のレーザ光の入射面に球面レンズ３１ａを設けるのは、レーザ光の集光ビームの直径を小さくし、測定分解能を向上させるためである。

[0034] フローセル体３１に球面レンズ３１ａを設けることで、レンズ系２３ｃを通過したレーザ光を測定点Ｐで集束させるとき、球面レンズ３１ａに入射するレーザ光の入射角は０度となる。このため、ＮＡ(開口数)は、球面レンズ３１ａを設けない場合に比べて、向上する。

具体的には、フローセル体３１を通過するレーザ光の集光ビームのＮＡは、（媒質の屈折率）×ｓｉｎθで表される。ここで、θはレーザ光のフロー

セル体３１への入射角度である。このとき、球面レンズ３１ａでは、レンズ系２３ｃを通過したレーザ光は常に入射角度０度で入射するのでθは変化せず、球面レンズ３１ａを通過したときＮＡは媒質の屈折率×ｓｉｎθの値になる。一方、図５に示す従来例のように、球面レンズ３１ａがない場合、レーザ光がフローセル体３１の側面に入射する入射角度は、レーザ光の光軸の部分を除いて、０度ではない。このため、フローセル体３１を通過する集光ビームにおけるＮＡはスネルの法則よりｓｉｎθとなる。以上より、本発明では、フローセル体３１をレーザ光が通過するとき、フローセル体３１の境界面でＮＡが従来のように低下しないように、球面レンズ３１ａを設ける。

球面レンズ３１ａを設けることにより、下記式に従って、従来に比べてのＮＡを増大させることができる。これによって集光ビームの直径を小さくすることができる。下記式は、レーザ光がガウシアン分布を想定した式である。

[0035] 集光ビームの直径ε＝　４×λ×ｆ／（πＤ）＝０．６４λ／ＮＡ

ここで、λはレーザ光の波長、ｆはレンズ焦点距離、Ｄはレンズ系２３ｃの開口径である。

[0036] このように、レーザ光が入射する、フローセル体３１の側面に球面レンズ３１ａを設けることで、集光ビームの直径を小さくすることができる。これによって、タンパク質等の細胞内の局所情報を調べる程度に測定分解能を向上することができる。

なお、本実施形態の球面レンズ３１ａは、直方体形状をしたフローセル体３１の１つの側面に設けるが、受光部２６の蛍光の測定分解能を向上させるために、蛍光が通過し受光部２６に至るフローセル体３１の別の側面に球面レンズ３１を設けてもよい。この場合においても、測定点Ｐの位置に球面レンズ３１の焦点位置が来るように配置する。

[0037] 本発明では、レーザ光の集光ビームのサイズを小さくするために、ＮＡを従来より高くする。このために、球面レンズ３１ａをフローセル体３１の側面に設けるが、ＮＡを高くすることにより、下記式に示されるように、焦点

深度が浅くなる。これによって測定分解能の低下が懸念される。

焦点深度ｚ＝０．６４／（ＮＡ）^{2}

[0038] そこで、本発明では、フローセル体３１に形成される流路３１ｂの断面形状が以下のように制限されることが好ましい。

すなわち、フローセル体３１に設けられた流路３１ｂの断面形状は、図４（ｂ）に示すように矩形形状であり、レーザ光は、光軸がこの矩形形状の一辺に平行になるように照射される。このとき、流路３１ｂの矩形形状の、レーザ光の光軸に平行な一辺の長さL_1に対する、この一辺に対して直交する他辺の長さL_2の比率が、１～２．５である。このような比率の矩形形状の流路３１ｂを形成することで、レーザ光の光軸方向において試料１２が通過する位置を規制することができる。このため、ＮＡを高くすることで浅くなった焦点深度の不都合を、流路３１ｂの矩形形状の比率を１～２．５とし、試料１２の流れる位置を規制することにより、解決することができる。また、測定する試料１２の平均サイズ（直径）に対して長さL_1を３０～２００倍とすることが好ましい。

フローサイトメータ１０は以上のように構成される。

[0039] このようなフローセル体３１を有するフローサイトメータ１０では、試料１２にレーザ光を照射し、そのとき発する蛍光を測定する、以下の蛍光検出方法が行われる。

試料１２を、フローセル体３１に設けられた流路にシース液を用いて流す。そして、フローセル体３１の表面に設けられた球面レンズ３１ａを用いて収束させたレーザ光を、流路中の測定点を通過する試料１２に対して照射させる。球面レンズ３１ａは、試料１２の測定点を通り、試料１２の移動方向と垂直な面で仮想的に切断したとき、断面が測定点を中心とした円形形状の一部分を成しているレンズである。このとき、レーザ光の照射された試料１２の発する蛍光を受光して受光信号を出力する。出力した受光信号から、蛍光強度の出力値を出力する。

なお、球面レンズ３１は、測定点を焦点位置とする球体形状の一部分を成

している。

[0040] なお、本実施形態では、フローセル体３１に球面レンズ３１ａを設けたが、本発明においては、これに限定されない。フローセル体３１のレーザ光が入射する表面には、試料１２の測定点Ｐを通り、試料１２の移動方向と垂直な面で仮想的に切断したとき、断面が円形形状の一部を成しているレンズを用いるとよい。例えばシリンドリカルレンズであってもよい。

[0041] 以上、本発明の蛍光検出装置および蛍光検出方法について詳細に説明したが、本発明は上記実施形態に限定されず、本発明の主旨を逸脱しない範囲において、種々の改良や変更をしてもよいのはもちろんである。

請求の範囲

[1] 測定対象物にレーザ光を照射し、そのとき発する蛍光を測定する蛍光検出装置であって、

測定対象物が流れる流路が形成されたフローセル体と、

流路中の測定点を通過する測定対象物に対してレーザ光を照射するレーザ光源部と、

レーザ光の照射された測定対象物の蛍光を受光して受光信号を出力する受光部と、

前記受光部から出力した受光信号から、蛍光強度の出力値を出力する処理部と、を有し、

前記フローセル体の表面には、レーザ光の光路を横切るようにレンズが設けられ、このレンズは、測定対象物の測定点を通る、測定対象物の移動方向と垂直な面で仮想的に切断したとき、前記レンズの断面が前記測定点を中心とした円形形状の一部分を成していることを特徴とする蛍光検出装置。

[2] 前記レンズは、前記測定点を焦点位置とする球体形状の一部分を成す球面レンズである請求項１に記載の蛍光検出装置。

[3] 前記ブローセル体に形成される前記流路の断面形状は、矩形形状であり、前記レーザ光は、光軸がこの矩形形状の一辺に平行になるように照射され、

前記矩形形状の、前記レーザ光の光軸に平行な一辺の長さに対する、この一辺に対して直交する他辺の長さの比率が、１～２．５である請求項１または２に記載の蛍光検出装置。

[4] 前記レーザ光の光軸に平行な一辺の長さは、測定対象物の平均直径に対して３０倍以上２００倍以下の長さである請求項３に記載の蛍光検出装置。

[5] 前記フローセル体と前記レンズは同じ材料で構成されている請求項１～４のいずれか1項に記載の蛍光検出装置。

[6] 前記測定点と前記受光部との間の蛍光の光路中の前記フローセル体の表面に、レンズの断面が前記測定点を中心とした円形形状の一部分を成した別のレンズが設けられている請求項１～５のいずれか１項に記載の蛍光検出装置

。

[7] 測定対象物は細胞であり、

前記レーザ光は、前記測定点において細胞内の一部分を照射し、

前記受光部は、細胞内のタンパク質が発する蛍光を受光する請求項１～６のいずれか１項に記載の蛍光検出装置。

[8] 測定対象物にレーザ光を照射し、そのとき発する蛍光を測定する蛍光検出方法であって、

測定対象物を、フローセル体に設けられた流路に流すステップと、

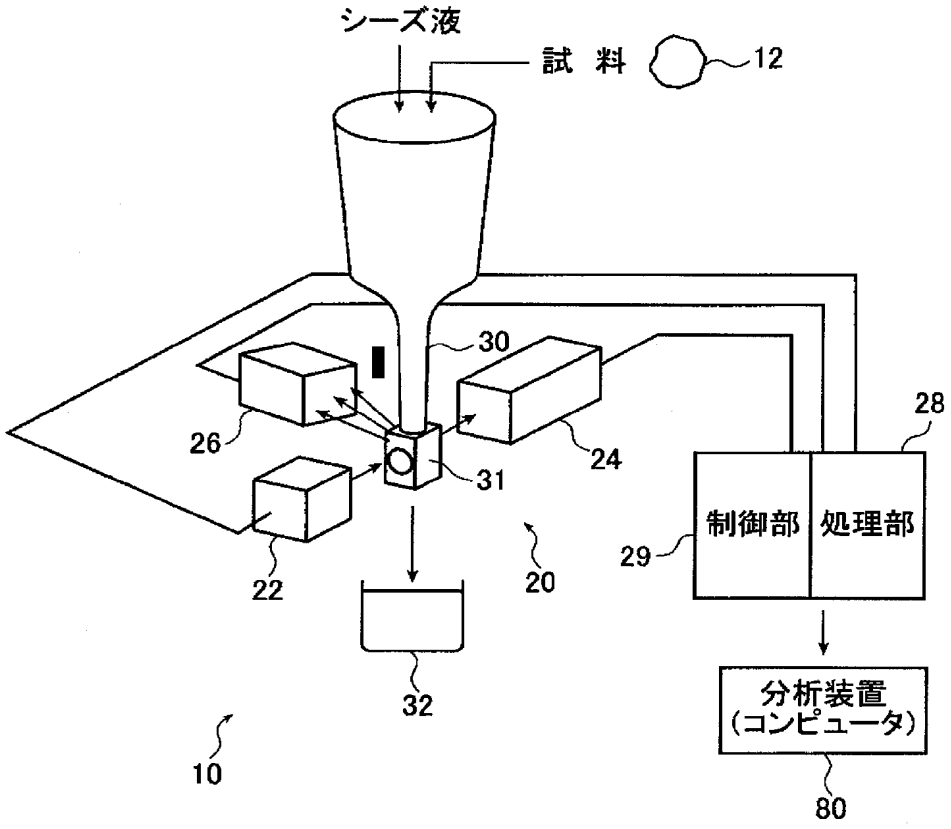
測定対象物の測定点を通り、測定対象物の移動方向と垂直な面で仮想的に切断したとき、断面が前記測定点を中心とした円形形状の一部分を成している、前記フローセル体の表面に設けられたレンズを用いて収束させたレーザ光を、前記流路中の測定点を通過する測定対象物に対して照射させるステップと、

レーザ光の照射された測定対象物の蛍光を受光して受光信号を出力するステップと、

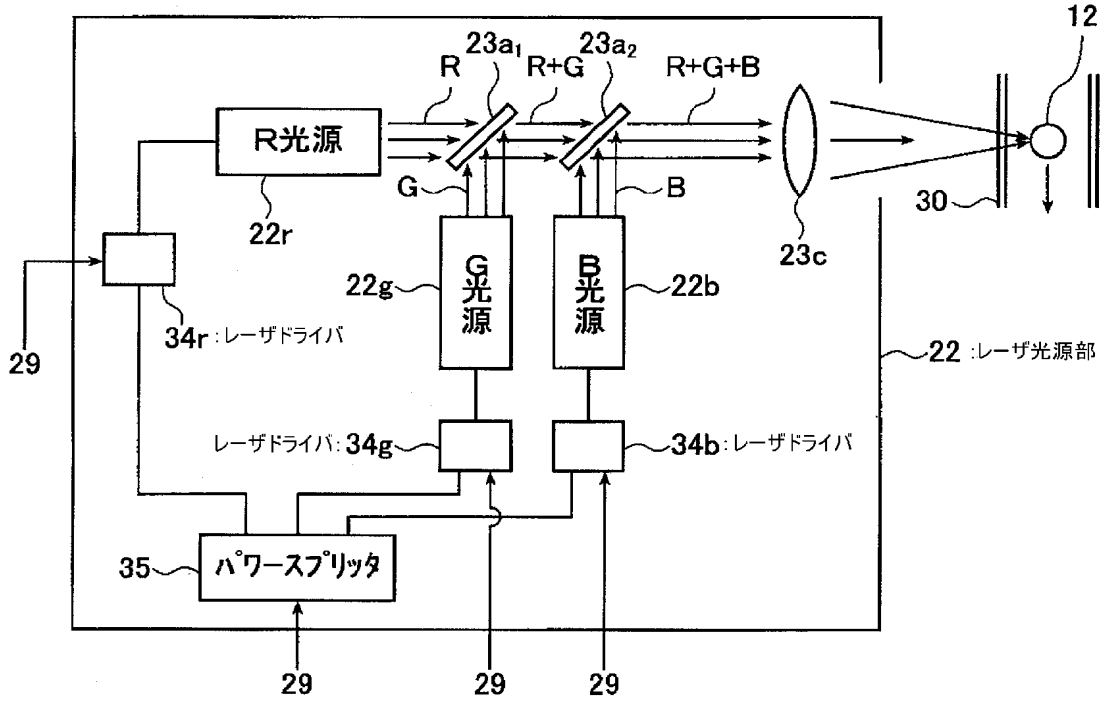
出力した前記受光信号から、蛍光強度の出力値を出力するステップと、を有することを特徴とする蛍光検出方法。

[9] 前記レンズは、前記測定点を焦点位置とする球体形状の一部分を成す球面レンズである請求項８に記載の蛍光検出方法。

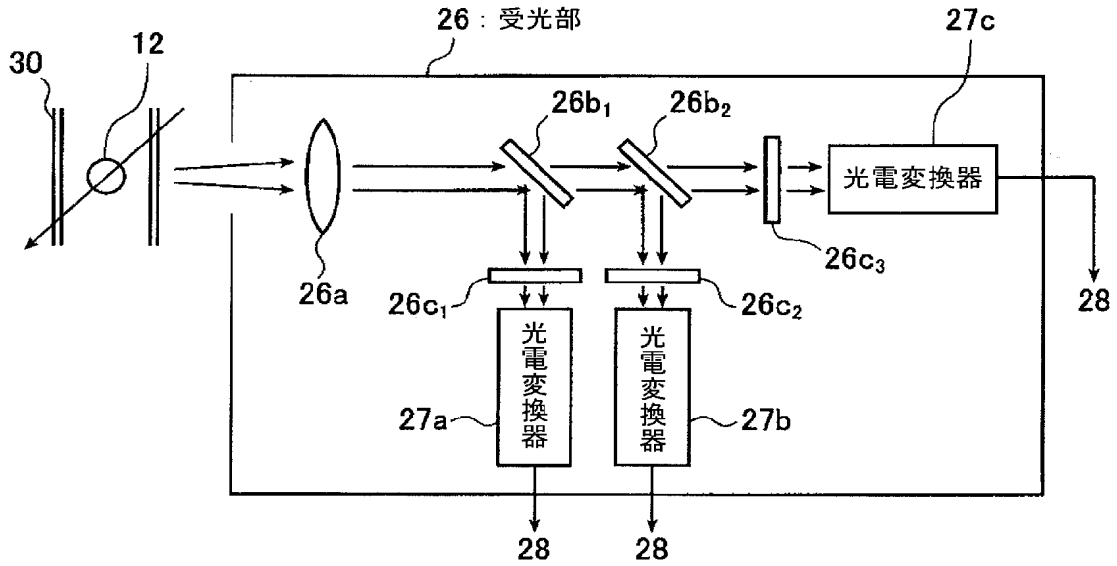
[図1]


シース液
試 料
12
30
26
24
28
31
制御部
処理部
29
22
20
32
分析装置
(コンピュータ)
10
80

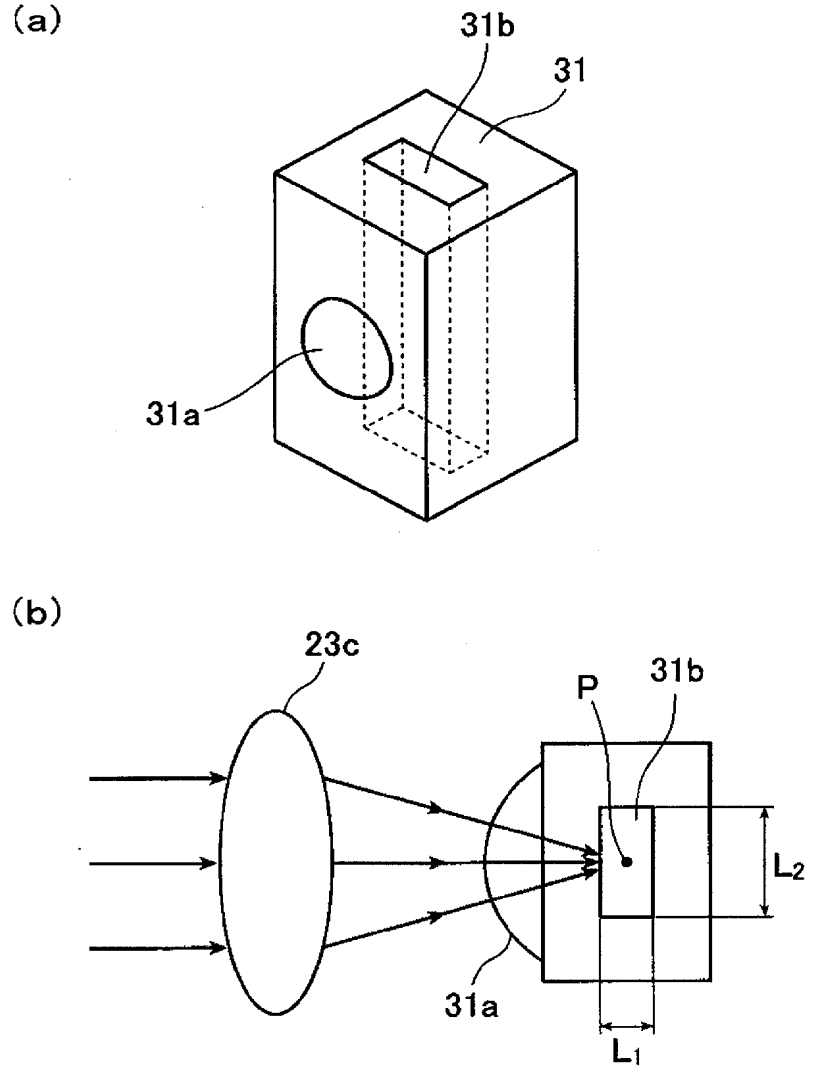
[図2]


12
R
23a1
R+G
23a2
R+G+B
R光源
G
B
22r
30
23c
22g
G光源
B光源
22b
34r:レーザドライバ
29
22:レーザ光源部
レーザドライバ:34g
34b:レーザドライバ
35
パワースプリッタ
29
29
29

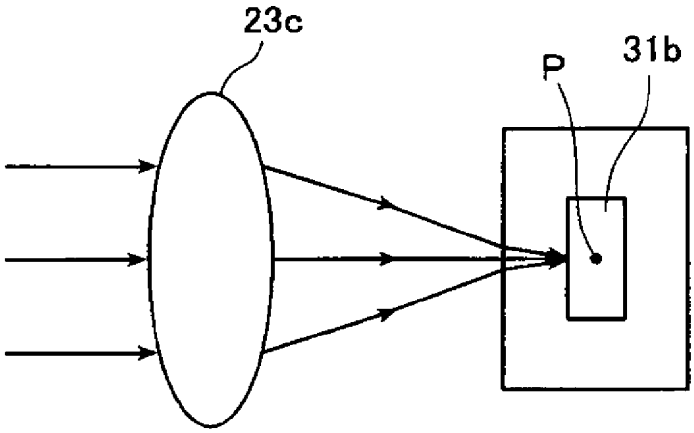
[図3]


26：受光部
27c
30
12
26b1
26b2
光電変換器
26c3
26a
26c1
26c2
28
光電変換器
光電変換器
27a
27b
28
28

[図4]


(a)
31b
31
31a
(b)
23c
P
31b
L2
31a
L1

[図5]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/000423

A. CLASSIFICATION OF SUBJECT MATTER
G01N15/14(2006.01)i, *G01N21/05*(2006.01)i, *G01N21/64*(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N15/00-15/14, G01N21/05, G01N21/64

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2009
Kokai Jitsuyo Shinan Koho 1971-2009 Toroku Jitsuyo Shinan Koho 1994-2009

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP 1-109245 A (Hitachi, Ltd.), 26 April, 1989 (26.04.89), Page 2, lower left column, line 6 to lower right column, line 6; Figs. 1 to 7 (Family: none)	1-9
Y	JP 2821191 B2 (Kowa Co., Ltd.), 05 November, 1998 (05.11.98), Claim 2; Figs. 1 to 4 (Family: none)	1-9
A	JP 1-242939 A (Kowa Co., Ltd.), 27 September, 1989 (27.09.89), Full text; all drawings & US 5017008 A & EP 334536 A2 & DE 68913058 D	1-9

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 April, 2009 (20.04.09)	28 April, 2009 (28.04.09)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/000423

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 2004-530868 A (Waters Investments Ltd.), 07 October, 2004 (07.10.04), Full text; all drawings & US 2005/0046834 A1 & EP 1373856 A & WO 2002/071029 A1	1-9
A	JP 10-221244 A (Tosoh Corp.), 21 August, 1998 (21.08.98), Full text; all drawings (Family: none)	1-9
A	JP 7-500191 A (Sapidyne, Inc.), 05 January, 1995 (05.01.95), Full text; all drawings & US 5372783 A & EP 606460 A & WO 1994/003104 A1	1-9

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ２００９／０００４２３

Ａ．　発明の属する分野の分類（国際特許分類（ＩＰＣ））

Int.Cl.　G01N15/14(2006.01)i, G01N21/05(2006.01)i, G01N21/64(2006.01)i

Ｂ．　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Int.Cl.　G01N15/00 - 15/14,
G01N21/05,
G01N21/64

最小限資料以外の資料で調査を行った分野に含まれるもの

日本国実用新案公報	１９２２－１９９６年
日本国公開実用新案公報	１９７１－２００９年
日本国実用新案登録公報	１９９６－２００９年
日本国登録実用新案公報	１９９４－２００９年

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

Ｃ．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求項の番号
Y	JP 1-109245 A（株式会社日立製作所）1989.04.26, 第２頁左下欄第６行～右下欄第６行、第１図～第７図（ファミリーなし）	1-9
Y	JP 2821191 B2（興和株式会社）1998.11.05, 請求項２、第１図～第４図（ファミリーなし）	1-9
A	JP 1-242939 A（興和株式会社）1989.09.27, 全文全図 & US 5017008 A & EP 334536 A2 & DE 68913058 D	1-9

☑　Ｃ欄の続きにも文献が列挙されている。　　☐　パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
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国際調査機関の名称及びあて先 日本国特許庁（ＩＳＡ／ＪＰ） 郵便番号１００－８９１５ 東京都千代田区霞が関三丁目４番３号	特許庁審査官（権限のある職員）　２Ｊ　３６０６ 渡▲辺▼　純也 電話番号　０３－３５８１－１１０１　内線　３２５２

様式ＰＣＴ／ＩＳＡ／２１０（第２ページ）（２００７年４月）

Ｃ（続き）．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求項の番号
A	JP 2004-530868 A（ウォーターズ・インヴェストメンツ・リミテッド）2004.10.07，全文全図 & US 2005/0046834 A1 & EP 1373856 A & WO 2002/071029 A1	1-9
A	JP 10-221244 A（東ソー株式会社）1998.08.21，全文全図（ファミリーなし）	1-9
A	JP 7-500191 A（サピダイン・インコーポレーテッド）1995.01.05，全文全図 & US 5372783 A & EP 606460 A & WO 1994/003104 A1	1-9

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(54) **Title**: AN INTEGRATED CYTOMETRIC SENSOR SYSTEM AND METHOD

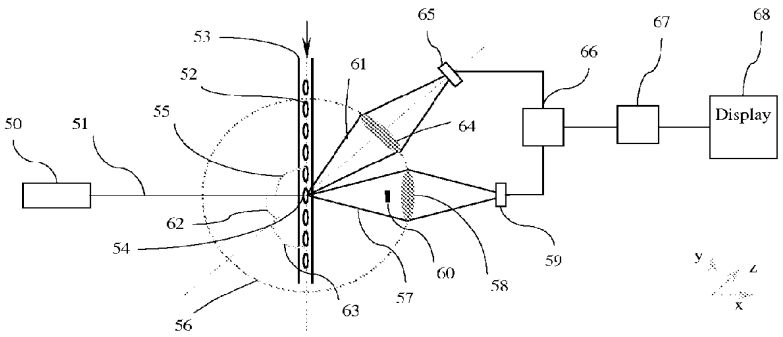


Figure 9

(57) **Abstract**: The invention provides a flow cytometric system comprising a first sensor positioned axially to a light source; a channel comprising means for receiving a sample target and interposed between said first sensor and light source; and a second sensor placed at an angle to said first sensor adapted to sense side scattering and/or fluorescent components and said first sensor is adapted to sense a forward scattering component in response to light illuminating the sample target in said channel. In another embodiment the invention provides for a wide dynamic range sensor comprising a plurality of photodiode pixels; wherein at least one or more of said photodiode pixels are voltage biased in one or more of the following modes: photon counting, normal, linear avalanche or Geiger modes, for wide dynamic sensor range operation. By altering the reverse bias voltage, thus putting each photodiode into one of normal, avalanche or Geiger mode, the dynamic range of incident scattering and fluorescent power to which the filter cell array is sensitive to is greatly increased, thus increasing the operational sensitivity and specificity of the cytometric instrument.

WO 2011/026942 A2

Title

An Integrated Cytometric Sensor System and Method

Field of the Invention

This invention relates generally to an integrated cytometric sensor system and method. In particular the invention relates to a highly integrated and low cost electronic optical sensor device, methods and algorithms for enumeration of microscopic cells, particles or molecules distributed in a sample. The invention also relates to a wide dynamic range optical sensor for use in a flow cytometer.

Background to the Invention

Flow cytometry is a powerful method of analysis to determine the cellular/biological content of various types of samples, and in particular samples that contain living cells. In clinical applications, flow cytometers are useful for myriad of applications including lymphocyte counting and classification, for immunological characterization of leukaemias and lymphomas, and for cross-matching tissues for transplants.

In most flow cytometry techniques, cells in a fluid solution are caused to flow individually through a light beam, usually produced by a laser light source or a monochromatically filtered LED source. As light strikes each cell, the light is scattered and the resulting scattered light is analyzed to determine the type of cell. The cell may also optionally be labelled with a marker linked to a fluorescent molecule, which fluoresces when light strikes it and thereby reveals the presence of the marker on the cell. In this fashion, information about the surface components of the cell can be obtained. Examples of such fluorescent molecules include FITC (fluorescein isothiocyanate), TRITC (tetramethyl rhodamine isothiocyanate), Texas Red (sulforhodamine 101), and PE (phycoerythrin). Intracellular components of the cell, such as nucleic acids, may be stained, and subsequently detected by fluorescence. Examples of such compounds include ethidium bromide, propidium iodide, YOYO-1, YOYO-3, TOTO-1, TOTO-3, BO-PRO-1, YO-PRO-1, and TO-PRO-1. In addition, smaller molecules or proteins in the plasma may be detected using stained microbeads or quantum dots.

Light scattering measurements are widely used in flow cytometry to measure cell sizes and to distinguish among several different types of cells. It is known that incident light is scattered by cells at small angles (approximately 0.5-20 degrees) from the axis of the incident light that interrogates the cells, and that the intensity of the scattered light is proportional to the cell volume. The light scattered at small angles is referred to as forward scattered light. Forward scattered light is useful in determining cell size, thus aiding in distinguishing which cell type is being detected.

The ability to measure cell sizes is influenced by the wavelength employed and the precise range of angles over which light is collected. For example, material within the cells having a strong absorption at the illuminating wavelength may interfere with the size determination because cells containing this material produce smaller forward scatter signals than would otherwise be expected, leading to underestimates of cell size. In addition, differences in refractive index between the cells and the surrounding medium may also influence the small-angle scatter measurements.

Different cell types may be further distinguished on the basis of the amount of orthogonal light scatter (or right angle side scatter) they produce. Cells having a high degree of granularity, such as blood granulocytes, scatter incident light at high angles much more than cells with low granularity, such as lymphocytes. As a result, forward and side scatter measurements are commonly used to distinguish among different types of blood cells, such as red blood cells, lymphocytes, monocytes, and granulocytes.

Much prior art exists for cytometer instruments including improvements to the light source, see for example WO2006104699, assigned to Beckman Coulter Inc., and WO0129538, assigned to Beckton Dickinson. Methods to reduce instrument cost, as disclosed in US 2007117158, Coumans *et al.* Methods to provide increases in multi-parametric detection EP 0737855, Beckton Dickinson, and methods to enable a portable cytometer instrument are disclosed by US2003/0142291, assigned to Honeywell International Inc., and WO2007103969 and US2007127863, assigned to Accuri Instruments Inc.

To obtain meaningful information about the numbers and types of cells in the sample or of the concentration of markers on cell surfaces, the samples must be calibrated with respect to the amount of light scatter or fluorescence associated with standardized populations of the cells. Calibration of the instrument is typically accomplished by passing standard particles through the instrument, and measuring the resulting scatter or fluorescence typically using synthetic standard materials (e.g., polystyrene microbeads). These microbeads are made to be extremely uniform in size, and to contain precise amounts of fluorescent molecules to serve in calibrating the photodetectors used in detection of fluorescent probes. US5380663 (A), assigned to Caribbean Microparticles Inc., discloses one such method for calibrating a flow cytometer, which employs combined populations of fluorescent microbeads and a software program matched to each combined population of microbeads.

A significant amount of training is required to learn how to use these instruments properly, and these instrument systems can require service several times a year. As a result, the high costs associated with purchasing, using and maintaining these high-end instruments make them financially inaccessible to small clinics and doctor's offices. Physicians can get access to these instruments by utilizing commercial laboratories that will collect the blood samples from the clinic or physician's office and transport the samples back to the laboratory for analysis on their high end haematology systems, which are typically based on flow cytometer techniques. The results are then sent to the doctor. This process is very time-consuming, and more often than not the doctor will not have the results until the next day, at the least, or up to a week, typically.

Many haematology analyzers now incorporate flow cytometric techniques and other advances, which make the instrument relatively compact and more efficient through incorporation of semiconductor microelectronics where applicable; however, in the end these systems are functionally based on the Coulter instruments used by clinical laboratories in the late 1970's and early 1980's. While the low-end cytometer based haematology analyzers have reduced in price to $30,000 - $50,000, they are still beyond the budget of many small-to-medium sized hospital laboratories and physician offices,

lack the flexibility and wide application support that the higher-end cytometers possess and still come with high maintenance and support costs.

Photomultiplier Tubes (PMT's) have been the incumbent photodetector of choice, within flow cytometers, for reasons of high dynamic range and sensitivity. However, a number of problems exist in that these PMT's are expensive (~$1,000 per channel), require very high voltage operation (>1,000V), which leads to large and expensive power management electronics, and require high maintenance.

Recent advances in solid-state photodiode detectors have started to replace the forward scatter PMT. PCT patent publication number WO 01 94938, assigned to Idexx Lab Inc., describes a lensless cytometer whereby a photodiode is used to collect forward scatter. No optical filters are in the light path between the flow system and photodiode. Hence, light at all wavelengths is detected by the photodetector, even fluorescence. US2007097364 and US2007207536 describe a cytometer and fluorescent biosensor, respectively, using photodiode arrays.

In addition, recent advances in avalanche- and Geiger-mode photodiode detectors (referred to as Silicon Photomultipliers, or SiPM detectors for short) are showing much promise in replacing the side scatter and fluorescence PMT's which require much more sensitivity and dynamic range, and therefore specificity, than the forward scatter PMT's. The main advantages are their much lower cost (<$50) and size, lower voltage operation (<50V) and higher photon detection efficiency. However, they still suffer from low dynamic range, leading to lower sensitivity and specificity compared with the PMT, and large dichroic filters and beam shaping optics are still needed. US 2006250604 (A1) describes one such cytometer based haematology analyzer, which uses avalanche photodiodes.

The use of dichroic filtering as a method to discriminate light across the UV, Visible and IR spectra is well known. JP 58044406 and US5341238 describe dichroic filtering techniques on glass and oxide semiconductor surfaces, respectively. SiPM's, as with

PMT's, cannot discriminate different wavelengths of light. So the large and costly dichroic filters ($300-500 each) remain.

US patent publication number US 2007145236, Kiesel *et al.*, discloses an integrated circuit (IC) that includes a photosensor array, some cells of which are reference cells that photosense throughout an application's energy range, while other cells of which are subrange cells that photosense within respective subranges. The subrange cells can receive photons in their respective subranges from a transmission structure that has laterally varying properties, such as due to varying optical thickness. The reference cells may be uncoated or may also receive photons through a transmission structure such as a gray filter. Subrange cells and reference cells may be paired in adjacent lines across the array, such as rows. Where photon emanation can vary along a path, quantities of incident photons photosensed by subrange cells along the path can be adjusted based on quantities photosensed by their paired reference cells, such as with normalization. However a problem with this sensor is that it is not suitable for cytometric systems as the sensing of the radiation emitted is performed along a path whereas cytometers detects the radiation emitted from a target point.

US Patent publication number US2002/154315, Myrick, discloses an optical filter systems and optical transmission systems, an optical filter compresses data into and/or derives data from a light signal. The filter way weight an incident light signal by wavelength over a predetermined wavelength range according to a predetermined function so that the filter performs the dot product of the light signal and the function. The approach outline by Myrick is akin to a CCD camera, with many pixels. However this filter is not suitable for use in a cytometric system.

US Patent publication number US2007/084990, Coates, discloses an integrated spectral sensing engine featuring energy sources and detectors within a single package that includes sample interfacing optics and acquisition and processing electronics. The miniaturized sensor is optimized for specific laboratory and field-based measurements by integration into a handheld format. Design and fabrication components support high volume manufacturing. Spectral selectivity is provided by either continuous variable optical filters or filter matrix devices. Coates relates to a Spectrophotometer or

Spectrometer, which are specific to measuring fluids or solutions which is not suitable for cytometer applications.

In summary, an objective of the present invention is to provide a highly sensitive, low-cost optical sensor with integrated optical filtering and high dynamic range, to enable low-cost, portable and easily maintainable flow cytometer to overcome the above mentioned problems.

Summary of the Invention

According to the invention there is provided, as set out in the appended claims, a flow cytometric system comprising a first sensor positioned axially to a light source; a channel comprising means for receiving a sample target and interposed between said first sensor and light source; and a second sensor placed at an angle to said first sensor adapted to sense side scattering and/or fluorescent components and said first sensor is adapted to sense a forward scattering component in response to light illuminating the sample target in said channel.

The integrated cytometric system of the present invention provides integrated optical filtering and high dynamic range, to enable low-cost, optically efficient, miniaturised, portable and easily maintainable flow cytometer. Incumbent PMT arrangements require numerous dichroic mirrors and filters to split the wanted wavelengths for detection by separate PMT's, resulting in long and inefficient optical signal paths. The numerous optical components of such systems, each generate optical noise, reduce optical transmission, and any misalignment due to mechanical movement all contribute to sub-optimal optical capture. The integrated cytometric sensor arrangement allows for its placement adjacent the detection zone, and its parallel optical detection paths, each requiring a single filter, provides for a more efficient optical capture arrangement.

Any misalignment of the incumbent PMT optics, due to mechanical movement from transportation or operation, further reduces its optical capture efficiency. The integrated cytometric sensor offers alignment-free optics, as both the filter and detector arrays are integrated into a single package, and each parallel optical path requires only one filter,

thus maximising optical transmission, minimising optical noise and removing any mis-alignment errors due to mechanical movement.

With its small size, additional integrated cytometric sensors can be placed about the detection zone to either increase optical gain by capturing more scattered and fluorescent light. Since the integrated cytometric sensor requires low-voltage operation (~30V) compared with high-voltage PMT based systems (~1,000V), the electronic design and power consumption of the resulting diagnostic instrument is much advanced.

In one embodiment the first or second sensor comprises a plurality of photodiode pixels;

a plurality of optical filters positioned on top of said photodiode pixels,

each optical filter comprises a set filter characteristic and co-operates with one or more of said plurality of photodiode pixels to define a filter cell; and

said filter cells are adapted to detect different wavelengths of light, when light is incident on said sensor, wherein different detected wavelengths are representative of specific biological targets.

In one embodiment the second sensor is positioned orthogonal to said first sensor.

In one embodiment a third sensor can be placed at an angle to said first or second sensor. The third sensor can be placed orthogonal to said first sensor and directly opposite said second sensor to sense side scattering and fluorescent components at 180 degrees to scattering and fluorescent components incident on said second sensor to provide further information.

In one embodiment at least one or more of said photodiode pixels are voltage biased in one or more of the following modes: normal, avalanche or Geiger modes, for wide dynamic sensor range operation. By altering the reverse bias voltage, thus putting each photodiode into one of normal, avalanche or Geiger mode, the dynamic range of incident scattering and fluorescent power to which the filter cell array is sensitive to is greatly increased, thus significantly increasing the resultant instrument sensitivity and specificity.

The invention significantly advances the art to develop such a highly integrated cytometric sensor, by using arrays of SiPM's, with each SiPM element having monolithically integrated optical filters that are separately configured in normal, avalanche and Geiger modes to overcome issues of low dynamic range, sensitivity and specificity.

In one embodiment at least one filter cell comprises a metal-dielectric based arrangement. The metal-dielectric filter comprises integrated metal wires, separated by insulating dielectric layers, arranged in metal grids to form Fabry- Perot cavities.

In one embodiment at least one filter cell comprises a thin-film based arrangement. The thin film layer may comprise areas of different dielectric constants allowing filter cells with different defined filter characteristics across the sensor.

In one embodiment the defined filter characteristics are provided by an array of different dichroic filter materials, with a distribution of different dielectric constants.

The invention embeds a plurality of either patterned thin film filters, or metal-dielectric filters, or both, deposited on a photodiode pixel array, each pixel biased in either, photon counting, normal, linear avalanche or Geiger mode, in an embodiment that integrates multiple dichroic filters and photodetectors (photodiodes) into one monolithic solid state sensor with high photon sensitivity and dynamic range, thus advancing the miniaturisation, operation and cost reduction of analytical instruments.

In one embodiment the invention provides a transparent window cap comprising additional thin film filters to compliment the filter characteristics monolithically deposited on the sensor.

In one embodiment the filter cells are adapted to detect different wavelengths of light when light is incident on said sensor, said different wavelengths are dependent on the scattering and fluorescent signal components representative of specific biological targets.

In one embodiment the set filter characteristics comprises one or more of the following filters: band-pass, high-pass (long-pass), low-pass (short-pass), out-of-band and/or band-stop filters.

In one embodiment a first sensor is positioned axially to an incident light source generated from either a laser beam or monochromatically filtered LED, to sense forward scattering components. A second sensor can be placed orthogonal to said first sensor to sense side scattering and fluorescent components to provide further information on the biological target.

In one embodiment a second sensor is placed at an angle to said first sensor to sense side scattering and fluorescent components to provide further information.

In one embodiment a beam stop is positioned between the forward scatter sensor and light source, to minimise the saturating effects within the sensor from the optically high-power laser incident directly on to the sensor.

This sensor of the present invention incorporates multiple optical filters, with different pass/stop-band characteristics across visible and near-infrared spectra, monolithically integrated on an array of photodiodes.

The invention relates to the integration of multiple optical filters and photodiodes (currently separate components in analytic instruments) into a highly integrated solid-state cytometric sensor; and embodiments into analytical instruments such as flow cytometers, scanning cytometers or fluorimeters which significantly simplifies, miniaturises and lowers their operation and cost.

The invention also provides for the use of these cytometric sensors in a typical cytometer instrument, and the data signal path from sensor output through to instrument display.

In one embodiment at least one filter cell output is post-processed with fluorescent biomarker compensation algorithms by conditioning the filter output by predetermined fractions to compensate for fluorescent interference from other biomarker profiles.

In one embodiment, the filter cell outputs are amplified and over-sampled into the digital domain for post-processing and later interpretation for user-readable display. Such post-processing algorithms may include calibration algorithms, gain optimising algorithms or signal conditioning algorithms. These post-processing algorithms may be implemented in a digital signal processor, hardwired into an ASIC, or integrated into the filter cell ASIC, using CMOS integration techniques.

In a further embodiment there is provided an integrated sensor, comprising:

a plurality of photodiode pixels;

a plurality of optical filters positioned on top of said photodiode pixels, wherein each optical filter comprises a set filter characteristic and co-operates with one or more of said plurality of photodiode pixels to define a filter cell; and

said filter cells are adapted to detect different wavelengths of light, when light is incident on said sensor.

In another embodiment there is provided a method of detecting different wavelengths representative of specific biological targets in a flow cytometric system comprising the steps of:

providing a plurality of photodiode pixels;

arranging a plurality of optical filters positioned on top of said photodiode pixels, wherein each optical filter comprises a set filter characteristic and co-operates with one or more of said plurality of photodiode pixels to define a filter cell; and

detecting different wavelengths of light, when light is incident on said filter cells, wherein a detected wavelength is representative of a specific biological target.

In another embodiment there is provided a cytometric system comprising a first sensor positioned axially to a light source; a channel comprising means for receiving a sample target and interposed between said first sensor and light source; and a second sensor

placed at an angle to said first sensor adapted to sense side scattering and/or fluorescent components and said first sensor is adapted to sense a forward scattering component in response to light illuminating the sample target in said channel.

In a further embodiment there is provided a wide dynamic range optical sensor, for use in a flow cytometric system, comprising:

a plurality of photodiode pixels;

wherein at least one or more of said photodiode pixels are voltage biased in one or more of the following modes: photon counting, normal, linear avalanche or Geiger modes, for wide dynamic sensor range operation.

In one embodiment the wide dynamic range sensor comprises a plurality of optical filters positioned on top of said photodiode pixels.

In one embodiment each optical filter comprises a set filter characteristic and cooperates with one or more of said plurality of photodiode pixels to define a filter cell.

In one embodiment the wide dynamic range sensor comprises a transparent window cap comprising additional thin film filters.

In another embodiment of the present invention there is provided a method of analysing a sample target in a flow cytometric system comprising the steps of:

positioning a first sensor axially to a light source;

receiving a sample target in a channel, said channel interposed between said first sensor and light source; and

positioning a second sensor at an angle to said first sensor adapted to sense side scattering and/or fluorescent components and said first sensor is adapted to sense a forward scattering component in response to light illuminating the sample target in said channel.

In one embodiment a plurality of axially and orthogonally placed sensors may be placed along the sample path for each incident light source generated by one laser beam split into a plurality of light sources.

In one embodiment a plurality of axially and orthogonally placed sensors may be placed along the sample path for each incident light source generated by a plurality of lasers.

There is also provided a computer program comprising program instructions for causing a computer program to carry out the above method and control the sensor according to the invention which may be embodied on a recording medium, carrier signal or read-only memory.

Brief Description of the Drawings

The invention will be more clearly understood from the following description of an embodiment thereof, given by way of example only, with reference to the accompanying drawings, in which:-

Figure 1 illustrates a plan view of the cytometric sensor showing an array of filter cells with one of the filter cells exploded to show individual photodiode pixels and biasing arrangement, according to the invention;

Figure 2 illustrates a cross section of the sensor design from Figure 1 of a packaged sensor, according to the invention;

Figure 3 illustrates a detailed perspective view of a thin-film based filtering approach;

Figure 4 illustrates a detailed perspective view of a metal-dielectric based filtering approach;

Figure 5 shows the spectral characteristics of filter cells centred around fluorescent marker emission peaks;

Figure 6 shows the spectral characteristics of filter cells using an out-of-band blocking filter in a window cap;

Figure 7 describes the data signal path from the output for the cytometric sensor stage through to a digital signal processor;

Figure 8 illustrates typical biomarker fluorescent profiles and the need for fluorescent compensation;

Figure 9 shows an embodiment of a miniaturised flow cytometer using the integrated cytometric sensors of the present invention;

Figure 10 shows an embodiment of a miniaturised flow cytometer using the integrated cytometric sensor of the present invention with two fluorescence and side scatter sensors;

Figure 11 shows an embodiment of a miniaturised flow cytometer using the integrated cytometric sensors of the present invention with two light sources each with an integrated cytometric sensor with distinct detection zones on a single flow channel; and

Figure 12 shows a graph illustrating dynamic range operation of the flow cytometer.

Detailed Description of the Drawings

Figure 1 illustrates a sensor design of a semiconductor die 1 with IO pads 2 surrounding a core area 3. This core comprises an X*Y array 4 of N filter cells each denoted F_i where $i = 1...N \leq X*Y$. Each filter cell has a defined filter characteristic and a photodetector design comprising a plurality of photodiode pixels 5 and areas for other non photo-sensitive circuitry 6, necessary for inter-connection and sampling circuits. In one embodiment the photodiodes may be biased in photon counting, normal, avalanche or Geiger mode of operation. In this arrangement each filter cell column is biased into one of these three modes by connecting biasing voltage rails 7, 8 to each filter cell in the array column. For example, by connecting a voltage greater than the reverse breakdown of the photodiode e.g. -35V, assuming a -30V breakdown, to the biasing rail marked 8, the filter cell in the centre of the illustration, and those above and below it, is biased into Geiger mode. Similarly, connecting a bias voltage below the breakdown e.g. -10V, to the rail marked 7, will configure the filter cells in the left-most column into avalanche mode. Other embodiments may have separate biasing rails to enable greatest biasing flexibility. The photodiodes may be arranged in an array, as shown, or arranged in a different pattern, or even in a random arrangement. The filtering component of each filter cell comprises either a metal-dielectric based arrangement, or a thin-film based arrangement, or both, as described later in Figure 2.

When a photodiode is reverse biased, a photon incident onto its pn (or PiN) junction region creates an electron-hole pair upon impact ionisation. The reverse bias drives this electron to the anode and, as long as the bias voltage is relatively low, does not further amplify within the photodiode, thus creating a current collected at the anode that is proportional to the number of incident photons. This operation is termed normal reverse bias mode. Once the photodiode reverse bias is increased towards, but remains lower than, the reverse breakdown voltage, electron-hole pairs created by incident photons create a cascade of further electron-hole pairs, thus amplifying the effect of the first electron-hole pair generated by the incident photon. This describes the avalanche mode of operation. This cascade effect is not self sustaining but still results in moderate amplification with current gains typically in the 10^1-10^3 range. This gain is higher the closer the reverse bias voltage approaches the reverse breakdown voltage. In Geiger mode, the photodiode is biased beyond its reverse breakdown voltage so that the incident photons create a rapid cascade of electron-hole pairs by impact ionisation. The high reverse-bias voltage sustains and amplifies the cascade. In this mode, the photodiode exhibits very high gain (typically $>10^5$) and is highly sensitive to single photons but can only quantify low incident photon numbers since the high gain quickly saturates to the voltage rails. When in Geiger mode the SPM can be also be configured as a photon counter (photon counting mode). For very low levels of light, when the frequency of photons hitting the sensor is so low that the current pulse created by an incident photon is extinguished before the next incident photon arrives, the SPM is seen to be able to detect individual photons. These pulses are counted using the signal acquisition electronics (such as a comparator connected to a digital counting circuit) to create a photon counter.

Once the output current pulse is generated, quenching circuits may be used to quickly bring the photodiode out of these modes, especially avalanche and Geiger modes, and back to the reversed biased state prior to the onset of the cascading effects from incident photons. The quicker the cascade can be 'quenched' the more incident photon events can be sensed or counted. Passive quenching circuits typically use capacitors and resistors to limit the current flowing through the photodiode in avalanche and Geiger modes thus terminating the cascading effects. Active quenching circuits typically sense

the increase in current and reduce the bias voltage across the photodiode bringing it out of the cascade.

By altering the reverse bias voltage, thus putting each filter cell into one of normal, avalanche or Geiger mode, the dynamic range of incident scattering and fluorescent power, to which the filter cell array is sensitive to, is greatly increased. For example, if two filter cells have the same filter characteristic, but one is in avalanche mode and the other is in Geiger mode, then the former can quantify high numbers of incident fluorescence/scattering photons without saturating, whereas the latter is sensitive to low numbers of incident fluorescence/scattering photons, even down to single photon detection.

It will be appreciated that the following sensor description shows the filter cells all integrated onto one semiconductor die. Equally, the cytometer apparatus later described may use a cytometric sensor with separate die, having one or more filter cells, mounted into one package.

Figure 2 illustrates a cross-section elevation of the cytometric sensor, cut along AA' of Figure 1. Figure 2 shows a semiconductor die 10 mounted on a substrate carrier 11, comprising a plurality of photodiode pixels 12. The first of two independent filtering components is built using monolithic metal interconnection layers 13 typical of standard semiconductor processes, whereby metal wires 14 separated by insulating dielectric, typically used for interconnection of the integrated electronic circuits, are arranged in layers of metal grids to form Fabry-Perot cavities which filter the light incident upon them. This metal-dielectric filter component is described in greater detail below in Figure 4.

The second independent filtering component comprises a plurality of thin film layers 15 monolithically built atop the die and metal interconnect layers, with each thin film layer having different dielectric constants. Each single thin film layer may comprise areas of different dielectric constants 16, allowing filter cells with different filter characteristics across the sensor die. This thin-film based filter component is described in greater detail

in Figure 3. The filter characteristic of each filter cell may be delivered using either the thin-film based approach, the metal-dielectric approach, or both.

The sensor design interfaces through IO pads 17 connected to the substrate carrier via wirebonds 18. Other methods of mounting the die that do not obscure the photodiodes may be used. The sensor die can be protected with a package 19 that has a transparent window cap 20. This window cap may either be transparent or may have deposited thin film filtering layers 21 to compliment the filter characteristics monolithically deposited on the sensor die. In this way the light 22 incident on the package can be filtered by a combination of the window cap and monolithic filters (thin films and/or metal-dielectric) before detection by the photodiode pixels.

Figure 3 describes the thin-film based filter component in more detail, where a section of a die on a semiconductor wafer is shown. A monolithic patterned dichroic filter array is built on the underlying semiconductor die, by combining microelectronic and microlithography techniques. Once the underlying photodiode 30 and metal interconnection wires 31 (in this case, these metal wires are used for normal electronic circuit interconnection, as opposed to forming part of a Fabry-Perot cavity) are built, the semiconductor wafer is coated with a photoresist. The assembly is masked, and the unmasked photoresist, after exposure to ultraviolet light, is developed to expose a predetermined section 32. That section of wafer is then over developed to expose the underlying wafer. Dichroic filter material is then deposited onto the wafer, and the photoresist layer is then removed, leaving only the dichroic filter material on the wafer, with well-defined edges, to cleanly separate it from other sections that are subsequently developed. Separate filter sections can be produced in a pattern to simultaneously measure scattering and fluorescent wavelengths. These filter sections are cast in patterns that overlay the photodiodes, thus defining the filter cell. The process is repeated to create an array of different dichroic filter materials, with a distribution of dielectric constants. The different shading patterns of these sections in Figure 3 depict filter cells with different dichroic filter material, each with different dielectric constants, and thus different filter characteristics.

Figure 4 describes the metal-dielectric filter component. As in Figure 3 the filter component is built on the underlying photodiode array 40 and the low-level metal interconnection wires 41. In modern semiconductor processes, the metal interconnection lines are comparable or smaller than the wavelength of visible light (400-700nm). At these scales, the metal interconnection layers 42, 43 are not as opaque to visible light as bulk metal, and variations in the pattern and inter-layer dielectric 44 thickness, control the probability that incident light at a particular wavelength will filter through 45 to the underlying photodiode, leading to the design of integrated metal-dielectric filters. The likelihood of the incident light filtering through to the photodiode is governed by the optical properties of the metal wires and dielectric layers, respective layer thickness and the geometry of the patterns implemented. This so-called surface-plasmon induced resonance phenomenon yields a peak transmittance, which occurs for a given wavelength depending on the type of metal, the dielectric surrounding and pattern geometry. Exemplary teachings on this surface-plasmon phenomenon can be found in "Extraordinary optical transmission through sub-wavelength hole arrays", Ebbesen *et al.*, Nature (London), Feb. 1998, vol. 391, pp667-669 and on metal-dielectric filters can be found in US2003103150 (A1). The use of such monolithically integrated metal-dielectric filters with photodiodes, to produce a colour sensitive photodetector, or so-called Integrated Color Pixel is known in the art.

Figure 5 shows a typical spectral characteristic of the filter cells required for cytometry applications. In this illustration, the light incident on the sensor of Figure 2 comprises light scattering at the lowest wavelength 50, from forward or side scattering generated from the laser beam interrogation of the cells, and a plurality of fluorescent light signals each with emission peaks at longer wavelengths 51 up to wavelength maxima 52. The filter characteristics of the filter cells discriminate incident light scattering components 53 and the plurality of fluorescence components 54 allowing the photodiode pixels to detect these wavelength discriminated signals incident upon them. This embodiment of the sensor uses filter cells with band-pass characteristics centred at differing wavelengths, normally coincident with the peak fluorescent emission of respective biomarkers. Other embodiments may use filter cells with different filter characteristics such as, but not limited to, high-pass, low-pass, and band-stop, depending on the biological target being diagnosed.

Figure 6 shows the spectral characteristics whereby the filter characteristics are defined by the combination of out-of-band blocking filter characteristics 60 and a plurality of in-band filter characteristics 61, 62, 63. The out-of-band blocking filter may be realised by deposited dielectric thin films on the window cap, as previously described in Figure 2 whereas the in-band filter characteristics are as a result of the monolithic filters integrated in the filter cells. In this embodiment, the light scattering component of the incident light is filtered by a short-pass filter cell 61. The fluorescent component with the longest wavelength is filtered by a long-pass filter cell 62. The intermediate wavelength fluorescent components are filtered using a plurality of band-pass filter cells 63. Another embodiment of the out-of-band blocking filter is the deposition of metal-dielectric filters, as already described, onto the window cap.

Figure 7 describes the electronic data signal path whereby the multiple filter cell outputs 70 from the Integrated Cytometric Sensor 71 are amplified 72 and digitized by an analog-to-digital converter 73. A custom ASIC 74 and digital signal processor 75 (DSP) configures and controls the integrated cytometric sensor, and implement signal conditioning and other post-processing algorithms, with the latter sending data output to the instrument display 76. These algorithms include, but are not limited to, glitch and dark count filtering, fluorescent biomarker compensation (described in Figure 8 described below) and control/configure algorithms for the photodiode bias voltage for optimum performance. These algorithms are typically partitioned across an ASIC and/or DSP. It will be appreciated that the sensor 71 can be combined with the electronic circuitry on a single integrated chip to improve the efficiency by providing finer feedback loops, thus improving overall performance.

When the photodiode detectors of the filter cells are biased in high-gain avalanche and Geiger modes, thermal or shot noise creates false output pulses known as dark current pulses. These dark current interferer pulses may be filtered using decision-driven gating methods. For example, no filter cell outputs due to scattering or fluorescent light are generated unless the biological targets intersect the laser beam. Typically, no fluorescence signals are produced unless light scattering is produced. Therefore, any dark current pulses may be filtered out when no light scattering is detected.

The need for fluorescent biomarker compensation is illustrated in Figure 8. In this figure, the characteristics for three filter cells are denoted F_1 80, F_2 81 and F_3 82. These filter characteristics discriminate the incident scattering and fluorescence on to the photodiodes, as already described. These are typically centred on the wavelengths of peak fluorescence of the fluorescently tagged biomarkers, at 83, 84, 85, with profiles denoted as 86, 87, 88, respectively. Here, the fluorescent profile 86 with peak fluorescence 83 interferes upon filter characteristic F_2. Similarly, the fluorescent profile 88 with peak fluorescence 85 interferes upon filter characteristic F_2. Since the emission profiles of these biomarkers are predefined, this interference may be minimised by reducing the F_2 filter cell output by predetermined fractions of the fluorescent interferers. Similarly, well defined fluorescent profiles of biomarkers may be used to implement calibration algorithms.

Figure 9 shows an embodiment of a miniaturised flow cytometer using two integrated cytometric sensors 59 and 65, illustrating operation of the invention. Here a laser source 50 generates a light beam 51 incident upon biological targets (e.g. cells, molecules, proteins) 52 flowing in a fluidic channel 53 in the direction as shown by the arrow. It will be appreciated that the flow channel can be a slot to receive a stationary sample. Equally, a monochromatically filtered LED source may be used in lieu of a laser source. The region where the laser beam is incident on the biological targets shall be defined as the detection zone 54 with the angle of incidence, φ, 55 normally 90°. As the biological targets flow across the laser beam, light scattering and fluorescent components are emitted in all directions 56 (basically 4Π Steradians). The light scattering component is emitted at a wavelength equivalent to the incident laser beam.

The fluorescent light components are emitted at a plurality of wavelengths defined by the properties of the fluorescent markers which may have been mixed with the biological targets. The emitted light component axial to the incident laser beam 57, denoted as the forward scattering component, is focused by the forward lens 58 on to the forward cytometric sensor 59. Other embodiments may not use this forward lens. A beam stop 60 may be placed axially between the detection zone and the cytometric sensor, in front of the forward lens, if used, to avoid the laser beam damaging the

forward scatter sensor when the biological targets are not interposed. Extra diagnostic information is collected by interrogating side scattering and fluorescent components 61 at an angle, Φ, 62 to the axial and an angle, Ψ, 63 to the fluidic channel. Φ and Ψ are normally 90° each.

This emission component is focused by the side lens 64 on to the side cytometric sensor 65. Other embodiments may not use this side lens. Both forward and side cytometric sensors interface to an ASIC 66 and DSP 67, whose operation is previously described with respect to Figure 7. The output information can be displayed on to a display unit 68 in user-readable format, e.g. a Graphic User Interface. Further cytometric sensors may be placed at varying angles {φ, Φ, Ψ} to, and distances from, the detection zone.

Figure 10 shows an embodiment of a miniaturised flow cytometer using three integrated cytometric sensors 59, 65 and 69, illustrating operation of the invention. This is similar to the embodiment shown in figure 9 with the addition of a cytometric sensor, 69, orthogonal to the flow channel as with sensor 65, but placed at 180 degrees to sensor 65, with the flow channel, 53, interposed between them . The scatter and fluorescent light components emitted are focused on to the both the cytometric sensors 65 and 69.

Figure 11 shows an embodiment of a miniaturised flow cytometer system using a plurality of miniaturised flow cytometers illustrating operation of the invention. This is similar to the embodiment shown in figure 9 with the addition of a second complete miniaturised cytometer interrogating the same flow channel, 53, but which is focussed on a second detection zone, 92, through which the sample flows after it passes through the detection zone, 54, for the first miniaturised flow cytometer. In this embodiment each miniaturised flow cytometer has a dedicated laser source, 50 and 93. However one could also use a single light source and distribute it to each miniaturised flow cytometer using a prism, mirror or other optical arrangement. It is clear that a plurality of such miniaturised flow cytometers can be assembled.

ASIC or microelectronic circuit assembly 66 and DSP 67 take the output signals from each SiPMs and the bias information from every SiPMs for each measurement and combine them to create a single wide dynamic range measurement. The dynamic range

of the combined measurement is significantly larger than the individual dynamic ranges of the SiPMs operating at a single bias condition.

Figure 12 shows an embodiment of such a combining action. Here the high dynamic range is shown, with the x axis 100 being the optical input power captured at the detection zone illustrated as a logarithmic scale and the y-axis 101 being the resultant measured output voltage. The figure shows two SiPM traces, the first 102 being the transfer function from a Geiger mode SiPM, and the second 103 being the transfer function from the linear mode SiPM. Separately, the dynamic range of the Geiger mode SiPM, as shown, is approximately 4 decades, calculated by the difference between where both its extremities saturate at 104 and 105, and the linear mode SiPM has a dynamic range, as shown, of approximately 4 decades, calculated by the difference between where both its extremities saturate at 106 and 107. In one embodiment, the separate SiPM outputs are digitised and sent into an FPGA for combining. The high dynamic range algorithm transposes the outputs along both the x-axis and y-axis to remove any discontinuities in the combined transfer function. While transposing along the x-axis by dx 108 is at the expense of a lower combined dynamic range, it ensures a smooth transition for the combined transfer function. The transposition distance along the y-axis, dy, is a function of the respective bias voltages and separate SiPM transfer functions. This is predetermined at calibration by sweeping the optical input power across the x-axis and, once transposed by dx, calculating a dy that overlaps both SiPM outputs.

It will be appreciated that the invention anticipates that any combination of SiPM bias modes is suitable, photon counting, normal, avalanche or Geiger. Additionally, one or more sensors which are biased in any one of these modes, but with different bias voltages within that mode of operation may also be utilised.

It is envisaged that the embodiment described with respect to Figure 9, 10 and 11 can be incorporated in a hand held device, for example the size of a typical personal digital assistant. The use of the integrated sensor hereinbefore described allows for fast detection (in real time) of a biological target in a sample.

It will be appreciated that the invention can be incorporated in other similar analytical instruments. This invention enables the various embodiments of such instruments presented herein. Such instruments include, for example, and without limitation, immunoassay analyzers, clinical haematology analyzers, flow and scanning cytometers, fluorimeters, and chemistry analyzers. The specific biological targets can be in a fluid sample or a non-fluidic sample, so long as the sample can be subjected to a light source and detection of different wavelengths can be achieved.

It will be appreciated that the invention illustrates a single laser source in Figure 9, 10 and 11 use of second laser (or more) can be provided to provide a wider range of bio-compatibility and also to provide additional improved compensation methods.

The embodiments in the invention described with reference to the drawings comprise a computer apparatus and/or processes performed in a computer apparatus. However, the invention also extends to computer programs, particularly computer programs stored on or in a carrier adapted to bring the invention into practice and control operation of the cytometric sensor. The program may be in the form of source code, object code, or a code intermediate source and object code, such as in partially compiled form or in any other form suitable for use in the implementation of the method according to the invention. The carrier may comprise a storage medium such as ROM, e.g. CD ROM, or magnetic recording medium, e.g. a floppy disk or hard disk. The carrier may be an electrical or optical signal which may be transmitted via an electrical or an optical cable or by radio or other means.

The invention is not limited to the embodiments hereinbefore described but may be varied in both construction and detail.

Claims

1. A flow cytometric system comprising:

a first sensor positioned axially to a light source;

a channel comprising means for receiving a sample target and interposed between said first sensor and light source; and

a second sensor placed at an angle to said first sensor adapted to sense side scattering and/or fluorescent components and said first sensor is adapted to sense a forward scattering component in response to light illuminating the sample target in said channel.

2. The flow cytometric system as claimed in claim 1 wherein the first or second sensor comprises:

a plurality of photodiode pixels;

a plurality of optical filters positioned on top of said photodiode pixels,

each optical filter comprises a set filter characteristic and co-operates with one or more of said plurality of photodiode pixels to define a filter cell; and

said filter cells are adapted to detect different wavelengths of light, when light is incident on said sensor, wherein different detected wavelengths are representative of specific biological targets.

3. The flow cytometric system as claimed in claim 1 or 2 wherein the second sensor is positioned orthogonal to said first sensor.

4. The flow cytometric system as claimed in any preceding claim comprising a third sensor placed at an angle to said first or second sensor.

5. The flow cytometric system of claims 2, 3 or 4 wherein at least one or more of said photodiode pixels are voltage biased in one or more of the following modes: normal, avalanche or Geiger modes, for wide dynamic sensor range operation.

6. The flow cytometric system as claimed in any of claims 2 to 5 wherein at least one filter cell comprises a metal-dielectric based arrangement.

7. The flow cytometric system of claim 6 wherein said metal-dielectric filter comprises integrated metal wires separated by insulating dielectric, are arranged in metal grids separated by insulating dielectric layers, to form Fabry- Perot cavities.

8. The flow cytometric system of any preceding claim wherein at least one filter cell comprises a thin-film based arrangement.

9. The flow cytometric system of claim 8 wherein said thin film layer comprises areas of different dielectric constants allowing filter cells with different defined filter characteristics across the sensor.

10. The flow cytometric system of claim 9 wherein said defined filter characteristics are provided by an array of different dichroic filter materials, with a distribution of different dielectric constants.

11. The flow cytometric system of any preceding comprising a transparent window cap comprising additional thin film filters to compliment the filter characteristics of said optical sensors.

12. The flow cytometric system as claimed in any of claims 2 to 11 wherein said filter cells are adapted to detect different wavelengths of light when light is incident on said sensor, said different wavelengths are dependent on scattering and fluorescent signal components representative of specific biological targets in said target sample.

13. The flow cytometric system in any of claims 2 to 12 wherein the set filter characteristics comprises one or more of the following filters: band-pass, high-pass, low-pass, long-pass, short-pass, out-of-band and/or band-stop filters.

14. The flow cytometric system in any of claims 2 to 13 wherein at least one filter cell output is post-processed for fluorescent biomarker compensation by conditioning the filter output by predetermined fractions to compensate for fluorescent interference.

15. The flow cytometric system of any preceding claim wherein the light source comprises a laser beam or monochromatically filtered LED.

16. The flow cytometric system as claimed in any preceding claim comprising a beam stop positioned between the sensor and light source.

17. A wide dynamic range optical sensor in a flow cytometric system, comprising:

a plurality of photodiode pixels;

wherein at least one or more of said photodiode pixels are voltage biased in one or more of the following modes: photon counting, normal, linear avalanche or Geiger modes, for wide dynamic sensor range operation.

18. The wide dynamic range optical sensor as claimed in claim 17 comprising a plurality of optical filters positioned on top of said photodiode pixels.

19. The wide dynamic range optical sensor as claimed in claim 18 wherein each optical filter comprises a set filter characteristic and co-operates with one or more of said plurality of photodiode pixels to define a filter cell.

20. The wide dynamic range optical sensor as claimed in claims 18 or 19, comprising a transparent window cap comprising additional thin film filters.

21. An integrated cytometric sensor in a flow cytometric system, comprising:

a plurality of photodiode pixels;

a plurality of optical filters positioned on top of said photodiode pixels,

characterised in that:

each optical filter comprises a set filter characteristic and co-operates with one or more of said plurality of photodiode pixels to define a filter cell; and

said filter cells are adapted to detect different wavelengths of light, when light is incident on said sensor, wherein different detected wavelengths are representative of specific biological targets.

22. A method of analysing a sample target in a flow cytometric system comprising the steps of:

positioning a first sensor axially to a light source;

receiving a sample target in a channel, said channel interposed between said first sensor and light source; and

positioning a second sensor at an angle to said first sensor adapted to sense side scattering and/or fluorescent components and said first sensor is adapted to sense a forward scattering component in response to light illuminating the sample target in said channel.

Drawings

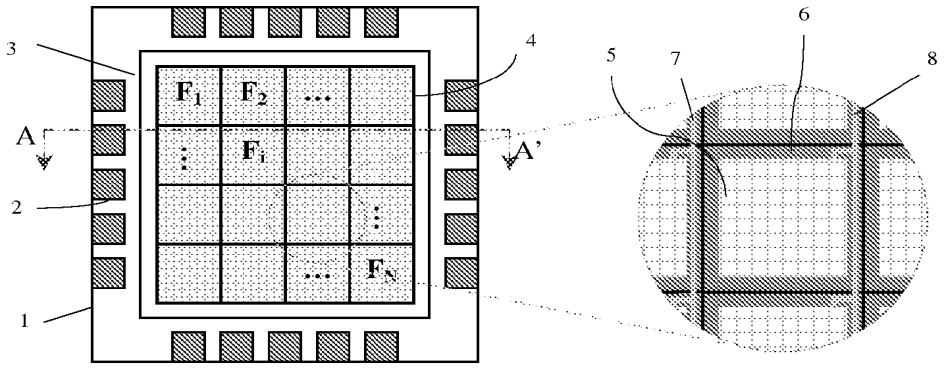


Figure 1

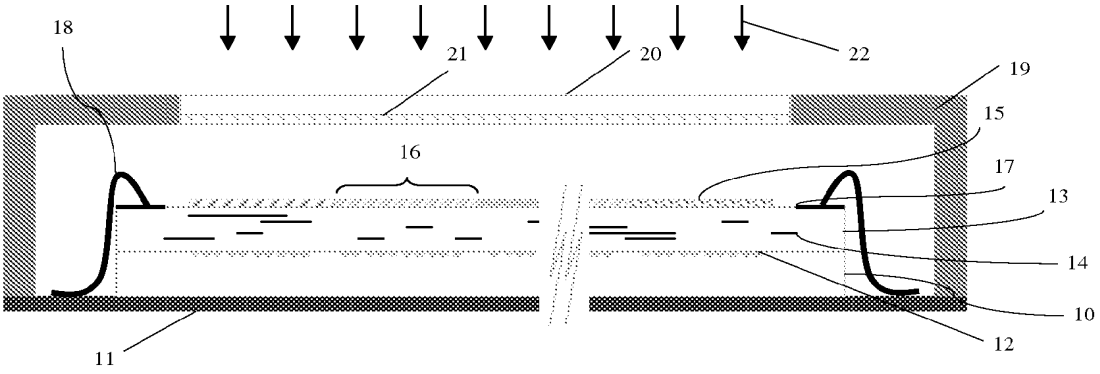


Figure 2

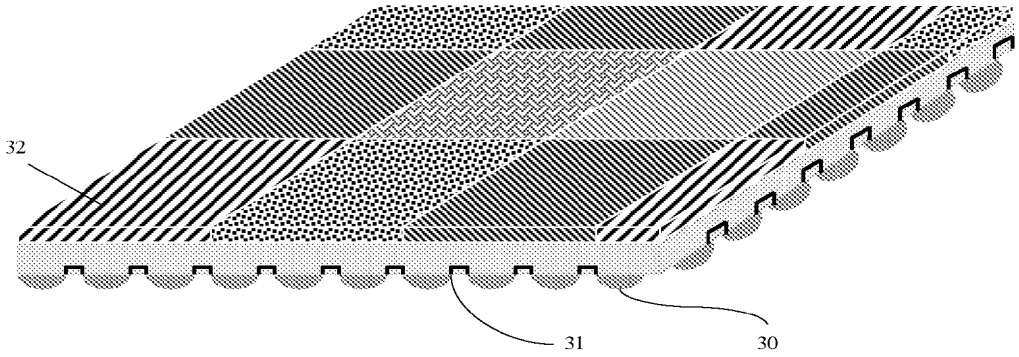


Figure 3

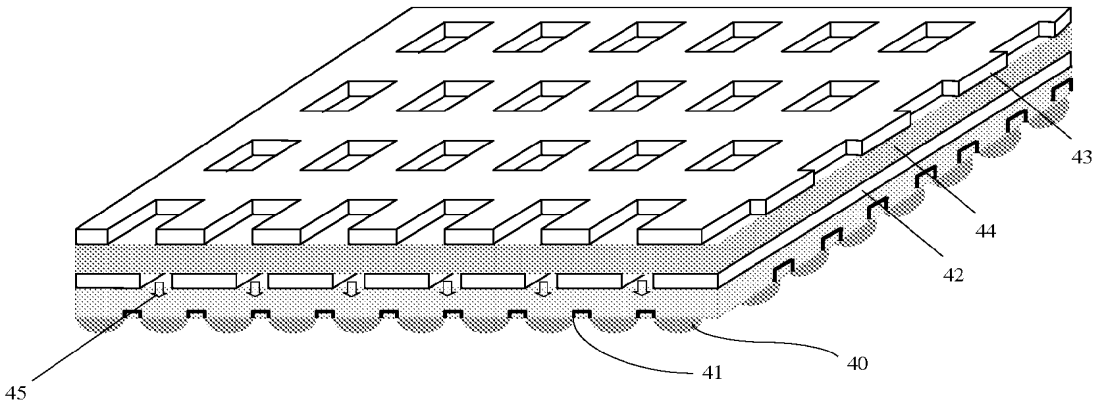


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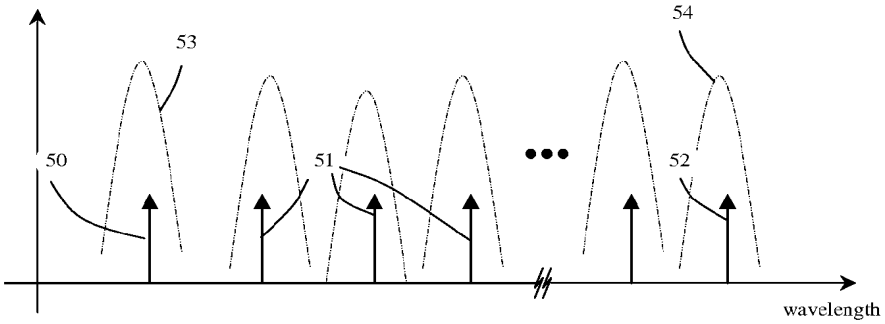


Figure 5

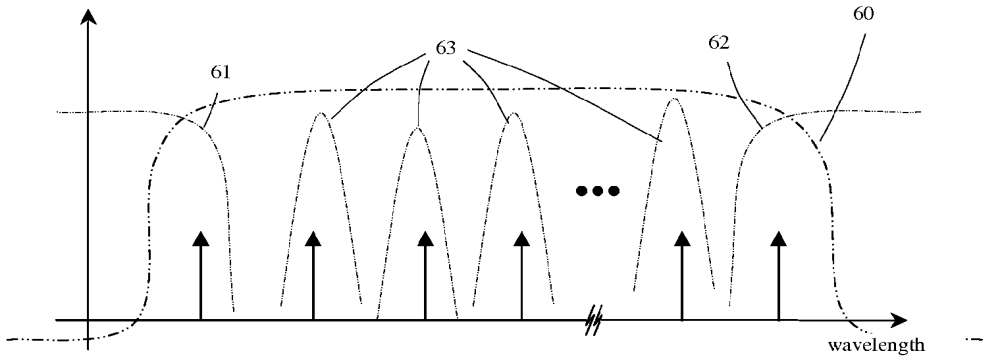


Figure 6

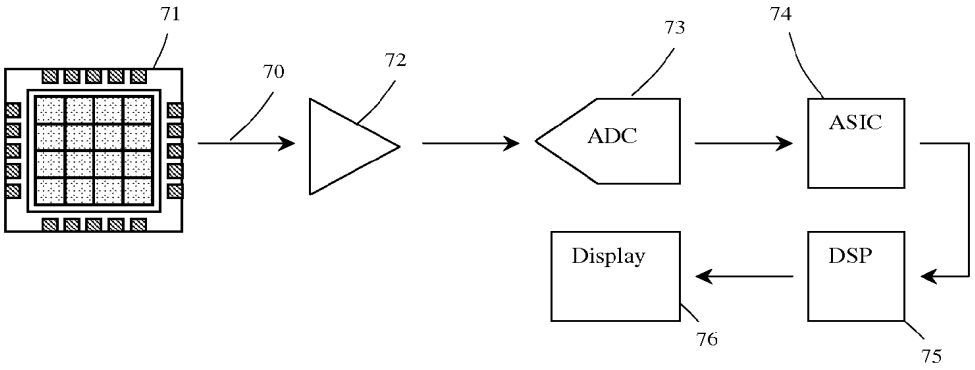


Figure 7

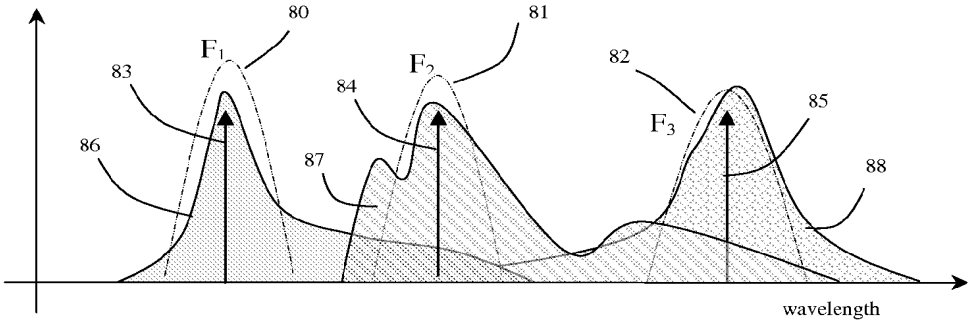


Figure 8

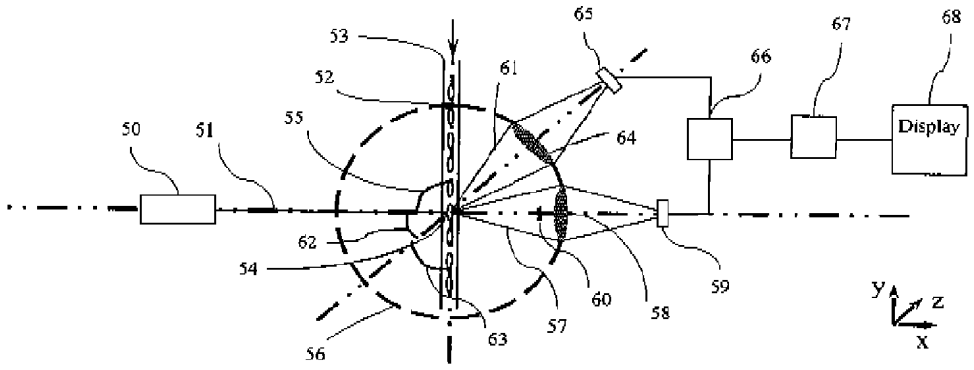


Figure 9

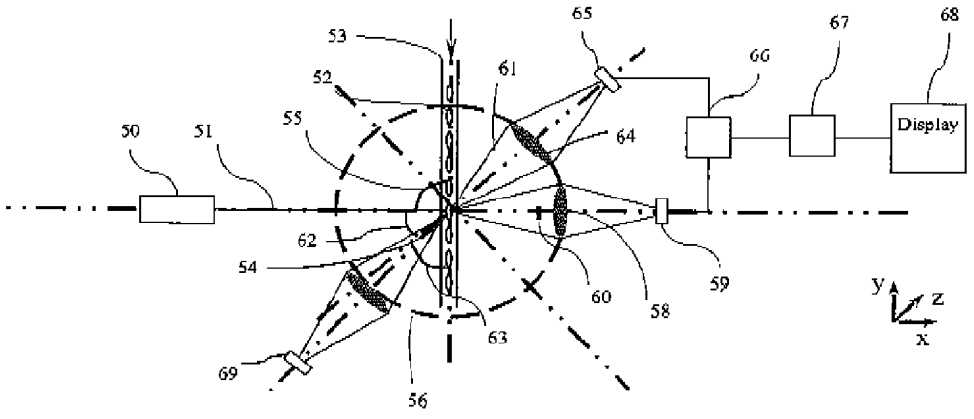


Figure 10

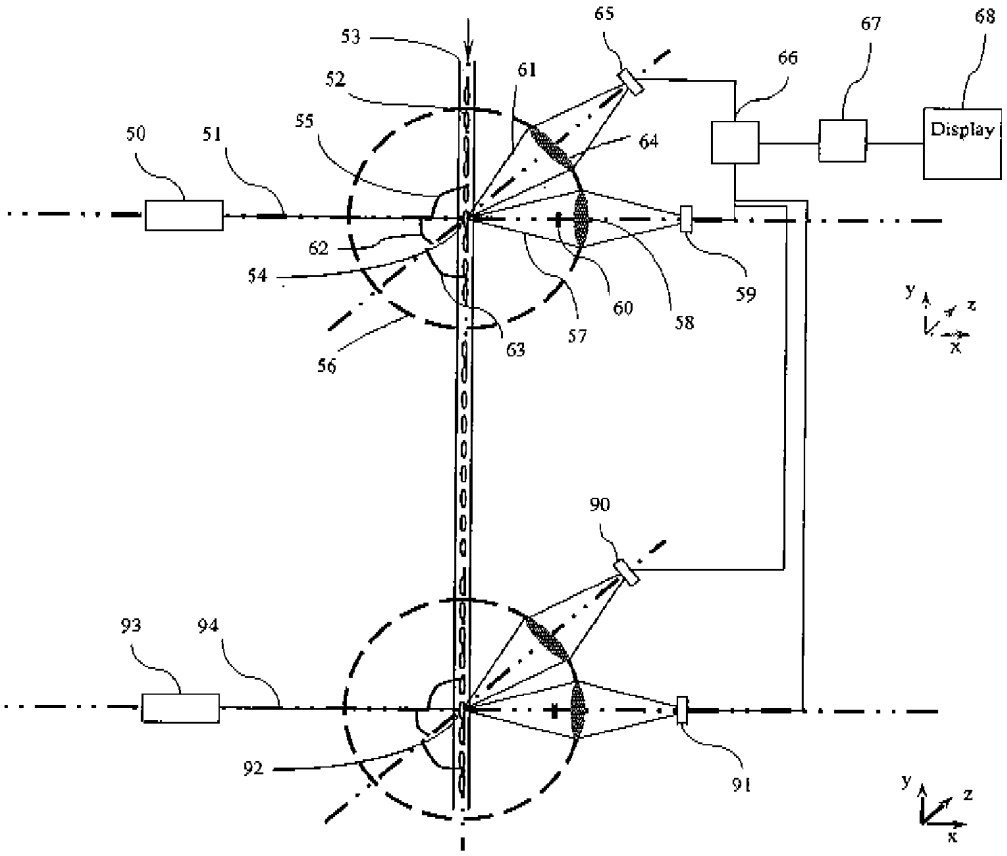

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Figure 11

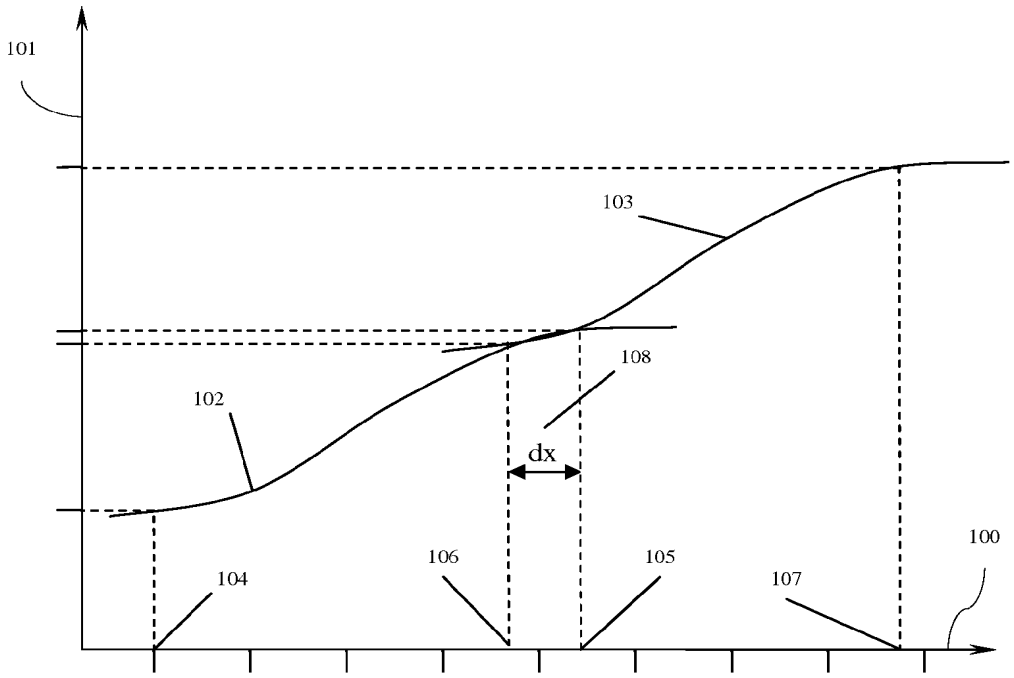


Figure 12

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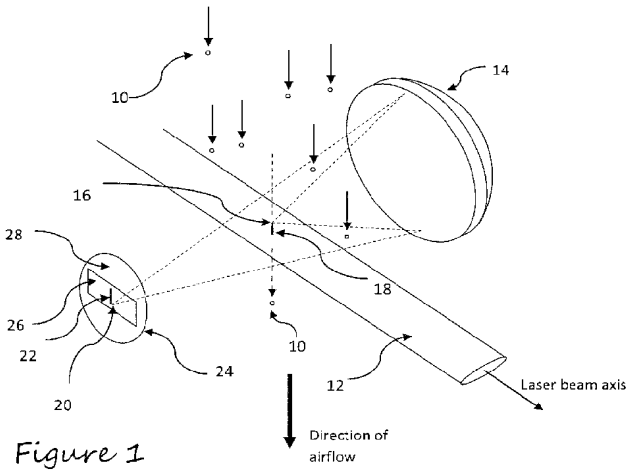


Figure 1

(57) Abstract: An apparatus for the detection of a fluid-borne particle (10) in an optically defined particle sensing zone, the apparatus comprising: i) a scattering chamber (40); ii) a means for providing a sample of fluid (34), containing the fluid-borne particle, in the form of a flow through the optically defined particle sensing zone;iii) A means for generating a beam of radiation (12) through the optically defined particle sensing zone;iv)a single reflector or refractor (14) means having a primary focus (16) within the optically defined particle sensing zone and a secondary focus (20) located outside the beam of radiation; v) a detector means comprising a first photosensitive detection area (26) a second photosensitive detection area (29); vi) a means for deriving area from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means wherein the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radation within the optically defined particle sensing zone to the detection means located at the secondary focus of the single reflector or refractor means and the optically sensing zone comprises a first and a second zone.

WO 2012/056217 A1

An Improved Low Cost Apparatus and Method for the Detection of a Fluid-Borne Particle

Field of the Invention

The present invention relates to a low cost apparatus and method for the detection of a fluid-borne particle. It is particularly applicable for the detection of a fluid-borne particle in an optically defined sensing zone.

Background

In many fields of environmental monitoring, workplace monitoring, pollution control, and occupational health monitoring, it is necessary to continuously measure the aerosol content of a local environment. Where real-time high-sensitivity measurements are desired, instruments are used that are capable of measuring the sizes of individual particles at high rates (typically thousands of particles per second) so that an accurate assessment of both the concentration of particles and their size distribution can be achieved.

The instruments that are commonly used for this purpose are 'optical particle counters' (OPC). These use an intense light source (usually a laser) to illuminate a narrow column of sample air drawn through a sensing chamber by an electrical air-pump. The air column is sufficiently narrow that the volume of air illuminated by the laser beam – often referred to as the particle 'sensing zone' - rarely contains more than a single particle, and the pulse of light scattered by this particle as it crosses the laser beam is recorded as a particle count, the magnitude of the pulse being indicative of the particle size according to a calibration function. The calibration function depends on factors such as the laser wavelength and beam power and the solid angular range over which the scattered light from the particle is recorded.

There are many excellent commercial varieties of OPC manufactured by companies such as Met One Instruments (USA), Grimm Aerosol Technik GmbH (Germany), and Casella Measurement Ltd., (UK). In all these instruments, the air-pump provides the necessary suction to draw the ambient air through a narrow sample tube and deliver the resulting column of air through the laser beam. The pump itself must be powerful enough to generate the required pressure drop inside the sensing chamber to draw the

external air in through the sample tube. Furthermore, since the pumps are normally required to be protected from particulate contamination in the air, they are generally preceded in the air-flow by a high efficiency particle filter and this too will present a flow impedance which the pump must overcome. These features of conventional OPC's result in several drawbacks; firstly, the pumps used must overcome the pressure drops in the sample tube and particle filter, and this has implications for pump size and current consumption (and, by extension, for battery-life in standalone of hand-held units); secondly, the particle filters need to be periodically replaced (incurring further cost to user); and thirdly, the air sample handling system comprising sample inlet tube, vent tube, interconnecting tubing and filter, etc.), can be relatively complex to manufacture and assemble, with implications for final unit cost.

WO2008140816 (UNGER) describes a compact low cost particle sensor wherein the flow of fluid is directed to a detection zone via a series of baffles. The detection zone is basically a detector located directly under the laser beam which collects radiation scattered by particles passing through the beam. A problem with this device is that the detector is not able to distinguish the number or size of particles passing through the, rather large, detection zone. This device is only able to detect that at least one particle has passed through the detection zone, and in this regard it is little more than a portable smoke detector. This device cannot be used to accurately count the number of particles passing through the detection zone

There is a need for an apparatus and method of aerosol monitoring, having single particle sensitivity, which requires no air-pump or particle filter, and which is mechanically very simple, thus offering the prospect of manufacture using a small number of parts.

GB 0917444.2 (University of Hertfordshire) describes one type of apparatus for the detection of a fluid-borne particle in an optically defined sensing zone wherein a first reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation in the optically defined sensing zone into the first detector means and wherein the second reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation in the optically defined sensing zone in to the second detector means.

Summary of the Invention

According to a first aspect of the present invention there is provided an apparatus for the detection of a fluid-borne particle in an optically defined particle sensing zone, the apparatus comprising:

i) a scattering chamber;

ii) a means for providing a sample of fluid, containing the fluid-borne particle, in the form of a flow through the optically defined particle sensing zone;

iii) a means for generating a beam of radiation through the optically defined particle sensing zone;

iv) a single reflector or refractor means having a primary focus within the optically defined particle sensing zone and a secondary focus located outside the beam of radiation ;

v) a detector means comprising a first photosensitive detection area and a second photosensitive detection area;

vi) a means for deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means

wherein the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation within the optically defined particle sensing zone to the detection means located at the secondary focus of the single reflector or refractor means.

Single means only one.

Preferably the detector means comprises a single detector means wherein the first photosensitive detection area and the second photosensitive detection area are integral. The detector can be 'integral' in that both photosensitive areas are on the same piece of silicon, but they must still be electrically separate from one another. In this way the detectors are like a single 'chip' but fabricated to have the required inner and outer photosensitive areas which are electrically separate.

Alternatively the detector means comprises two detector means wherein the first detector means comprises the first photosensitive detection area and the second detector means comprises the second photosensitive detection area. In this alternative the two detector areas are discrete 'chips', ie: one with a hole in the middle into which the second detector fits.

Preferably the first photosensitive detection area is located wholly inside the second photosensitive detection area.

Preferably the second photosensitive detection area wholly surrounds the first photosensitive detection area.

Preferably the first photosensitive detection area is in the shape of a rectangle. The length (or width) of the rectangle governs the length of the laser beam (measured along the beam axis) that a particle can be detected in to the left or right to the primary focus of the single reflector or refractor. The centre of the length (or width) of the rectangle is coincident with the secondary focus of the single reflector or refractor means where light scattered from a particle at the primary focus of the single reflector or refractor means is reflected or refracted to. The height of the rectangle corresponds to be slightly larger than the path traced out by a particle as it passes through the laser beam at the primary focus of the single reflector or refractor means (i.e. the height of the rectangle is slightly greater than the thickness of the laser beam along its minor axis for a laser beam with an elliptical cross section, the difference being a result of the magnification of the optical system, namely the distance from the detector to the reflector or refractor surface divided by the distance of the particle to the reflector or refractor surface).

Preferably the second photosensitive detection area is in the shape of a circle

Preferably the optically defined sensing zone comprises a first zone and a second zone wherein the second zone is smaller than the first zone and wherein the second zone is located wholly inside the first zone. The first zone of the optically defined sensing zone corresponds to the cross-sectional area of the laser beam orthogonal to the fluid flow direction within which a particle will scatter light to the single reflector or refractor with this light subsequently being reflected or refracted by the single reflector or refractor means to the first photosensitive detection area of the detector means. The second zone of the optically defined sensing zone corresponds to the cross-sectional area of

the laser beam orthogonal to the fluid flow immediately outside the first zone within which a particle will scatter light to the single reflector or refractor with this light subsequently being reflected or refracted by the single reflector or refractor means to the second photosensitive detection area of the detector means.

Preferably the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation within the first zone of the optically defined sensing zone to the first photosensitive detection area of the detection means.

Preferably the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation within the second zone of the optically defined sensing zone to the second photosensitive detection area of the detection means.

Preferably the means for deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means is adapted such that only particles passing through the first zone of the optically defined sensing zone are counted and/or sized.

Preferably the means for deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means is adapted such that particles detected only by the first photosensitive detection area are counted and/or sized.

Preferably the means for deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means is adapted such that particles detected by both the first photosensitive detection area and at a predetermined intensity by the second photosensitive detection area are counted and/or sized.

Preferably the criterion for counting a particle and thus the predetermined intensity as stated above is calculated by comparing the intensity of radiation from the particle detected in the first photosensitive detection area and the intensity of radiation detected from the particle in the second photosensitive detection area and wherein when the intensity of radiation detected in the second photosensitive detection area is equal to or

less than 25% of the intensity radiation detected in the first photosensitive detection area then the measurement is deemed 'valid' and the particle is counted and/or sized. More preferably when the intensity of radiation detected in the second photosensitive detection area is equal to or less than 20% of the intensity radiation detected in the first photosensitive detection area the particle is counted and/or sized, still more preferably when the intensity of radiation detected in the second photosensitive detection area is equal to or less than 15% of the intensity radiation detected in the first photosensitive detection area the particle is counted and/or sized, yet more preferably when the intensity of radiation detected in the second photosensitive detection area is equal to or less than 10% of the intensity radiation detected in the first photosensitive detection area the particle is counted and/or sized, even more preferably when the intensity of radiation detected in the second photosensitive detection area is equal to or less than 5% of the intensity radiation detected in the first photosensitive detection area the particle is counted and/or sized. In each case, the sizing of the particle is typically achieved by comparing the magnitude of the electrical signal from the first photosensitive detection area to a calibration function based on the light scattering of particles of know sizes.

As this detector assembly requires only a single reflector or refractor means and a single detector means this may have the effect of providing a low cost apparatus for counting and sizing particles which operates by providing an optically defined rather than physically defined (by for example, a narrow fluid delivery tube) particle sensing zone in which particles are detected. The dual optical sensing zone allows not only for particles to be detected but also to be counted and sized accurately. In situations where there is an existing airflow (eg: the apparatus is attached to a moving vehicle or aircraft or is moved by hand through the air) in a direction relative to the broad axis of the elliptical cross-section laser beam, the apparatus is able to operated without the need of a pump to draw a sample through a narrow chamber, as in conventional optical particle counters. Given that there is no pump and that the apparatus parts are simple, light and cheap to construct, this apparatus provides a low cost hand held device with single particle sensitivity. Furthermore, as this detector assembly requires only a single reflector or refractor means and a single detector means this may have the further effect of allowing the single reflector or refractor means and single detector means combination to be placed at any angle in the plane containing the broad axis of the laser beam, providing the mirror or detector do not obstruct the beam of radiation.

Preferably the apparatus further comprises focussing means adapted to focus the beam of radiation such that the beam of radiation has an elliptical cross-section. More preferably the major axis of the elliptical cross-section is orthogonal to the direction of fluid flow.

Preferably the beam of radiation is generated using a diode laser.

Preferably the means for providing a sample of fluid in the form of a flow through the optically defined sensing zone comprises an open ended chamber, referred to as a scattering chamber, of a diameter large compared to the optically defined sensing zone which is typically located at or close to the centre of the chamber through which fluid can freely flow. By selecting a scattering chamber diameter which is larger than the diameter of the optical sensing zone, the air, or other fluid sample, can freely flow through the scattering chamber. There is therefore no need for the air, or other fluid sample, to be sucked through the scattering chamber as in prior art devices, but whilst still retaining the desired single particle sensitivity without a pump.

In cases where there is no natural air or fluid flow in the environment, the means for providing a sample of fluid in the form of a flow through the optically defined sensing zone preferably comprises a fan. This may have the effect that a flow of air can be provided cheaply and with low weight and low power usage to help draw air, or other fluid sample through the apparatus. Alternatively the apparatus could be "waved" (i.e. moved through the air, for example manually by the user or attached to a moving vehicle) through the air to generate a flow of air or other fluid. Alternatively the apparatus can be placed in an environment wherein the fluid sample will flow through the optically defined sensing zone, such as an environment of sample air moving relative to the apparatus by convection or within an air-conditioning duct.

Preferably the single reflector or refractor means comprises a mirror. More preferably the mirror comprises a concave mirror. Still more preferably the concave mirror comprises an elliptical concave mirror. This allows the mirror to be used to not only to reflect the scattered radiation towards the single detector means, but also to focus the radiation so that a greater sample of scattered radiation is detected from the particle.

Preferably the single reflector means is integrally formed in the scattering chamber. This is advantageous as there are fewer parts to produce and producing fewer parts is cheaper resulting in a lower cost apparatus.

Preferably the scattering chamber is formed from an injection moulded plastics material. This is advantageous as injection moulding of plastics is a cheap process to run to mass produce identical items, resulting in a lower cost apparatus.

Preferably a single concave surface is formed integrally within the scattering chamber. Still more preferably a single concave elliptical surface is formed integrally within the scattering chamber.

Preferably the single concave surface is provided with a reflective material to form the single reflector or refractor means. This is advantageous as the mirror is formed internally to the scattering chamber, in the correct position, in a single step and coated with a reflective material. This results in a lower cost apparatus due to reduced parts and also because the mirrors are automatically positioned correctly.

According to a second aspect of the present invention there is provided a method for the detection of a fluid-borne particle in an optically defined particle sensing zone, the method comprising:

i) providing a sample of fluid, containing the fluid-borne particle, in the form of a flow through the optically defined sensing zone;

ii) directing a beam of radiation that illuminates the optically defined sensing zone and with uniform intensity of radiation;

iii) detecting radiation scattered by the fluid-borne particle as it passes through the beam of radiation in the optically defined sensing zone.

Preferably the method employs an apparatus for the detection of a fluid-borne particle in an optically defined particle sensing zone, the apparatus comprising:

i) a scattering chamber;

ii) a means for providing a sample of fluid, containing the fluid-borne particle, in the form of a flow through the optically defined particle sensing zone;

iii) a means for generating a beam of radiation through the optically defined particle sensing zone;

iv) a single reflector or refractor means having a primary focus within the optically defined particle sensing zone and a secondary focus located outside the beam of radiation;

v) a single detector means comprising a first photosensitive detection area and a second photosensitive detection area;

vi) a means for deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means

wherein the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation within the optically defined particle sensing zone to the detection means located at the secondary focus of the single reflector or refractor means.

According to a third aspect of the present invention there is provided a method for the detection of a fluid-borne particle in an optically defined particle sensing zone, the method comprising:

i) providing an apparatus having; a scattering chamber, a means for generating a beam of radiation, a single reflector or refractor means, a single detector means comprising a first photosensitive detection area and a second photosensitive detection area;

ii) providing a sample of fluid in the form of a flow through the optically defined particle sensing zone;

iii) directing the beam of radiation to illuminate the optically defined particle sensing zone with uniform intensity of radiation; and

iv) deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means;

wherein the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation within the optically defined particle sensing zone to the detection means located at the secondary focus of the single reflector or refractor means.

According to a fourth aspect of the present invention there is provided a method for the detection of a fluid-borne particle in an optically defined particle sensing zone, the method comprising:

i) providing an apparatus according to the first aspect of the present invention;

ii) providing a sample of fluid in the form of a flow through the optically defined particle sensing zone;

iii) directing a beam of radiation that illuminates the optically defined particle sensing zone with uniform intensity of radiation; and

iv) deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means.

The present invention seeks to provide a method of aerosol monitoring (having single particle sensitivity) which requires no air-pump or particle filter, and which is mechanically very simple, thus offering the prospect of manufacture using a small number of injection-moulded plastic parts. The air-movement may be achieved using a small electrical fan (far lower current consumption than a rotary-vane or diaphragm air-pump), by exploiting the natural movement of the ambient air (e.g.: wind, convection flow, etc.), or by simply moving the sensor unit itself through the air by hand or by attaching to a moving vehicle (car, train, aircraft, etc).

The present invention is based on the principle of optically defining a particle sensing zone rather than mechanically defining the zone as in conventional OPCs. As mentioned above, OPCs typically use a narrow airflow delivery tube to confine the sample airflow to a narrow column, typically ~1mm in diameter, which is directed through a narrow ribbon-shaped laser beam such that the intersection between the beam and the airflow – the sensing zone – is sufficiently small that it very rarely contains more than a single particle even when measuring high-concentration aerosols. Avoiding 'particle coincidences' where two or more particles occupy the sensing zone at any instant is crucial to obtaining unbiased aerosol size and concentration data.

In the present Invention, the sample air does not pass through a narrow delivery tube as in conventional OPCs, but instead passes through a far wider chamber that may or may not have cylindrical tube-like geometry. The particles carried by the air moving though this chamber are not constrained to move in single file by any mechanical tube

or other device. The single-particle sensing zone is instead defined by suitable optical components.

Brief Description of the Drawings

A preferred embodiment of the present invention will now be more particularly described, by way of example only, with reference to the accompanying drawings, wherein:

Figure 1 shows a perspective view of the particle detection apparatus according to a first embodiment of the present invention;

Figure 2 shows a perspective view of the first and second photosensitive areas of the light detector means of the particle detection apparatus of Figure 1 for a particle traversing the beam a distance x upstream from the primary focal point of the reflector or refractor means;

Figure 3 shows a perspective view of the first and second photosensitive areas of the light detector means of the particle detection apparatus of Figure 1 with a particle traversing the beam a distance y closer to reflector or refractor means than the primary focal point of the reflector or refractor means but still such that the light scattered by the particle falls entirely within the first photosensitive area of the detector means.

Figure 4 shows a perspective view of the first and second photosensitive areas of the light detector means of the particle detection apparatus of Figure 1 with a particle traversing the laser beam a distance y' (where y' is greater than y in Figure 3) closer to reflector or refractor means than the primary focal point of the reflector or refractor means such that the light scattered by the particle no longer falls entirely within the first photosensitive area of the detector means;

Figure 5 shows a perspective view of the first and second photosensitive areas of the detector means of the particle detection apparatus of Figure 1 indicating the extent of the first zone of the optically defined particle sensing zone within the laser beam;

Figure 6 shows a perspective view of the particle detection apparatus according to a second embodiment of the present invention wherein the laser beam passes through a hole provided in the reflector or refractor means;

Figure 7 shows a cross-sectional view of the scattering chamber which houses the particle detection apparatus of Figure 1 according to a third embodiment; and

Figure 8 shows a cross-sectional view of the scattering chamber which houses the particle detection apparatus of Figure 1 according to a fourth embodiment.

Description of the Preferred Embodiments

The preferred embodiment requires a flow of particles 10 carried in a moving airstream to pass through a laser beam 12, where the axis of the laser beam is orthogonal to the direction of the airflow, as in Figure 1. The particles 10 are not constrained and may pass anywhere through the laser beam 12. It is well known that the size of a particle may be determined by measuring at some angle the intensity of light scattered by the particle as it passes through the beam. However, the non-uniform intensity profile of the beam (typically Gaussian) means that a particle passing through the edge of the beam will be illuminated with a lower intensity of light than an identically-sized particle passing through the centre of the beam. It will thus scatter less light and will be measured as smaller than the particle passing through the centre of the beam.

Because of this potential source of particles sizing error, it is necessary to optically define a small sensing zone within the central part of laser beam where the intensity variation is small, so that only particles passing through this zone will be 'seen' by the detection system. Thus, all particles will experience essentially the same intensity of illuminating light and will therefore be accurately sized.

The preferred embodiment for achieving this optical sensing zone is shown in Figure 1. The laser beam 12 is focused through a cylindrical lens (not shown) such that it forms a flat, elliptical cross-section, with the major axis of the ellipse being orthogonal to the direction of the airflow carrying the particles 10. Positioned approximately in the same horizontal plane as the major axis of the flattened laser beam is reflector or refractor means, which in the present embodiment is an elliptical mirror 14. The mirror is positioned such that its primary focus 16 lies on the axis of the laser beam 12. The

angle of the mirror axis to the laser beam 12 axis may be typically about 90 degrees (both being essentially perpendicular to the direction of the fluid flow), although the mirror could be at other angles to the beam without detriment to the performance of the apparatus. An elliptical mirror is preferred to a spherical mirror as an elliptical mirror tends to suffer lower optical aberrations, and it is important to have low optical aberrations in this present embodiment. However it is not essential that the mirror is elliptical and in an alternative a spherical mirror can be employed. In a further alternative a spherical or elliptical lens may be employed.

Consider a particle 10 that passes through the laser beam 12 exactly coincident with the primary focus 16 of the elliptical mirror 14. As it passes through the laser beam 12, the particle 10 will trace out a short vertical path 18 orthogonal to the laser beam 12 axis as shown in Figure 1, and will scatter light in all directions whilst travelling along this path. Some of this light will fall onto the elliptical mirror 14 and will be reflected to the secondary focus 20 of the elliptical mirror 14. The trajectory of the particle 10 through the laser beam 12 therefore results in a thin vertical line of light 22 (a "pencil line") at the secondary focus 20 of the elliptical mirror 14, as shown in Figure 1.

Positioned at this secondary focus 20 and facing the mirror 14 is a detection means, which is typically a photodetector 24. The photodetector 24, typically a photodiode, comprises two separate photosensitive areas, a first photosensitive area being an inner rectangular area 26 surrounded by a second photosensitive area being an outer area 28 (typically, but not essentially, circular). The first photosensitive area 26 is of a height just slightly larger than the length of the pencil line of light 22 scattered by the particle 10 as it passes through the laser beam 12. The photodetector 24 is positioned such that this line of light 22 exactly bisects the rectangular photosensitive area 26, as shown in Figure 1. As described below, the width of the rectangular photosensitive area 26 determines the size of the optical sensing zone centred upon the primary focus 16 of the elliptical mirror 14 in the laser beam 12.

Figures 2 to 5 illustrate the behaviour of this optical system for various particle trajectories through the laser beam. For clarity, only the laser beam 12 and the photodetector 24 are shown. Consider in Figure 2 a particle 10 passing through the laser beam 12 a very short distance 'x' upstream along the laser beam axis from the primary focus 16 of the elliptical mirror (to the left of the primary focus in Figure 2). The scattered line of light reflected from the mirror will fall onto the inner photosensitive

area 26 of the photodiode detector but to the right of the centre-line of that area, as shown in Figure 2.

It follows that if another particle was to subsequently pass through the laser beam slightly further upstream along the laser beam axis from the primary focus 16 of the elliptical mirror than the particle described above, the line of scattered light it produced on the photodiode 24 may be just beyond the end of the rectangular photosensitive area 26, instead falling onto the outer photosensitive area 28. Similarly, another particle passing through the laser beam 12 at a distance downstream along the laser beam axis from the elliptical mirror 14 primary focus 16 (to the right of the primary focus in Figure 2) will result in a line of scattered light falling on the rectangular photosensitive area 26 but in the left half of that detector area.

By this example, it can be seen that the width of the rectangular photosensitive area 26 effectively defines the length of the laser beam 12 through which particles can traverse and be detected by the rectangular photosensitive area 26. Particles traversing the laser beam slightly further upstream or downstream along the laser beam axis will instead result in light falling onto the outer photosensitive area 28. Particles passing through the laser beam even further upstream or downstream from the primary focus of the mirror will result in scattered light that passes beyond the edge of the outer photosensitive area 28 and is therefore not detected at all.

In a similar way, a particle that traverses the laser beam a lateral distance 'y' from the primary focus 16 of the elliptical mirror 14 (ie a distance y along the major axis of the beam's elliptical cross-section from the centre line of the laser beam, parallel with its longitudinal axis), as shown in Figure 3, will result in a pattern of scattered light on the photodetector 24 that is not a sharp pencil-line of light but rather, because it is out of focus, an approximately circular patch of light. Because the height of the rectangular photosensitive area is slightly greater than the normal height of the pencil-line of light (scattered by a particle passing through the exact primary mirror focus), it will still be all captured by the photosensitive area provided the lateral displacement 'y' of the particle trajectory is small.

However, if the lateral displacement of the particle trajectory from the mirror focus 16 is increased to y', the patch of scattered light will begin to extend above and below the rectangular photosensitive area 26 of the photodetector, as in Figure 4. The further the

particle trajectory is to the side of the axis of the laser, the higher the fraction of the scattered light that will therefore fall onto the outer photosensitive area 28 of the photodetector 24. The same thing happens if the particle trajectory is displaced laterally to the opposite side of the primary focus of the mirror 16.

The net result is that the size of the rectangular photosensitive area 26 effectively defines an optical 'sensing zone' 30 within the laser beam, as in Figure 5. All particles passing through this zone will scatter light that is wholly captured by the rectangular sensing area 26 of the photodiode 24, with none falling into the outer photosensitive area 28. The electrical output from area 26 may therefore be used to not only register (count) the particle, but also, since the beam intensity is designed to be uniform across the sensing zone 30, from the magnitude of the electrical signal, it can be used to estimate the particle size. Particles passing through the edge of this sensing zone 30 will scatter some light to the outer photosensitive area 28 of the photodiode 24 and if the output of this photosensitive area 28 reaches a pre-set fraction (say, 25%) of the coincident output falling onto the inner photosensitive area 26, the particle will be deemed to have crossed outside of the sensing zone 30 and will not be recorded.

The elliptical mirror 14 and photodetector 24, therefore provide an effective and efficient (in terms of fewest component parts required) method of optically defining a sensing zone within the illuminating laser beam. Such an arrangement will allow the accurate estimation of both the particle size distribution and, since the cross-sectional area of the sensing zone can be accurately determined, also the concentration of particles carried in the air flowing through the laser beam. No mechanical tubing or similar device to constrain the trajectories of the particles is required, and consequently nor is any mechanical air-pump required to draw air through such tubing. This represents a saving of cost and electrical power requirements in the measurement of airborne particles. Indeed, if the particles to be measured are carried in a natural airflow (wind or draught), the apparatus described herein would be capable of achieving this measurement without the need for any moving parts, with the benefit of enhanced lifetime and reliability over conventional 'pumped-air' particle measurement devices.

Figure 6 illustrates an alternative embodiment of the present invention wherein the elliptical mirror 114 is provided with a small hole 136 at its centre sufficient to allow the laser beam 112 to pass through unaffected whilst still ensuring the primary focus 116 of

the mirror 114 lies on the laser beam 112 axis. The above measurement methodology would still apply. It would simply require the mirror 114 to be slightly tilted so that the secondary focus 120 of the mirror 114 fell to the side of the laser beam 112 (ie: the photodetector 124 would not obstruct the laser beam 112). Such a configuration could be advantageous in areas where space requirements of the apparatus need to be minimised.

Figure 7 illustrates a cross-sectional view of the particle detector 42 which houses the particle detection apparatus of Figure 1 according to a third embodiment. In this embodiment, a small low-power electrical fan 34 causes particle-laden ambient air 38 to move through a scattering chamber 40 at a desired rate. The fan 34, in one alternative, is of a type commonly used to cool computer microprocessors, requiring a current of only a few milliamps; far less than the tens-of-milliamp currents required by conventional OPC air-pumps. The scattering chamber 40 has end-caps (not shown) that do not restrict the airflow but act as light baffles to prevent entry of ambient daylight or room-light. An alternative arrangement can be seen in Figure 8. The Figure 8 embodiment does not have a fan, but instead is adapted to be manually waved through the air by a user.

Typically, for normal ground level applications, the particles being detected by the apparatus would be in the important respirable size range of around 0.5 to 15μm in diameter.

The concentration of particles may be computed using the product of the air velocity through the sensing zone and the cross-sectional area of sensing zone. This velocity may be determined from a knowledge of the laser beam-depth, being the minor axis of its elliptical beam cross-section, and the time-of-flight of the particle through the beam, as measured from the duration of the electrical signal from detector. Typically for normal ground level applications the minor axis of the elliptical cross-section of the beam of radiation is around 100μm and the major axis is around 2mm.

The maximum measurable particulate concentration will be reached when the probability of more than one particle being within the sensing zone reaches an unacceptable level, typically ~2%. The beam depth and sensing zone cross-section are selected such that this would not occur for concentrations less than typically a thousand particles per millilitre of air.

The apparatus as described offers several advantages over conventional OPCs;

- Mechanical simplicity. The absence of internal pipework, filters, and tubing common in conventional OPC units means that the invention could be fabricated at a low manufacturing cost, especially if specialist plastic injection moulded parts (including mirrored surfaces) were employed. In such a case, the scattering chamber comprises two semi-cylindrical parts, the first part with integral an integral elliptical mirrored surface and the second part with the detection means. The integral mirrored surface would be formed by injection moulding the scattering chamber with an integral concave surface which would then be provided with a coating of a reflective material.

- An air fan (if provided) would be capable of moving large volumes of air, required in efficient ambient monitoring, with very low power consumption, since it would be operating against minimal pressure drop. This could significantly extend the battery life of a standalone or hand-held unit compared to that in a conventional OPC with comparable sample air flow-rate. Indeed, the fan would not be necessary at all in some cases. For example, where the airflow through the sensor unit was generated by movement of the unit itself through the air, such as 'waving' a hand-held unit at arm's length. Such a 'no moving parts' implementation of the invention would be not only be even cheaper to manufacture than the fan-assisted version of the invention but would also offer improved reliability, it having no moving parts to wear out.

- In addition as no pump is required, the scattering chamber does not need to be air-tight. In conventional OPC devices where a pump is required to suck the air through the narrow mechanically defined sensing zone, the scattering chamber needs to be air-tight because of the air pressure differential required to cause the air to flow through the narrow mechanically defined sensing zone is significant. In the present invention there is virtually no air pressure differential needed as the scattering chamber is large and open-ended and the sensing zone is defined optically rather than mechanically. So air-tightness of the scattering chamber is unnecessary. This means that the scattering chamber can be formed from low dimensional tolerance plastic components rather than

the high precision (usually metal) components needed for an air-tight chamber. This allows for a cheaper and lighter apparatus to be constructed.

- Further in addition, if a fan is employed the fan is located below the sensing zone such that any particles depositing on the fan blades have already been detected.

- The apparatus would require no periodic replacement of internal filter units, making it more suitable than conventional OPCs in remote or hostile monitoring environments where manual filter replacement is undesirable on grounds of inaccessibility or safety hazard.

CLAIMS:

1. An apparatus for the detection of a fluid-borne particle in an optically defined particle sensing zone, the apparatus comprising:

i) a scattering chamber;

ii) a means for providing a sample of fluid, containing the fluid-borne particle, in the form of a flow through the optically defined particle sensing zone;

iii) a means for generating a beam of radiation through the optically defined particle sensing zone;

iv) a single reflector or refractor means having a primary focus within the optically defined particle sensing zone and a secondary focus located outside the beam of radiation ;

v) a detector means comprising a first photosensitive detection area and a second photosensitive detection area;

vi) a means for deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means

wherein the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation within the optically defined particle sensing zone to the detection means located at the secondary focus of the single reflector or refractor means and wherein the optically defined sensing zone comprises a first zone and a second zone wherein the first zone is smaller than the second zone and wherein the first zone is located wholly inside the second zone.

2. An apparatus according to claim 1 wherein the detector means comprises a single detector means wherein the first photosensitive detection area and the second photosensitive detection area are integral.

3. An apparatus according to claim 1 wherein the detector means comprises two detector means wherein the first detector means comprises the first photosensitive detection area and the second detector means comprises the second photosensitive detection area.

4. An apparatus according to any preceding claim further comprising a focussing means adapted to focus the beam of radiation such that the beam of radiation has an elliptical cross-section at the point at which is passes through the particle sensing zone.

5. An apparatus according to claim 4 wherein the major axis of the elliptical cross-section is orthogonal to the direction of fluid flow.

6. An apparatus according to any preceding claim wherein the beam of radiation is generated using a diode laser.

7. An apparatus according to any preceding claim wherein first photosensitive detection area is located wholly inside the second photosensitive detection area.

8. An apparatus according to any preceding claim wherein the second photosensitive detection area wholly surrounds the first photosensitive detection area.

9. An apparatus according to any preceding claim wherein the first photosensitive detection area is in the shape of a rectangle.

10. An apparatus according to any preceding claim wherein the second photosensitive detection area is in the shape of a circle

11. An apparatus according to any preceding claim wherein the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation within the first zone of the optically defined sensing zone to the first photosensitive detection area of the detection means.

12. An apparatus according to any preceding claim wherein the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation within the second zone of the optically defined sensing zone to the second photosensitive detection area of the detection means.

13. An apparatus according to any preceding claim wherein the means for deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means is adapted such that particles detected only by the first photosensitive detection area are counted and/or sized.

14. An apparatus according to any preceding claim wherein the means for deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means is adapted such that particles detected by both the first photosensitive detection area and at a predetermined intensity by the second photosensitive detection area are counted and/or sized.

15. An apparatus according to any of claim 14 wherein the predetermined intensity is calculated by comparing the intensity of radiation from the particle detected in the first photosensitive detection area and the intensity of radiation detected from the particle in the second photosensitive detection area and wherein when the intensity of radiation detected in the second photosensitive detection area is equal to or less than 25% of the intensity radiation detected in the first photosensitive detection area the particle is counted and/or sized

16. An apparatus according to any preceding claim wherein the means for providing a sample of fluid in the form of a flow through the optically defined sensing zone comprises an open ended chamber of a large diameter compared to the optically defined sensing zone through which fluid can freely flow.

17. An apparatus according to any of claims 1 to 16 wherein the means for providing a sample of fluid in the form of a flow through the optically defined sensing zone comprises a fan.

18. An apparatus according to any preceding claim wherein the single reflector or refractor means comprises a mirror.

19. An apparatus according to claim 18 wherein the mirror comprises a concave mirror.

20. An apparatus according to claim 19 wherein the concave mirror comprises an elliptical concave mirror.

21. An apparatus according to any preceding claim wherein the single reflector means is integrally formed in the scattering chamber.

22. An apparatus according to any preceding claim wherein the scattering chamber is formed from an injection moulded plastics material.

23. An apparatus according to any preceding claim wherein a single concave surface is formed integrally within the scattering chamber.

24. An apparatus according to claim 23 wherein a single concave elliptical surface is formed integrally within the scattering chamber.

25. An apparatus according to claim 23 or claim 24 wherein the single concave surface is provided with a reflective material to form the single reflector or refractor means.

26. A method for the detection of a fluid-borne particle in an optically defined particle sensing zone, the method comprising:

i) providing an apparatus having; a scattering chamber, a means for generating a beam of radiation, a single reflector or refractor means, a single detector means comprising a first photosensitive detection area and a second photosensitive detection area;

ii) providing a sample of fluid in the form of a flow through the optically defined particle sensing zone;

iii) directing the beam of radiation to illuminate the optically defined particle sensing zone with uniform intensity of radiation; and

iv) deriving data from the radiation detected by the first photosensitive detection area and second photosensitive detection area of the detection means;

wherein the single reflector or refractor means is adapted to direct radiation scattered from the fluid borne particle passing through the beam of radiation within the optically defined particle sensing zone to the detection means located at the secondary focus of the single reflector or refractor means and wherein the optically defined sensing zone comprises a first zone and a second zone wherein the first zone is smaller than the second zone and wherein the first zone is located wholly inside the second zone.

27. A method for the detection of a fluid-borne particle in an optically defined particle sensing zone, the method comprising:

i) providing an apparatus as claimed in any of claims 1 to 25

ii) providing a sample of fluid in the form of a flow through the optically defined particle sensing zone;

iii) directing a beam of radiation that illuminates the optically defined particle sensing zone with uniform intensity of radiation; and

iv) deriving data from the radiation detected by the first and second photosensitive detection areas of the detector means.

28. An apparatus or method substantially as hereinbefore described with reference to any suitable combination of the accompanying figures.

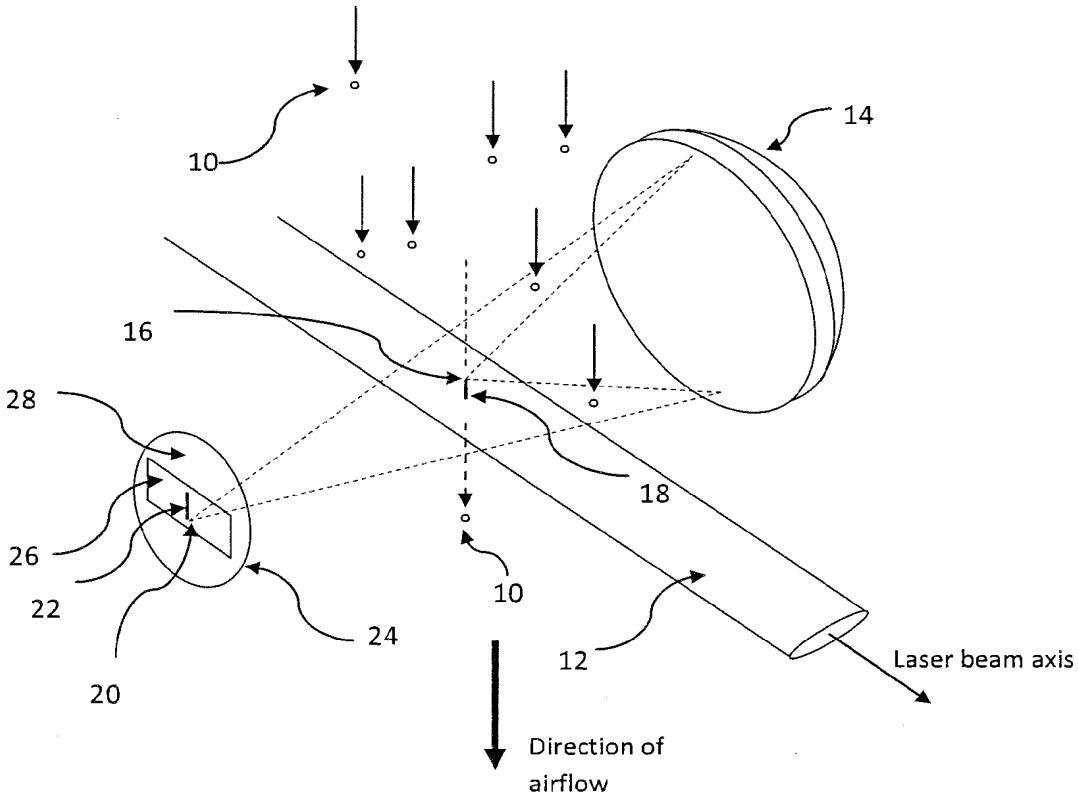

10
14
16
28
18
26
22
10
24
12
Laser beam axis
20
Direction of airflow

Figure 1

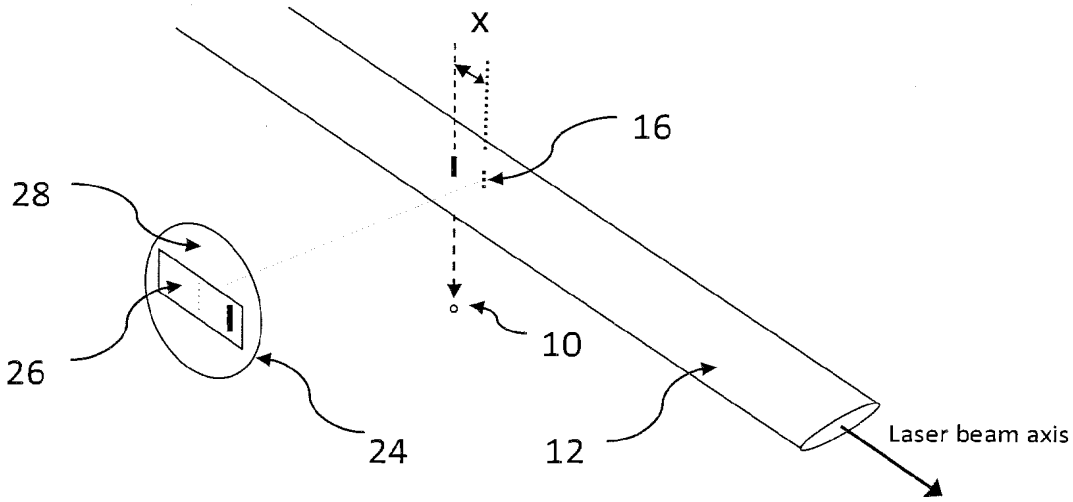


Figure 2

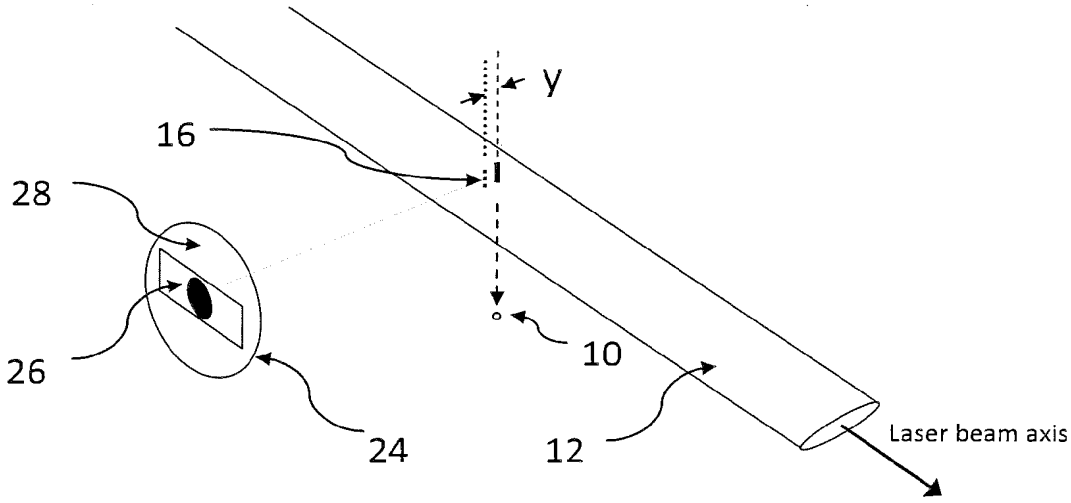


Figure 3

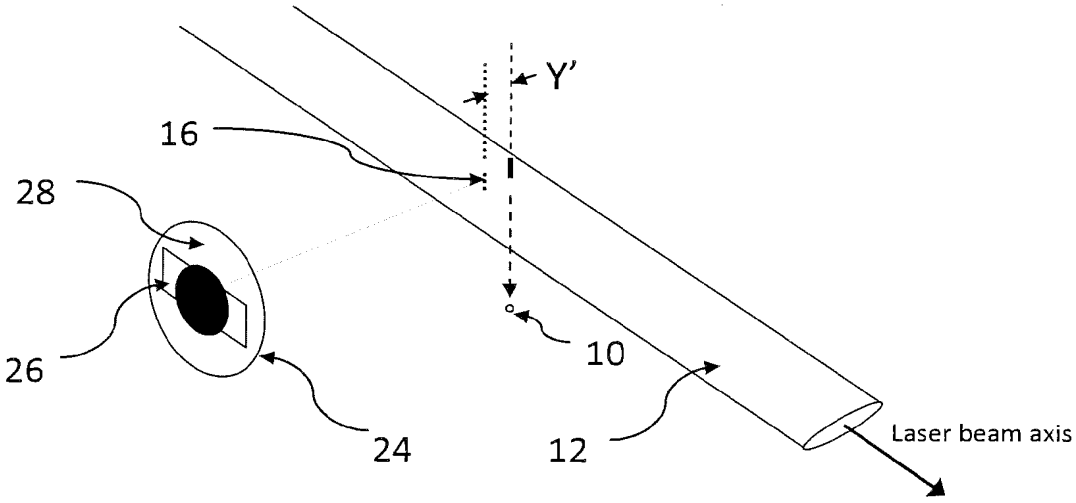


Figure 4

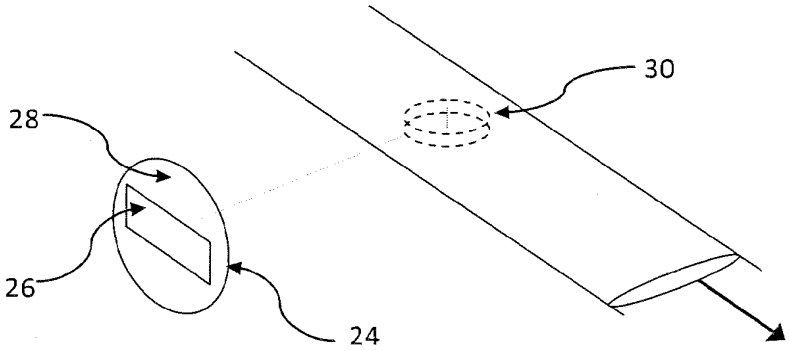


Figure 5

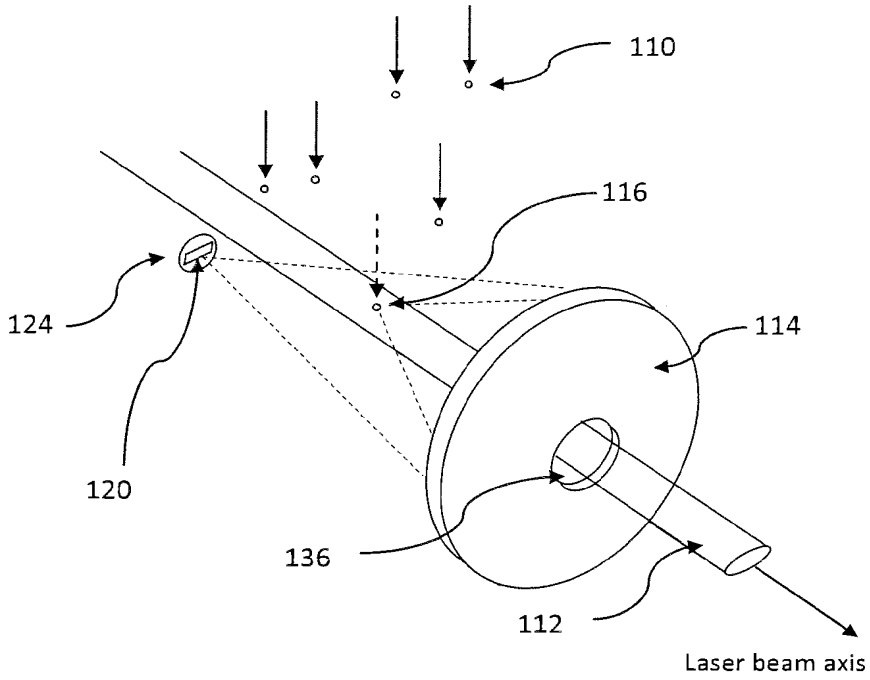


Figure 6

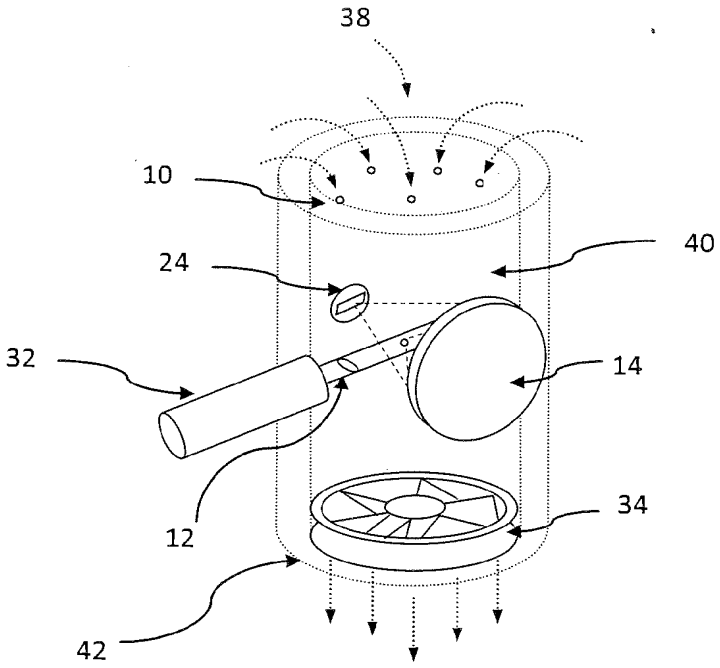


Figure 7

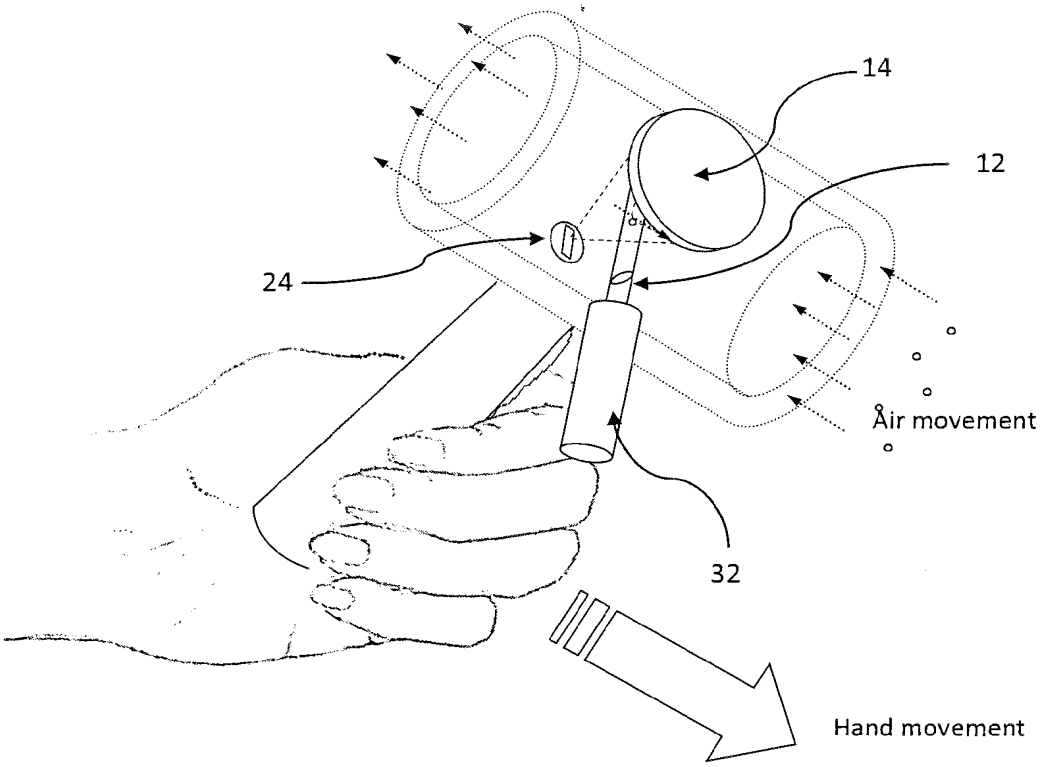


Figure 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2011/052028

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N15/14 G01N15/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 140 395 A (KREIKEBAUM GERHARD) 20 February 1979 (1979-02-20) sentences 6-13, 26-29 column 2, lines 36-51 column 2, line 60 - column 3, line 5 column 3, lines 26-29 column 3, line 56 - column 4, line 2 column 4, line 31 - column 6, line 28 column 7, line 31 - column 8, line 6 column 9, line 64 - column 11, line 33 claim 1 figures 1-3 ----- -/--	1-3, 6-17,22, 26

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 December 2011	30/12/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Traversa, Marzia

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2011/052028

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 28
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 28

The subject matter of claim 28 is defined only with reference to the figures, therefore it does not meet the requirements of Rule 6.2 (a) PCT according to which claims shall not rely on references to the description or drawings. A meaningful search for claim 28 is then not possible.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2) declaration be overcome.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 467 189 A (KREIKEBAUM GERHARD [US] ET AL) 14 November 1995 (1995-11-14) column 2, line 56 - column 3, line 4 column 3, lines 62-64 column 4, lines 62-67 column 5, lines 21-25 column 7, line 26 - column 8, line 14 column 8, lines 55-56 figures 1-3,7	1,6, 17-27

X	US 2007/010974 A1 (NICOLI DAVID F [US] ET AL) 11 January 2007 (2007-01-11) paragraph [0006] paragraph [0162] paragraph [0163] paragraph [0272] figure 11	1,4-6, 17,22,26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2011/052028

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4140395 A	20-02-1979	CA 1075488 A1	15-04-1980
		US 4140395 A	20-02-1979
US 5467189 A	14-11-1995	NONE	
US 2007010974 A1	11-01-2007	NONE	

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(25) Filing Language: English

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61/557,334 8 November 2011 (08.11.2011) US

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(74) Agents: **MILES, Kirk, M.** et al.; The Webb Law Firm, One Gateway Center, 420 Fort Duquesne Boulevard, Suite 1200, Pittsburgh, Pennsylvania 15222 (US).

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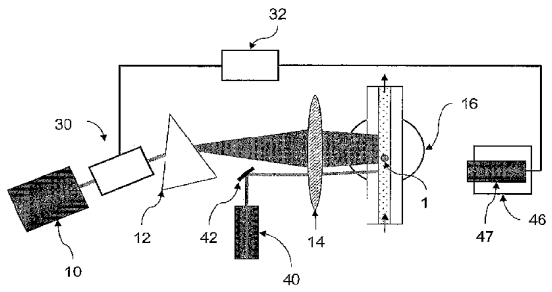
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(54) **Title**: ABSORBANCE SPECTRUM SCANNING FLOW CYTOMETRY

Fig. 3B



(57) **Abstract**: The present invention provides systems and methods for analyzing the excitation spectra of fluorescent particles in a flowing stream. The system uses a white light laser and color separation optics to provide a spatially-distributed, continuous color-spectrum excitation light system that is used to illuminate a region of a flowing stream. A particle that passes through the detection region traverses the full dispersed spectrum of excitation light, and the fluorescence emissions from the particle are continuously measured as it passes through the detection region. The measured fluorescence emissions at each wavelength of excitation light, which changes through full spectrum of the excitation light as the particle passes through the detection region, provides the excitation spectrum of the particle.

WO 2012/177367 A2

ABSORBANCE SPECTRUM SCANNING FLOW CYTOMETRY

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to the field of optical analysis of fluorescent particles in fluid streams.

DESCRIPTION OF RELATED ART

Flow-type particle analyzers, such as flow cytometers, are well known analytical tools that enable the characterization of particles on the basis of optical parameters such as light scatter and fluorescence. In a flow cytometer, for example, particles, such as molecules, analyte-bound beads, or individual cells, in a fluid suspension are passed by a detection region in which the particles are exposed to an excitation light, typically from one or more lasers, and the light scattering and fluorescence properties of the particles are measured. Particles or components thereof typically are labeled with fluorescent dyes to facilitate detection, and a multiplicity of different particles or components may be simultaneously detected by using spectrally distinct fluorescent dyes to label the different particles or components. Typically, a multiplicity of photodetectors, one for each of the scatter parameters to be measured, and one for each of the distinct dyes to be detected. The data obtained comprise the signals measured for each of the light scatter parameters and the fluorescence emissions.

Cytometers further comprise means for recording the measured data and analyzing the data. For example, typically, data storage and analysis is carried out using a computer connected to the detection electronics. The data typically are stored in tabular form, wherein each row corresponds to data for one particle, and the columns correspond to each of the measured parameters. The use of standard file formats, such as an "FCS" file format, for storing data from a flow cytometer facilitates analyzing data using separate programs and machines. Using current analysis methods, the data typically are displayed in 2-dimensional (2D) plots for ease of visualization, but other methods may be used to visualize multidimensional data.

The parameters measured using a flow cytometer typically include the excitation light that is scattered by the particle along a mostly forward direction, referred to as forward scatter

(FSC), the excitation light that is scattered by the particle in a mostly sideways direction, referred to as side scatter (SSC), and the light emitted from fluorescent molecules in one or more channels (range of frequencies) of the spectrum, referred to as FL1, FL2, etc., or by the fluorescent dye that is primarily detected in that channel. Different cell types can be identified by the scatter parameters and the fluorescence emissions resulting from labeling various cell proteins with dye-labeled antibodies.

Flow cytometers are commercially available from, for example, BD Biosciences (San Jose, CA). Flow cytometry is described at length in the extensive literature in this field, including, for example, Landy et al. (eds.), Clinical Flow Cytometry, Annals of the New York Academy of Sciences Volume 677 (1993); Bauer et al. (eds), Clinical Flow Cytometry: Principles and Applications, Williams & Wilkins (1993); Ormerod (ed.), Flow Cytometry: A Practical Approach, Oxford Univ. Press (1997); Jaroszeski et al. (eds.), Flow Cytometry Protocols, Methods in Molecular Biology No. 91, Humana Press (1997); and Shapiro, Practical Flow Cytometry, 4th ed., Wiley-Liss (2003); all incorporated herein by reference.

The data obtained from an analysis of cells (or other particles) by multi-color flow cytometry are multidimensional, wherein each cell corresponds to a point in a multidimensional space defined by the parameters measured. Populations of cells or particles are identified as clusters of points in the data space. The identification of clusters and, thereby, populations can be carried out manually by drawing a gate around a population displayed in one or more 2-dimensional plots, referred to as "scatter plots" or "dot plots, of the data. Alternatively, clusters can be identified, and gates that define the limits of the populations, can be determined automatically. A number of methods for automated gating have been described in the literature. See, for example, US Patent Numbers 4,845,653; 5,627,040; 5,739,000; 5,795,727; 5,962,238; 6,014,904; 6,944,338, each incorporated herein by reference.

In a typical laser-based flow cytometer, the excitation wavelengths available are limited by the availability of a suitable laser. Wavelength-selectable, single-wavelength excitation sources have been described for use in flow cytometry. For example, U.S. Patent No. 4,609,286 (Sage) describes a flow cytometer that uses a dispersion prism to select a wavelength from a spectrally rich light source for use as the excitation source. The light source is dispersed by the prism such that the wavelength can be selected using a slit to allow only light of essentially a single wavelength through, block all other wavelengths. The desired wavelength can be selected by physically moving the slit to correspond to the desired wavelength in the spectrum.

Telford et al, 2009, Cytometry A 75(5):450-459, describes the use of a supercontinuum white light laser as an excitation source in flow cytometry. The supercontinuum white light laser emits continuously over a wide bandwidth ranging from the near-ultraviolet to the infrared, thus appearing white to the human eye. Telford et al. describe interposing an acoustooptical filter or a coated bandpass filter in front of the beam to isolate particular wavelength ranges, permitting the user to select bandwidths of interest from the supercontinuum. The resulting excitation source can be used to select any single excitation wavelength and bandwidth by using a filter with the desired color transmission requirements.

In a typical flow cytometer, fluorescence emissions are measured in a multiplicity of detection channels (each defined as a range of frequencies within the spectrum), wherein the emissions in each channel are measured using a single photodetector. Thus, each detector provides a single measure of a range of frequencies. Typically, the detector channels are selected such that each channel is optimized to detect emissions from one of the distinct dyes. Alternatively, the emission light can be measured using an array of detection channels such that each dye emissions are measured in more than one channel.

Robinson et al., in Advanced Biomedical and Clinical Diagnostic Systems III, edited by Tuan Vo-Dinh et al., Proc. of SPIE Vol. 5692 (SPIE, Bellingham, WA, 2005): 3579-365, describes a flow cytometer detection system in which the emitted light is dispersed by diffraction grating onto a 32 channel PMT detector. Thus, fluorescence emissions are measured in 32 narrow, adjacent detection channels that together span a region of the spectrum. Instead of a single fluorescence intensity value for each dye, the data obtained using this system comprise, for each dye, intensity values for a multiplicity of adjacent detection channels. The set of measurements obtained from a dye across a multiplicity of spectrally adjacent detection channels depends on the emission spectrum of a dye.

BRIEF SUMMARY OF THE INVENTION.

The present invention provides systems and methods for analyzing the excitation spectra of fluorescent particles in a flowing stream.

The system of the present invention uses a white light laser and color separation optics to provide a spatially-distributed, continuous color-spectrum excitation system that is used to illuminate a region of a flowing stream. The spectrum of the excitation light is spread out along the length of flow stream such that the spectrum is spread over a region that is significantly larger than the diameter of a particle to be detected. A particle flowing through

the detection region will, at any one point, be excited by only a small range of wavelengths, but will be exposed to the full spectrum of the excitation light as it traverses the entire detection region. As a fluorescent particle travels through the excitation beam and is thereby excited by a continuously changing wavelength of the excitation light, a detector measures the fluorescence emissions from the particle, which vary in intensity according to the excitation efficiency at each wavelength. The result is a scan of the excitation spectrum of the particle.

Spatial expansion (spreading) of the excitation light spectrum is achieved by passing the light through color separation optics, e.g., a color dispersion element, such as a prism or diffraction grating. The color separation optics are oriented such that the spectrum is spread along the flow path, covering an elongated detection region. A particle flowing in the stream will be exposed to continuously varying wavelength light as it passes through the detection region.

In general, the spectrum can be spread along the flow path such that a moving particle will sweep through the excitation light spectrum from short to long wavelength, or from long to short wavelength. Preferably, the spectrum is spread along the flow path such that a moving particle will sweep through the excitation light spectrum from short to long wavelength.

In one embodiment, a single, broad-band detector is used to measure the emissions from the particles excited by the continuous-spectrum excitation beam as the particle passes through the detection region. Thus, the particle emissions are measured continuously as the particle is excited sequentially by the full spectrum of excitation light. The range of wavelengths detected by the single detector preferably is selected to be in a range in which the fluorescent particle emits, but is outside the excitation spectrum. Preferably, the detector is configured to measure all wavelengths that are longer than the longest excitation wavelength using an appropriate long-pass filter.

In another embodiment, the excitation light source includes a continuously variable, wavelength-selectable bandpass filter (tuneable filter) positioned between the white-light laser excitation light source and the color separation optics. The filter allows selecting any essentially single-color (a narrow band of wavelengths) excitation beam from the range of colors provided by the white-light laser. The essentially single-color excitation beam will illuminate a single spot within the detection region. Because the spectrum is spread out over the detection region by the color separation optics, and the angle of dispersion is depends on

the color of the light, a change in the color of the excitation light selected by the filter also results in a change in the position of the illumination spot within the detection region.

The continuously variable, wavelength-selectable bandpass filter is configured to continuously vary the wavelength of the excitation light from one end of the spectrum to the other, which also results in a sweep of the illumination spot from one end of the detection region to the other. The rate of change of the filter is timed such that the illumination spot traversed the detection region at the same rate that a particle in the flow stream does. By initiating the spectral sweep when the particle enters the detection region, the particle will remain illuminated while it transverses the detection region, but with a continuously varying color of excitation light.

The continuously varying spectral sweep that is aligned with the particle flow enables sweeping through the spectrum using the particle motion, but limits the excitation light to just the particle and, perhaps, a limited surrounding region. This minimized that amount of stray excitation light that may result in signal noise in the detection channel.

In another embodiment, multiple broad-band detectors are used to measure the emissions from the particles excited by the continuous-spectrum excitation beam as the particle passes through the detection region. Each detection channel (range of frequencies measured by a detector) is selected such that each is outside at least a portion of the excitation spectrum, with successive detection channels staggered such that each starts with a longer wavelength and overlaps less of the excitation light spectrum.

The embodiments using staggered detection channels, preferably together with the tuneably filtered excitation light, enable detection of particle emissions that in wavelengths that overlap a portion of the excitation light spectrum. As a particle moves through the detection region and is illuminated with progressively longer wavelengths, detection channels that measure light of a wavelength longer that the wavelength of the excitation light can be used to detect particle emissions. When the excitation light wavelength is lengthened during the scan such that it overlaps a detection channel, that detection channel is no longer useful for measuring emission because the interference caused by the excitation light. At that point, emissions are detected by one or more of the remaining detection channels. Similarly, when the excitation light wavelength is lengthened during the scan such that it overlaps the second detection channel, that detection channel is no longer useful for measuring emission because the interference caused by the excitation light, and emissions are detected by one or more of the remaining detection channels. The final detection channel preferably measures all

wavelengths that are longer than the longest excitation wavelength using an appropriate long-pass filter.

In another embodiment, multiple band-pass detectors are used to measure the emissions from the particles excited by the continuous-spectrum excitation beam as the particle passes through the detection region. Each detection channel (range of frequencies measured by a detector), which detects light over a selected band of wavelengths, is selected such that the detection channels do not overlap. Preferably, each detection channel will correspond approximately to the excitation spectrum maximum of a fluorescent dye that is being used to label particles, such that each detection channel measures primarily the emissions from one of the dyes. This enables relatively independent measurements of the excitation spectra of each of the dyes used to label a multiply labeled particle.

In another aspect, the present invention provides methods of analyzing fluorescent particles using the instruments of the invention.

The present methods and instruments may be used to analyze particles labeled with one or more dyes. For a singly dyed particle, the dye can be identified from the excitation spectrum pattern by, for example, matching the measured excitation spectrum to previously measured excitation spectra from the dyes that are used in the assay. For a multiply dyed particle, the dyes can be identified from the excitation spectrum pattern by fitting the measured excitation spectrum to a combination of previously measured excitation spectra. Various known "goodness of fit" algorithms are suitable for fitting a measured spectrum to a combination of known spectra.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figures 1A, 1B, and 1C depict schematic representations of elements of the optical system of an embodiment of a flow-type particle analyzer of the present invention.

Figures 2A, 2B, and 2C depict the passage of a fluorescent particle through a flow channel and the excitation light that illuminates the particle.

Figures 3A, 3B, and 3C depict schematic representations of elements of the optical system of another embodiment of a flow-type particle analyzer of the present invention.

Figures 4A, 4B, 4C, and 4D depict the passage of a fluorescent particle through a flow channel and the excitation light that illuminates the particle.

Figure 5 depicts the relationship between the excitation and emission spectra of a fluorescent particle, the spectrum of the excitation light source use to excite the particle, and the detection channel used to detect particle emissions.

Figure 6 depicts the relationship between the excitation and emission spectra of a fluorescent particle, the spectrum of the excitation light source use to excite the particle, and two overlapping (staggered) detection channels used to detect particle emissions.

Figure 7 depicts the relationship between the excitation and emission spectra of a fluorescent particle, the spectrum of the excitation light source use to excite the particle, and two non-overlapping detection channels used to detect particle emissions, wherein each detection channel is selected to correspond approximately to the excitation maxima of a different dye.

DETAILED DESCRIPTION OF THE INVENTION.

The following definitions are provided for clarity. Unless otherwise indicated, all terms are used as is common in the art. All reference cited herein, both supra and infra, are incorporated herein by reference.

A "flow-type particle analyzer" is used herein to refers to any instrument that analyzes particles suspended in a flowing fluid stream by passing the particles past one or more optical detectors, and includes, for example, analyzing or sorting flow cytometers, hematology analyzers, and cell counters. The fluid particle-containing stream (flow stream) typically is passed through a channel in an optical cuvette in which optical analysis is carried out, although optical analysis can be carried out on a fluid stream in air, ejected from a nozzle. At least a portion, preferably all, of the cuvette and channel are optically transparent to enable optical detection of particles within the flow stream. For example, in a typical flow cytometer, optical detection is carried out by exciting fluorescently labeled particles using an excitation light from outside the cuvette, and the fluorescence emissions from the particles are detected using photodetectors positioned outside the cuvette. The optically transparent portion of a cuvette can be made from any suitable material, including fused silica, quartz, optical glass, or an optical grade plastic.

As used herein, "system" and "instrument" are intended to encompass both the hardware (e.g., mechanical and electronic) and associated software (e.g., computer programs) components.

A photodetector, as used herein, refers broadly to the photodetector itself and any associated optics and/or electronics. For example, signal amplification (photodetector gain) may be provided by a photodetector alone or by a separate signal amplifier that amplifies the output of the photodetector. The light measured by a photodetector may be limited by the use of filters, mirrors, lenses, or other optics. For this reason, "photodetector" is used herein to refer to either a photodector alone or a photodetector with accompanying signal amplifier(s) and optics, if present.

A "detector channel", "detection channel", or "channel" refers to the range of wavelengths that is detected by a specific photodetector. The range of wavelengths detected typically is determined by the use of frequency-dependent filters and/or dichroic mirrors, as is well known in the art. For clarity, the use herein of the term "channel" is distinguished from a secondary use in the field of flow cytometry to refer to a discrete subdivision of the range of intensity values detectable by a single detector.

In some embodiments, a single detector channel is used, wherein the detector channel is defined either by a band-pass filter that transmits light of wavelengths between two threshold wavelengths, or by a long-pass filter that only transmits light of wavelengths longer than some threshold wavelength.

In other embodiments, a plurality of detector channels defined by long-pass filters are used, wherein each transmits light of wavelengths longer than different threshold wavelength, such that the detector channels partially overlap.

In other embodiments, a plurality of non-overlapping detector channels, each defined by a band-pass filter, are used to facilitate the independent measurement of a plurality of spectrally distinct fluorescent dyes. The dyes and detector channels are selected such that, as much as is feasible, the emission maximum of each dye is within a different detector channel, i.e., such that each dye is matched to a detector channel optimized to detect light from that dye. However, due to the breadth of its emission spectrum, light from a given dye may be emitted within one or more other detector channels. The light emitted by a dye within a detector channel other than the detector channel that most closely matches the emission maxima of the dye is referred to herein as "spillover". The detector channel that most closely matches the emission maximum of a dye is referred to herein, with reference to the given dye, as the dye-detection channel or primary channel. All other detector channels are referred to, with reference to the given dye, as spillover channels or secondary channels. A dye and its dye-detection channel will be referred to as "corresponding" or "matched." With reference to a detection channel, the dye that corresponds to the detection channel is referred to as the

primary dye; other dyes that emit spillover into the detection channel are referred to as secondary dyes.

As used herein, the term "particles" refers to both to synthetic particles, such as microparticles or beads, and to particles derived from biological sources, such as eukaryotic cells, bacteria, viruses, or macromolecules. As used herein, a "population" of particles refers to a group of particles that possess essentially the same optical properties with respect to the parameters to be measured, such as cells of the same type (cell population), or synthesized beads that, within practical manufacturing tolerances, are of the same size, shape, and composition.

A fluorescent particle is used herein to any particle that exhibits a detectable fluorescence. The particle may be inherently fluorescent, or it may include at least one component that exhibits a detectable fluorescence, or it may be labeled with one or more fluorescent dyes. For example, cells typically are labeled with one or more fluorescent dyes using dye-antibody conjugates that bind to cellular subcomponents to enable detection and analysis, and the labeled cells are examples of fluorescent particles.

The term "MFI", as used herein, refers to the mean or median fluorescence intensity of a population of fluorescence particles. It will be understood that other statistical measures of the population fluorescence, such as truncated mean or truncated median fluorescence, may be used.

A spectrum refers to a continuum of color formed when a beam of white light (or broad-spectrum light source) is dispersed, such as by passage through a prism, so that its component wavelengths are arranged in order. With reference to a broad-spectrum or white-light excitation source, such as a white-light laser, as used herein, the spectrum refers to the continuum of wavelength (color) that is emitted by the excitation source. The "excitation source", as used herein, refers to the light-emitting component, such as a laser, and any associated beam shaping or filtering components, if present. The range of wavelengths emitted by the excitation source as a whole may modified or restricted relative to the range of wavelengths emitted by the light-emitting component alone. For example, the spectrum emitted by a white-light laser may be used in unmodified form, or the spectrum may be restricted by appropriate filtering to provide a spectrum over a desired range of wavelengths.

A table of approximate correspondences of wavelengths and colors in the visible spectrum is provided below.

Table of Color/Wavelengths

Wavelengths	Color
400 - 420 nm	Violet
420 - 440 nm	Indigo
440 - 490 nm	Blue
490 - 570 nm	Green
570 - 585 nm	Yellow
585 - 620 nm	Orange
620 - 780 nm	Red

White light excitation source

As used herein, a "white light excitation source" refers to a light source that emits a broad-band, essentially collimated light. Preferred white light excitation sources for use in the present invention are supercontinuum white light lasers.

Supercontinuum white light lasers are available from, for example, Fianium Ltd. (Southampton, UK). Examples include the FemtoPower FP1060 & FP532, the Fianium SC450, the Fianium SC450-8-VE supercontinuum lasers. The Fianium SC450 has 5 W total laser output and has a significant emission range from approximately 450 to 2000 nm. The Fianium SC450-8-VE supercontinuum laser has 8 W of total optical power and over 1.5 W in the visible wavelength range, in a single-mode beam.

Additional examples of supercontinuum white light lasers included the SuperK EXTREME Supercontinuum Fiber Laser Series (NKT Photonics A/S, Birkerød, Denmark), which provides a 400-2400 nm single mode spectrum, and the Koheras SuperK Extreme source with 5 W total laser output (Koheras A/S, Denmark), which provides an approximately 450 to 2000 nm spectrum.

The maximum range of wavelengths provided by the white light excitation source will depend on the range of wavelengths emitted by the laser. The actual range of wavelengths emitted by the white light excitation source may be further restricted using additional filters between the laser and the flow cell.

Color separation optics

Dispersion of the broad-spectrum (white-light) excitation source into its spectrum is achieved using color separation optics that includes a dispersion element, such as a prism or

diffraction grating, both of which are well known in the art. The dispersion of the excitation beam also results in a spatial expansion (spreading) of the excitation light in one direction, such that the spectrum can be spread out over a detection region that is significantly longer that a particle dimension.

In a preferred embodiment, one or more prisms are used to disperse the white light excitation beam into its constituent spectrum of colors. Prisms will generally disperse light over a much larger frequency bandwidth than diffraction gratings, making them useful for broad-spectrum spectroscopy. Furthermore, prisms, unlike gratings, do not suffer from complications arising from overlapping spectral orders.

A suitable dispersion prism is described in U.S. Patent No. 4,609,286, incorporated herein by reference.

In a preferred embodiment, the prism system is as described in European Patent Publication EP01403632, incorporated herein by reference. This prism-based cytometry excitation optics system was designed to work with several separate single-color excitation lasers. A set of dispersive prisms redirect the beams different wavelengths so that beams with separated inputs emerge from the final prism substantially overlapping in position an nearly parallel to one another. However, as used in the present invention with a single, broad-spectrum excitation beam, the prism-based cytometry excitation optics system will disperse the broad-spectrum beam into its spectrum, spread out parallel to the flow channel.

Tuneable Filter (TF)

A Tuneable Filter (TF) is a device whose spectral transmission can be electronically controlled by applying voltage, acoustic signal, etc. A variety of tuneable filters are well known in the art. In a preferred embodiment, the tuneable filter is an AcoustoOptical Tuneable Filters (AOTF). An Acousto-Optic Tunable Filter is a solid-state, electronically tunable bandpass filter which uses the acousto-optic interaction inside an anisotropic medium. The transmitted light is controlled using an radio-frequency (RF) frequency applied to the AOTF transducer. The light transmitted through the AOTF can be varied over a complete spectrum by varying the applied frequency corresponding to the wavelength range.

AOTF are available from, for example, Fianium Ltd. (Southampton, UK), Panasonic Industrial Company (Secaucus, NJ), and Crystal Technology, Inc. (Palo Alto, CA). A preferred AOTF is the available as part number 97-02838-01 from Crystal Technology, Inc, which allows selectable transmission over a range of wavelengths from 430-670 nm.

In embodiments that incorporate an AOTF, the actual range of wavelengths emitted by the white light excitation source is restricted by additional filters to be within the range of selectable transmission frequencies provided by the AOTF.

Programmable RF frequency generators capable of generating a rapidly varying RF signal suitable for controlling an AOTF are well known in the art.

Description Based on the Figures

Figures 1A-1C

Figures 1A-1C depict several views of the components of a system of the present invention. Figure 1A depicts a side, angled view, figure 1B depicts a side view, and figure 1C depicts a top view. Not all elements are visible in all views.

Particle **1** (visible in figures 1B and 1C) is carried through a flow channel in cuvette **2**. Light from supercontinuum white-light laser **10** is passed through a prism **12** and then focused onto the fluid stream flowing through the flow channel in cuvette **2** by focusing lens **14**. The prism disperses the excitation light into its spectrum of colors such that the width of the beam is broadened in a direction parallel to the flow channel. Thus, the wavelength of the excitation light that impinges on particle **1** varies continuously as particle **1** moves through the detection region (the region of the flow channel in cuvette **2** that is exposed to light from laser **10**). The prism does not affect the width of the excitation light beam transverse to the flow channel, as shown in the top view of figure 1C.

Fluorescence emissions from the particle are collected by lens **16** and directed towards photodetector **20**. The diameter of lens **16** is larger that the length of the detection region such that emissions are collected regardless of where the particle is within the detection region. A long-pass filter **18** is oriented in front of photodetector **20**. The long-pass filter block is selected to pass only wavelengths longer than the longest wavelength of the excitation light. Thus, the long-pass filter blocks the excitation light from being measured by the photodetector, and allows measurement of the particle emissions essentially without interference from the excitation light.

The detection optics (lens **16**, long-pass filter **18**, and photodetector **20**) are oriented to collect and measure fluorescence light emitted at a right angle from the direction of the excitation beam. Because the intensity of the excitation light is typically much greater than the intensity of fluorescence emissions, and because long-pass filters, in general, are not 100% effective at blocking light outside the selected range of wavelengths, an in-line

orientation would suffer from an undesirable level of background signal resulting from excitation light passing through the long-pass filter. By orienting the collection optics such that only fluorescence emissions at right angles to the excitation beam path are measured, this background is minimized. In this configuration, the band-pass filter functions primarily to exclude excitation light that is scattered by the particle at right angles, referred to in flow cytometry as side-scatter.

Figures 2A-2C

Figures 2A-2C depict the use of the system of Figure 1 to scan the excitation spectrum of a particle. As depicted, the direction of particle **1** (visible in figures 2B and 2C) as it passes through a flow channel in cuvette **2** is from the bottom to the top of the figure. The excitation beam that has been dispersed by the prism into spectrum of colors intersects the flow channel over a length of the channel referred to as the detection region. For illustration purposes, the excitation beam, depicted as the shaded area between the dashed lines, is shown as expanded into a spectrum ranging from a blue light to a red light. The actual range of wavelengths will depend on the range of wavelengths emitted by the excitation source. To facilitate the description, four discrete colors within the excitation beam are indicated, although the excitation beam actually varies continuously from the blue end of its spectrum at the bottom limit of the beam to the red end of its spectrum at the top limit of the beam. Thus, particle **1**, as it passes through the detection region of the flow channel, is exposed to the entire spectrum of the excitation light from the blue to the red.

Figure 2A depicts the start of the spectral scan of a fluorescent particle. The particle has just entered the detection region and is exposed to a wavelength of light that is in the blue end of the excitation beam's spectrum.

Figure 2B depicts an intermediate stage of the spectral scan. The particle has moved partway through the detection region such that it is exposed to a wavelength of light that is in between yellow and green.

Figure 2C depicts the end of the spectral scan. The particle has moved to such that it is about to exit the excitation beam. At this point, it is exposed to a wavelength of light that is in the red.

As the particle travels through the detection region and is excited by the continuously varying excitation beam wavelength, fluorescence emissions from the particle, measured in a static band of detection wavelengths, are collected by lens **16** and directed towards

photodetector **20** (both elements shown in figure 1). The resulting data provide a measure of the excitation spectrum of the particle over the range of wavelengths in the excitation beam.

At any given position within the detection region, particle **1** will be exposed to a small range of wavelengths that depends on the size of the particle, the size of the detection region, and the range of wavelength emitted by the excitation beam. Preferably, the excitation beam spectrum will be spread over a detection region that is significantly larger than the particle size. For example, if excitation beam is spread out by a prism to cover a 1 mm detection region, a cell that is 10 μm in diameters will be exposed to a band of wavelengths that is $1/100^{th}$ of the total excitation spectrum that is dispersed over the detection region. In practice, this can be treated as essentially single-wavelength excitation.

The width of the band of wavelengths that the particle is exposed to any one position can be calculated based on the size of the particle and the rate of change of the excitation wavelength per distance. Let P denote the particle size, let D denote the length of the detection region, which corresponds to the dimension of the expanded excitation beam, and let F1 and F2 denote the shortest and longest wavelength emitted by the excitation source, respectively. The spectrum covers a range wavelengths equal to the absolute value of the difference, |F1-F2|, spread out over a distance D, and the rate of change of the excitation wavelength per distance is |F1-F2| / D. Thus, the particle at a given location within the detection region is exposed to a range of wavelengths that is |F1-F2| / D * P. For example, if the spectrum covers a range of 200 nm, which is spread over a 1 mm detection region, a 10 nm particle will be exposed to only a 2 nm range of excitation light at any one point in the detection region.

Figures 3A-C

Figures 3A-C depict several views of components of an alternative embodiment of a system of the present invention. Figure 3A depicts a side, angled view, figure 3B depicts a side view, and figure 3C depicts a top view. Components that are identical to components depicted in figures 1A-C are labeled with the same number. As with figure 1, not all elements are visible in all views.

Particle **1** (visible in figures 3B and 3C) is carried through a flow channel in cuvette **2**. Light from supercontinuum white-light laser **10** is passed through a prism **12** and then focused onto the fluid stream flowing through the flow channel in cuvette **2** by focusing lens **14**. The prism disperses the excitation light into its spectrum of colors such that the wavelength of the excitation light that impinges on particle **1** varies continuously as particle **1**

moves through the detection region (the region of the flow channel in cuvette **2** that is exposed to light from laser **10**).

Fluorescence emissions from the particle is collected by lens **16** and directed towards photodetector **20**. The diameter of lens **16** is larger that the length of the detection region such that emissions are collected regardless of where the particle is within the detection region. A long-pass filter **18** is oriented in front of photodetector **20**. The long-pass filter block is selected to pass only wavelengths longer than the longest wavelength of the excitation light. Thus, the long-pass filter blocks the excitation light from being measure by the photodetector, and allows measurement of the particle emissions.

The system of figure **3** further comprises a triggering mechanism that allows detection when a particle is about to enter the detection region. Laser **40** emits a beam that is reflected by mirror **42** to be essentially parallel to, but upstream of, the excitation beam emitted by white-light laser **10**. Laser **40** preferably emits a wavelength of light that is outside the spectrum of laser **10**, e.g., a shorter wavelength, but may, alternatively, emit a wavelength that is within the spectrum of laser **10**. A particle that is about to enter the detection region is first exposed to the light from laser **40**. Photodetector **47** is positioned to measure forward-scatter light. Photodetector array **46** is positioned to measure side-scatter light and fluorescence emissions from particles excited by laser **40**.

As shown in the top view (figure 3C), the side-scatter and fluorescence emissions from a particle illuminated by laser **40** are collected by lens **16** and reflected toward detector array **46** using mirror **44**. Mirror **44** preferably reflects 100% of the light, but is positioned such that only emissions originating from the intersection of the beam from laser **40** with the flow stream are reflected. Light from the spectral scanning detection region misses the mirror and is detected by photodetector **20**. Alternatively, mirror **44** may be a dichroic mirror that reflects light of a given range of wavelengths.

Detector array **46** comprises one or more separate detectors that enable the detection of side-scatter light and/or fluorescence emissions. For example, in a preferred embodiment, detector array 46 comprises the detection optics described in US Patent Nos. 7,129,505 and 6,683,314, both incorporated by reference.

Detector array **46** is configured to measure emissions using detector channels that are distinct from, partially overlapping, or overlapping with the range of frequencies provided by the white light laser. Because the two excitation/detector systems are spatially separated, they are essentially independent. Thus, in general, the spectral scanning of the present invention can be implemented together with the standard excitation and detection optics used

in a typical flow cytometer, which may include multiple excitation lasers and multiple detector arrays.

The trigger is used to time the entry of the particle into the detection region. Preferably, the triggering is based on a scatter parameter, e.g., forward scatter, but in practice, any distinguishable signal, such as a fluorescence signal, upstream of the detection region is suitable for triggering.

An Acousto-Optic Tunable Filter (AOTF) **30** is positioned in the path of the excitation beam between laser **10** and the prism **12**. AOTF **30** is operably connected to controller **32**. AOTF **30** is a bandpass filter that can select and transmit a selected narrow band of wavelengths from the incoming light, under the control of an RF frequency applied to the AOTF by controller **32**. To simplify the explanation, the AOTF may be described as selecting a particular wavelength, although it will be understood that the AOTF actually selects a narrow band of wavelengths. Controller **32** is configured to provide a variable RF frequency to the AOTF, thus controlling the selected wavelength.

The width of the dispersed excitation beam filtered to a narrow band of wavelengths corresponds to a fraction of the width of the dispersed, full-spectrum excitation beam. Thus, at a given selected narrow band of wavelengths, only a small spot within the detection region is illuminated, and the location of the spot is determined by angle of dispersion by the prism. As each wavelength is dispersed by the prism at a different angle, varying the selected narrow band of wavelengths from one end of the spectrum to the other results in a concomitant movement of the illuminated spot from one end of the detection region to the other. The controller **32** is configured to change the narrow band of wavelengths selected by the AOTF in synchrony with the movement of the particle through the flow channel such that the particle is illuminated continuously as it passes through the detection region, albeit with a constantly varying wavelength of excitation light.

In order to synchronize the change in wavelength selected by the AOTF with the movement of the particle through the flow channel, the wavelength selection must be synchronized with the entry of the particle into the detection region, and the rate of change of the wavelength selection must be synchronized with the speed of the particle through the detection region. Entry of a particle into the detection region is timed by from the trigger signal (e.g., forward-scatter) obtained from the particle passing through the trigger laser beam just upstream of the detection region. The trigger signal is sent from detector **47** or **46** (depending on which is used as the trigger signal) to controller **32**, which the initiates a sweep through the RF signal that controls the AOTF's sweep through the selected wavelengths.

The time from the detection of the trigger to the initiation of the spectral sweep depends on the flow rate and distance from the trigger detection region to the spectral-scanning detection region. In practice, the system can be calibrated empirically.

Controller **32** is configured to change of the wavelength selected by the AOTF such that the movement of the illuminated spot with the detection region moves at the same speed as the particle. The speed of the particle (i.e., speed of the flow stream through the cuvette **2**) is instrument-dependent, and can be determined empirically using methods that are standard in the field of flow cytometry.

Figures 4A-D

Figures 4A-D depict the use of the system of Figure 3 to scan the excitation spectrum of a particle. As depicted, the direction of particle **1** as it passes through a flow channel in cuvette **2** is from the bottom to the top of the figure. The excitation beam that has been dispersed by the prism into spectrum of colors intersects the flow channel over a length of the channel referred to as the detection region. For illustration purposes, the excitation beam is shown as expanded into a spectrum ranging from a blue light to a red light. The actual range of wavelengths will depend on the range of wavelengths emitted by the excitation source. To facilitate the description, four discrete colors within the excitation beam are indicated, although the excitation beam actually varies continuously from the blue end of its spectrum at the bottom limit of the beam to the red end of its spectrum at the top limit of the beam. Thus, particle **1**, as it passes through the detection region of the flow channel, is exposed to the entire spectrum of the excitation light from the blue to the red.

Figure 4A depicts the detection of the particle by the forward-detector, **47**. The detection of the particle by the forward-scatter detector provides the trigger that indicates that particle 1 is about to enter the spectral-scanning detection region.

Figure 4B depicts the start of the spectral scan of a the fluorescent particle. The particle has just entered the detection region and is exposed to a wavelength of light that is in the blue end of the excitation beam's spectrum. The full excitation beam is filtered by the Acousto-Optic Tunable Filter (AOTF) **30** to provide a narrow band of excitation light at the shortest wavelength (approximately blue) in the spectrum of the full excitation beam. The narrow band of the filtered excitation beam is depicted as the shaded area between the dashed lines. Because the blue end of the excitation beam spectrum is dispersed at the greatest angle and forms the limit of the detection region, the narrow band intersects the flow stream at the beginning of the detection region.

Figure 4C depicts an intermediate stage of the spectral scan. The particle has moved partway through the detection region such that it is positioned in the detection region where a wavelength of light that is in between yellow and green intersects the flow stream. The narrow band of wavelengths selected by the AOTF has been changed in synchrony with the particle movement such that the particle is illuminated with a wavelength within this narrow band, and the other wavelengths within the full spectrum of the excitation beam have been filtered out.

Figure 4D depicts the end of the spectral scan. The particle has moved to such that it is about to exit the excitation beam. At this point, the particle is positioned in the detection region where a wavelength of light that is approximately red intersects the flow stream. The narrow band of wavelengths selected by the AOTF has been changed in synchrony with the particle movement such that the particle is illuminated with a wavelength within this narrow band, and the other wavelengths within the full spectrum of the excitation beam have been filtered out.

Thus, as described above, as the particle travels through the detection region, it is excited by the continuously varying excitation beam having a narrow band of wavelengths. The narrow excitation beam tracks the particle through the detection region. The physical movement of the excitation beam results from wavelength-dependent dispersion by the prism and the continuously varying wavelength selection by the AOTF.

As the particle travels through the detection region and is excited by the continuously varying excitation beam wavelength, fluorescence emissions from the particle, measured in a static band of detection wavelengths, are collected by lens **16** and directed towards photodetector **20** (both elements shown in figure 3). The resulting data provide a measure of the excitation spectrum of the particle over the range of wavelengths in the excitation beam.

Figure 5

Figure 5 depicts the excitation beam spectrum and the detector channel of one embodiment of the present invention, along with the excitation and emission spectra of a exemplary fluorescent particle, referred to herein as **FP1**. The excitation spectrum **510** and emission spectrum **520** of **FP1** are shown for illustrative purposes only, to aid in understanding the invention, and are not meant to represent the spectra of any particular fluorescent dye. The x-axis is wavelength, and the y axis is an arbitrary scale used to exemplify the normalized spectra. In this example, the fluorescent particle exhibits an

excitation peak at about 550 nm and an emission peak at about 625 nm (Stokes' shift of 75 nm).

The limits of the excitation beam spectrum (range of wavelengths emitted by the excitation source) and the detection channel (range of wavelengths used for the detection of particle fluorescence) are shown schematically as bars along the x-axis. In this example, it is assumed that the spectrum of the excitation beam ranges from 450 to 600 nm, which corresponds to a range in color from a blue to an orange (see table, below). The detector channel, which is defined by the transmission range of a long-pass filter (e.g., filter **18**, shown in figures 1 and 3), detects emissions of wavelength longer than about 630 nm.

As can be seen in figure 5, the detector channel measures the emissions over a significant portion of the total emission spectrum of the fluorescent particle, **FP1**. As the particle moves through the flow channel and is excited by a constantly changing excitation wavelength, the emissions from the particle are measured in the detector channel. In this example, the sweep through the spectrum of the excitation source provides essentially a scan of the full excitation spectrum of **FP1**, as the excitation spectrum of the **FP1** is largely encompassed within the range of wavelengths provided by the excitation beam source.

The use of a long-pass filter typically is preferable to the use of a band-pass filter because it maximizes the amount of fluorescence emission measured, although either can be used.

The long-pass filter that defines the detection channel excludes excitation light from reaching the detector. Because the detector channel is clearly separated from the excitation beam spectra, the excitation beam can be on at all times. Thus, embodiment shown in figure 5 can be used with either the instrument and methods shown in figures 1 and 2, or the instrument and methods shown in 3 and 4.

Figure 6

Figure 6 depicts the excitation beam spectrum and the detector channel of another embodiment of the present invention, along with the excitation and emission spectra of a second exemplary fluorescent particle, referred to herein as **FP2**. The excitation spectrum **610** and emission spectrum **620** of **FP2** are shown for illustrative purposes only, to aid in understanding the invention, and are not meant to represent the spectra of any particular fluorescent dye. The x-axis is wavelength, and the y axis is an arbitrary scale used to exemplify the normalized spectra. In this example, the fluorescent particle exhibits an

excitation peak at about 510 nm and an emission peak at about 585 nm (Stokes' shift of 75 nm).

The limits of the excitation beam spectrum (range of wavelengths emitted by the excitation source) and the detection channel (range of wavelengths used for the detection of particle fluorescence) are shown schematically as bars along the x-axis, below the particle spectra. In this example, it is assumed that the spectrum of the excitation beam ranges from 450 to 600 nm. The first detection channel, **640**, is identical to that of figure 5, and detects emissions of wavelength longer than about 630 nm. A second detection channel, **630**, defined by a second long-pass filter, detects all emissions of wavelength longer than about 550 nm. Thus, the second long-pass filter excludes only excitation light below 550 nm in wavelength from reaching the detector.

As can be seen in figure 6, the emission maximum of **FP2** is well to the left of the wavelength cutoff for detector channel **640**, and the fraction of the total emission of **FP2** that falls within detector channel **640** is only a small portion of the total emission spectrum of **FP2**. Although the emission from **FP2** can be measured using detector channel **640**, the detection sensitivity is expected to be reduced because most of the emission from **FP2** is blocked by the long-pass filter.

The second detector channel, **630**, detects emissions of wavelength longer than about 550 nm, and is configured to detect almost all the light emitted by **FP2**. However, detection channel **630** overlaps the emission spectrum of the excitation beam. Thus, if the full spectrum of the excitation beam were to be continually illuminating the flow channel, scatter from the portion of the excitation beam spectrum that overlaps detection channel **630** would interfere with the detection of **FP2** fluorescence in that channel.

The detection scheme shown in figure 6 preferably is used with the instrument and methods shown in figures 3 and 4. As described above, as the particle moves through the flow channel and is excited by a constantly changing excitation wavelength, only an essentially single wavelength is shining on the particle at any one time. The excitation beam sweeps from a wavelength of about 450 nm to about 600 nm, tracking the movement of the particle. During this sweep, while the excitation beam is under the wavelength cutoff of detection channel **630**, there is no excitation beam light that can scatter and compete with the detection of **FP2** fluorescence. Thus, detection channel **630** can be used to detect the emission from **FP2** during a scan of the **FP2** excitation spectrum over the range of 450-550 nm.

Once the excitation beam has reached the threshold of detection channel 630, detection channel 630 is no longer used to measure the emissions from **FP2**. However, detection channel 640 is still useable for detecting the emissions from **FP2**. The signal from **FP2** emissions measure in detection channel **640** preferably are amplified to compensate for the lower detection sensitivity. Thus, the excitation spectrum of **FP2** can be scanned over the full excitation beam spectrum, measured either in detection channel **640** (with lower sensitivity), or piecewise using detection channel **630** (with high sensitivity) over the range 450-550 nm, followed by completing the scan using detection channel **640** (with lower sensitivity).

Figure 6 describes a detection scheme using two overlapping detector channels to enable scanning excitation spectra with higher specificity over a broader range. Clearly, additional detector channels can be used. Each detection channel can increase the range of the excitation spectrum scan that is carried out with highly efficient detection of the emissions.

The multiple detector channels need not be overlapping. For example, detector channel 630 could also be defined by a band-pass filter that transmits light between 550 and 630 nm, which is the start of the next detector channel.

The detection scheme shown in figure 6 is also suitable for measuring the excitation spectrum of other fluorescent particles, such as **FP1**, described above and shown in figure 5. To measure the excitation spectrum of **FP1**, detection channel **630** either can be ignored, or can be used to improve the measurement of the emission of **FP1** during the first portion of the excitation scan. In general, by providing a set of staggered detection channels, the excitation spectrum of any fluorescent particles that has an excitation spectrum overlapping the spectrum of the white light laser can be measured. The information from the separate detection channels preferably is combined to obtain the most sensitive measurement of the dye emissions while the excitation light is varied across the spectrum.

Figure 7

Figure 7 depicts the excitation beam spectrum and the detector channels of another embodiment of the present invention, along with the excitation and emission spectra of a second exemplary fluorescent particle, referred to herein as **FP2**, and the excitation and emission spectra of a third exemplary fluorescent particle, referred to herein as **FP3**. The excitation spectrum **710** and emission spectrum **720** of **FP2** and the excitation spectrum **715** and emission spectrum **725** of **FP3**are shown for illustrative purposes only, to aid in

understanding the invention, and are not meant to represent the spectra of any particular fluorescent dye. The x-axis is wavelength, and the y axis is an arbitrary scale used to exemplify the normalized spectra. In this example, the fluorescent particle, **FP2**, exhibits an excitation peak at about 510 nm and an emission peak at about 585 nm (Stokes' shift of 75 nm), and the fluorescent particle, **FP3**, exhibits an excitation peak at about 550 nm and an emission peak at about 625 nm (Stokes' shift of 75 nm).

The limits of the excitation beam spectrum (range of wavelengths emitted by the excitation source) and the detection channel (range of wavelengths used for the detection of particle fluorescence) are shown schematically as bars along the x-axis, below the particle spectra. In this example, it is assumed that the spectrum of the excitation beam ranges from 450 to 600 nm. The first detection channel, **730**, defined by a band-pass filter, detects emissions of wavelength over a range of about 560-590 nm. This first detection channel corresponds approximately to the emission maximum of **FP2**, with the upper wavelength boundary selected so as to avoid encompassing significant emission from **FP3**. This first detection channel is also referred to as the **FP2**-channel. The second detection channel, **740**, defined by a second band-pass filter, detects emissions of wavelength over a range of about 625-710 nm. This second detection channel corresponds approximately to the emission maximum of **FP3**, with the lower wavelength boundary selected so as to avoid encompassing significant emission from **FP2**. This second detection channel is also referred to as the **FP3**-channel.

As can be seen in figure 7, the **FP2**-channel measures a significant fraction of the emissions of **FP2**, but measures on a small faction of the emissions of **FP3**. Similarly, the **FP3**-channel measures a significant fraction of the emissions of **FP3**, but measures on a small faction of the emissions of **FP2**. The selection of the two detection channels to correspond to the two dye excitation maxima enables relatively independent measurement of the two dye emissions, which further enables relatively independent measurement of the two dye excitation spectra.

The detection scheme shown in figure 7 preferably is used with the instrument and methods shown in figures 3A-C and 4A-D. As described above, as the particle moves through the flow channel and is excited by a constantly changing excitation wavelength, only an essentially single wavelength is shining on the particle at any one time. The excitation beam sweeps from a wavelength of about 450 nm to about 600 nm, tracking the movement of the particle. During this sweep, while the excitation beam wavelength is under the wavelength of the **FP2**-detection channel **730**, there is no excitation beam light that can

scatter and compete with the detection of **FP2** fluorescence. Thus, the **FP2**-detection channel **730** can be used to detect the emission from **FP2** during a scan of the **FP2** excitation spectrum over the range of 450-560 nm, which represents most of the range of wavelengths over which **FP2** exhibits significant excitation.

Because the FP3-channel is outside the range of the excitation light wavelengths, no scattered excitation beam light is detected in the **FP3**-channel. Thus, the **FP3**-detection channel **740** can be used to detect the emission from **FP3** during a scan of the **FP3** excitation spectrum over the full sweep of excitation light wavelengths. Because the excitation spectrum of FP3 extends beyond the range of excitation light wavelengths. this provides a significant portion of, but not all of, the excitation spectrum of **FP3**.

Figure 7 describes an example of a detection scheme using detector channels that are matched to the particular dyes that will be used in the assay. The selection of the detection channels that correspond to the dye excitation maxima enables relatively independent measurement of the dye emissions, which further enables relatively independent measurement of the dye excitation spectra. Although two dyes are shown in Figure 7, this detection scheme can be applied to the analysis of a higher number of spectrally distinct dyes by using a correspondingly higher number of detection channels, each matched to the emissions of a particular dye.

An advantage of this embodiment of the invention is that it facilitates that analysis of particles labeled with multiple dyes. For example, as is typically the case in cell analysis by flow cytometry, a cell may be labeled with a number of distinct dyes, each bound to a different cellular protein. The multiply labeled cell will exhibit a total excitation spectrum that results from the combination of the dyes. By using detectors that are matched to the individual dyes and, therefore, enable relatively independent measurements of the dye emissions, the excitation spectrum can be separated more easily into its components corresponding to the excitation spectrum of each of the dyes in the combination.

Methods for mathematically separating (deconvolving) a spectrum that is the convolution of a plurality of spectra into the component spectra are known in the art (see, for example, Robinson et al. *supra*). Such methods can be used to calculate the contribution from each of component dyes in the present methods and, thus, to identify the labels present on a particle, such as a cell.

We Claim:

1. A flow-type particle analyzer for measuring the excitation spectrum of fluorescent particles in a fluid stream, comprising:

a) an excitation light source that emits a collimated broad-spectrum excitation light beam,

b) excitation optics that focuses and directs the excitation light beam onto a detection region in said fluid stream, wherein said excitation optics include a dispersion element that disperses the spectrum of the excitation light beam, and wherein said dispersion element is oriented such that the spectrum is dispersed over the detection region of said cuvette channel, such that a particle passing through cuvette said channel will traverse the dispersed spectrum of the excitation light beam; and

c) detection optics that measure light emitted from particles in the detection region.

2. The flow-type particle analyzer of claim 1, further comprising an optical cuvette having an cuvette channel extending through said cuvette, wherein said fluid stream is passed through said cuvette channel, and whercin said detection region in the fluid stream is in to said cuvette channel.

3. The flow-type particle analyzer of claim 1, wherein said excitation light source contains a white-light laser.

4. The flow-type particle analyzer of claim 3, wherein said excitation light source further contains filter elements that restrict the range of wavelengths emitted by the white-light laser.

5. The flow-type particle analyzer of claim 1, wherein said detection optics comprises a photodetector configured to measure a range of wavelengths that are longer than the wavelengths in the excitation light spectrum that is directed onto the detection region.

6. A flow-type particle analyzer for measuring the excitation spectrum of fluorescent particles in a fluid stream, comprising:

a) an excitation light source that emits a collimated broad-spectrum excitation light beam,

b) excitation optics that focuses and directs the excitation light beam onto a detection region in said fluid stream, wherein said excitation optics include a dispersion element that disperses the spectrum of the excitation light beam, and wherein said dispersion element is oriented such that the spectrum is dispersed over the detection region of said cuvette channel, such that a particle passing through cuvette said channel will traverse the dispersed spectrum of the excitation light beam;

c) detection optics that measure light emitted from particles in the detection region.

d) a second excitation source that emits a second beam that intersects the flow stream upstream of the detection region;

e) second detection optics that measure light emitted from the intersection of said beam and said flow stream, configured to detect particles based on the light emitted and provide a trigger signal when a particle is detected;

f) a tuneable filter positioned in the excitation light beam between the excitation light source and the dispersion element, configured to allow selection of a narrow band of wavelengths that is passed by the filter, wherein said selection is under the control of a control signal;

g) a controller configured to receive said trigger signal and to provide a control signal to said tuneable filter, such that the selected narrow band of wavelengths that is passed by the filter is changed through the spectrum of the excitation light source when a particle is passing through said detection region.

7. The flow-type particle analyzer of claim 6, wherein said excitation light source contains a white-light laser.

8. The flow-type particle analyzer of claim 7, wherein said excitation light source further contains filter elements that restrict the range of wavelengths emitted by the white-light laser.

9. The flow-type particle analyzer of claim 6, wherein said detection optics includes a filter that excludes light of wavelengths that overlap the range of wavelengths provided in the excitation light spectrum that is directed onto the detection region.

10. The flow-type particle analyzer of claim 6, wherein said detection optics comprises a plurality of photodetectors, each photodetector containing a filter that excludes

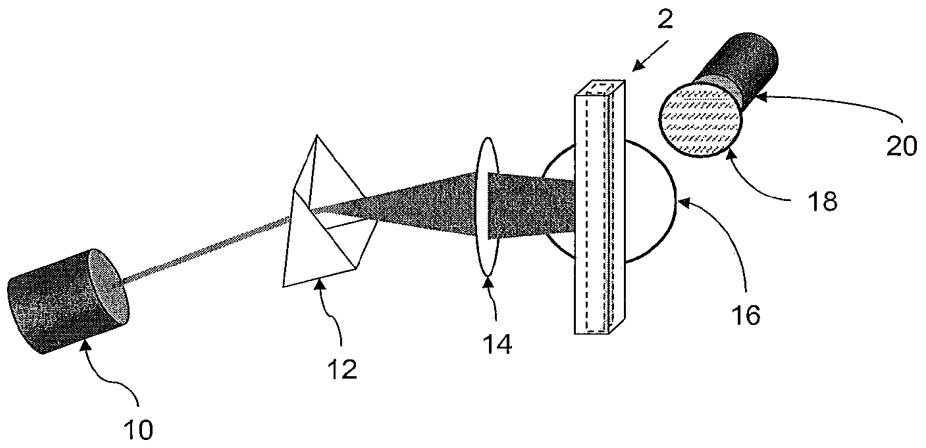
light of wavelengths that overlap the range of wavelengths provided in the excitation light spectrum that is directed onto the detection region.

11. The flow-type particle analyzer of claim 6, wherein said detection optics comprises a plurality of photodetectors, each configured to measure a range of wavelengths that are longer than at least or portion of the wavelengths in the excitation light spectrum that is directed onto the detection region.

12. The flow-type particle analyzer of claim 6, wherein said detection optics comprises a plurality of photodetectors, each configured to measure a range of wavelengths defined by a bandpass filter, wherein the ranges of wavelengths measured by said plurality of photodetectors do not overlap.

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Fig. 1A



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Fig. 1B

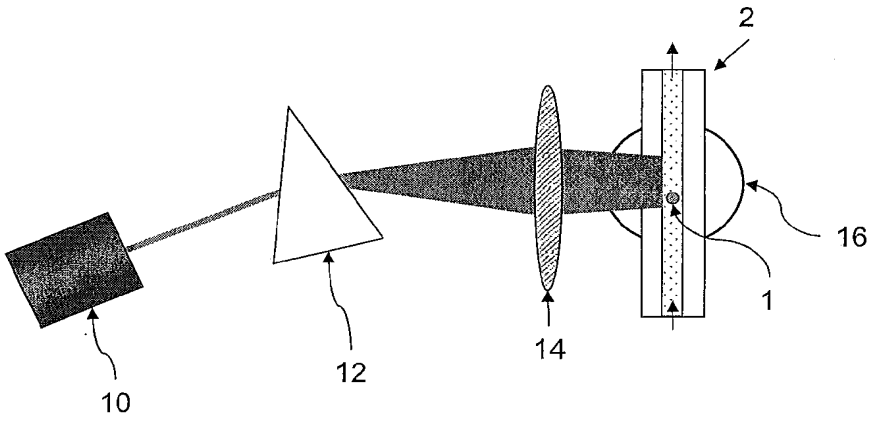
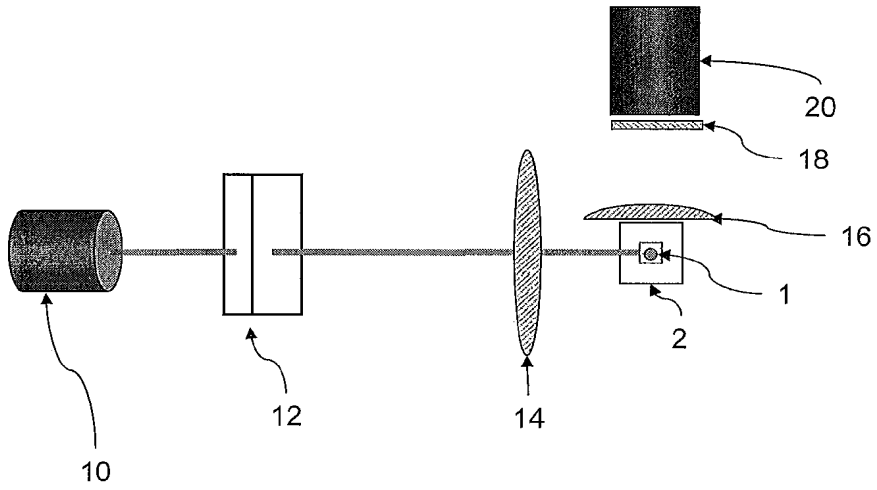
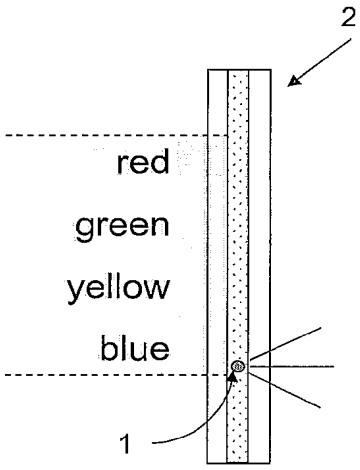


Fig. 1C



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Figure 2A



Start Spectral Scan

Figure 2B

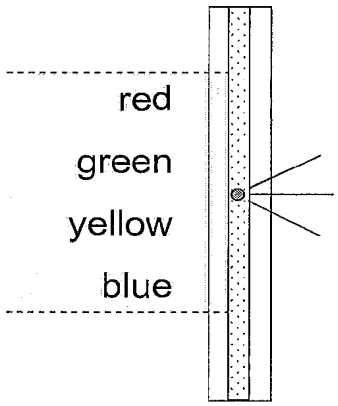
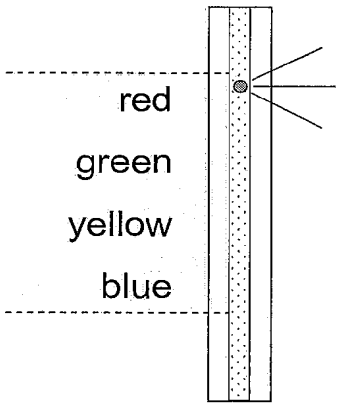


Figure 2C



End Spectral Scan

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Fig. 3A

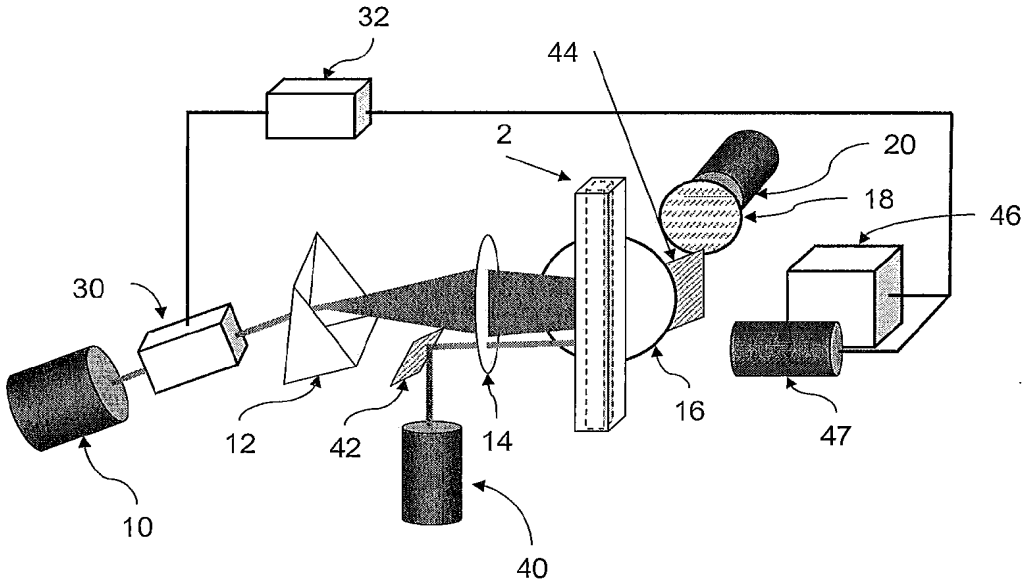
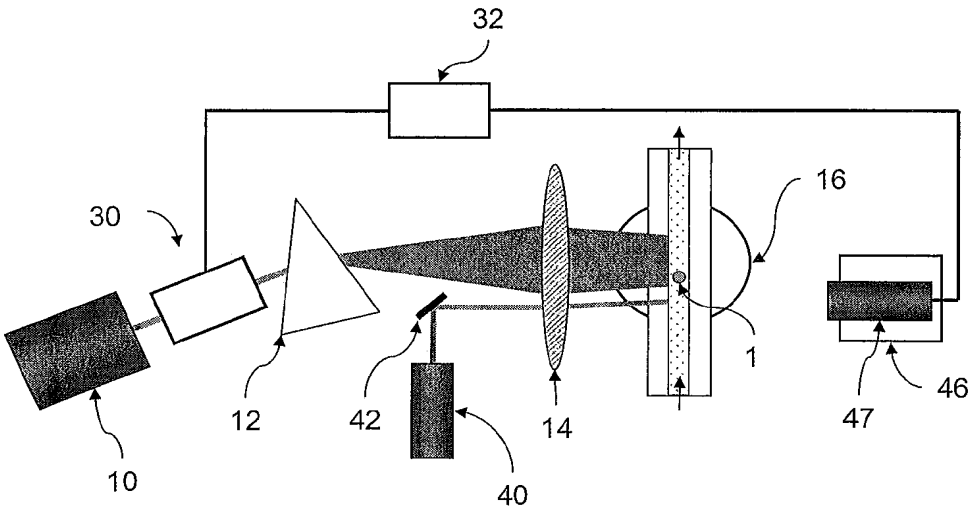


Fig. 3B



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Fig. 3C

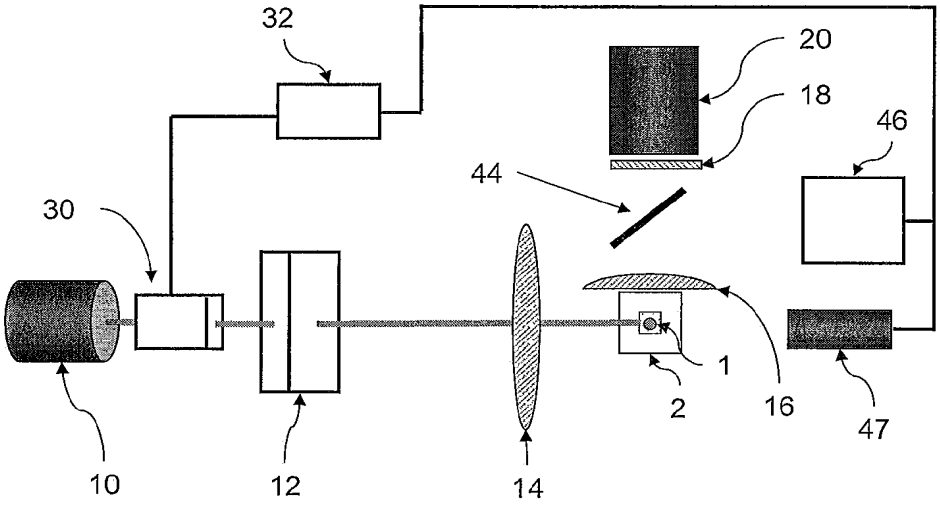


Figure 4A

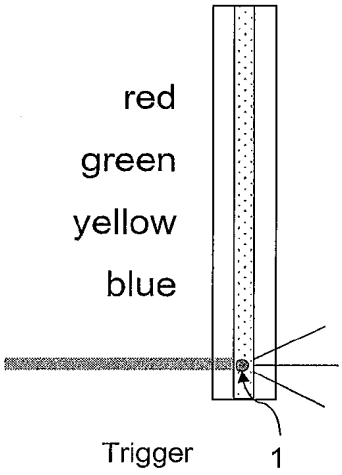


Figure 4B

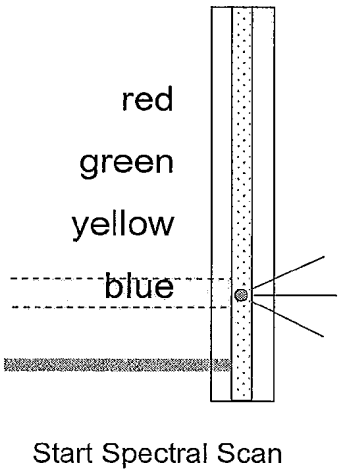


Figure 4C

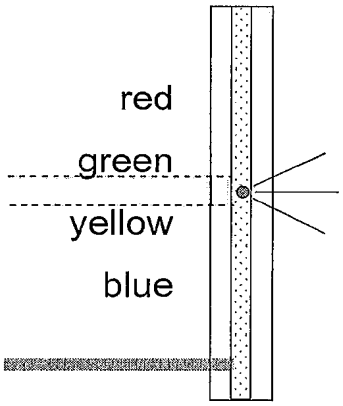
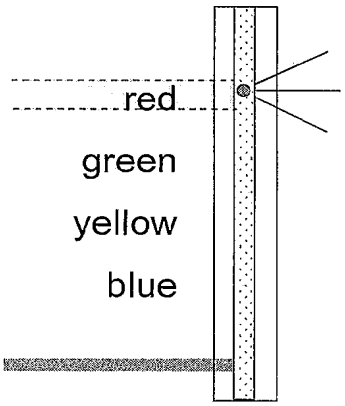


Figure 4D



End Spectral Scan

Figure 5

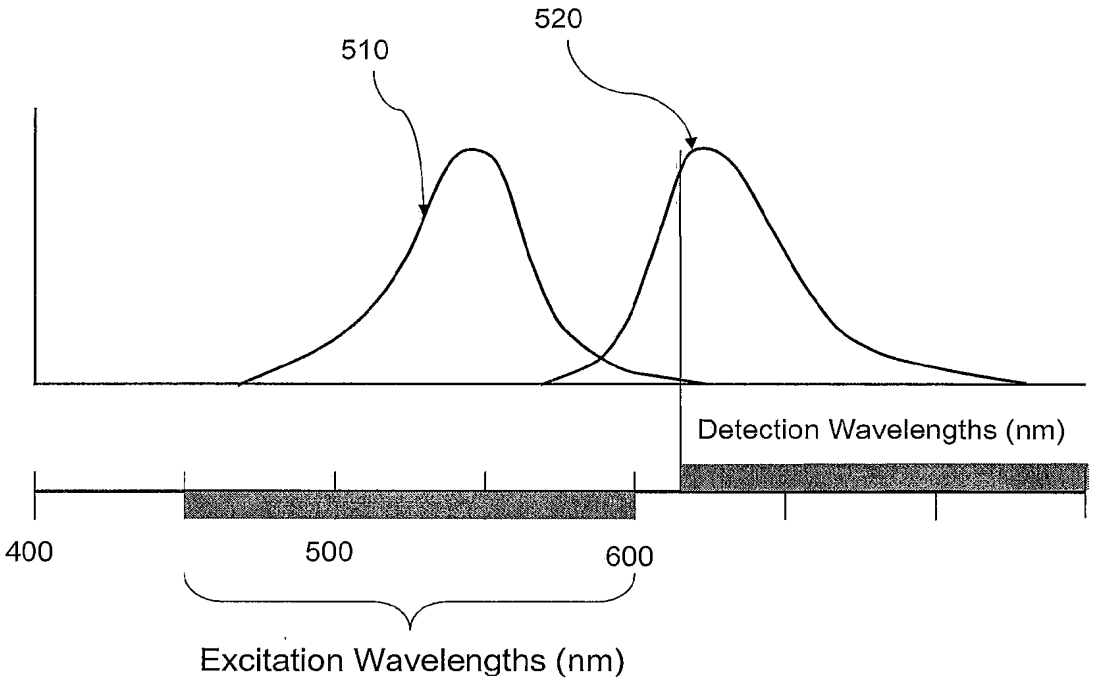
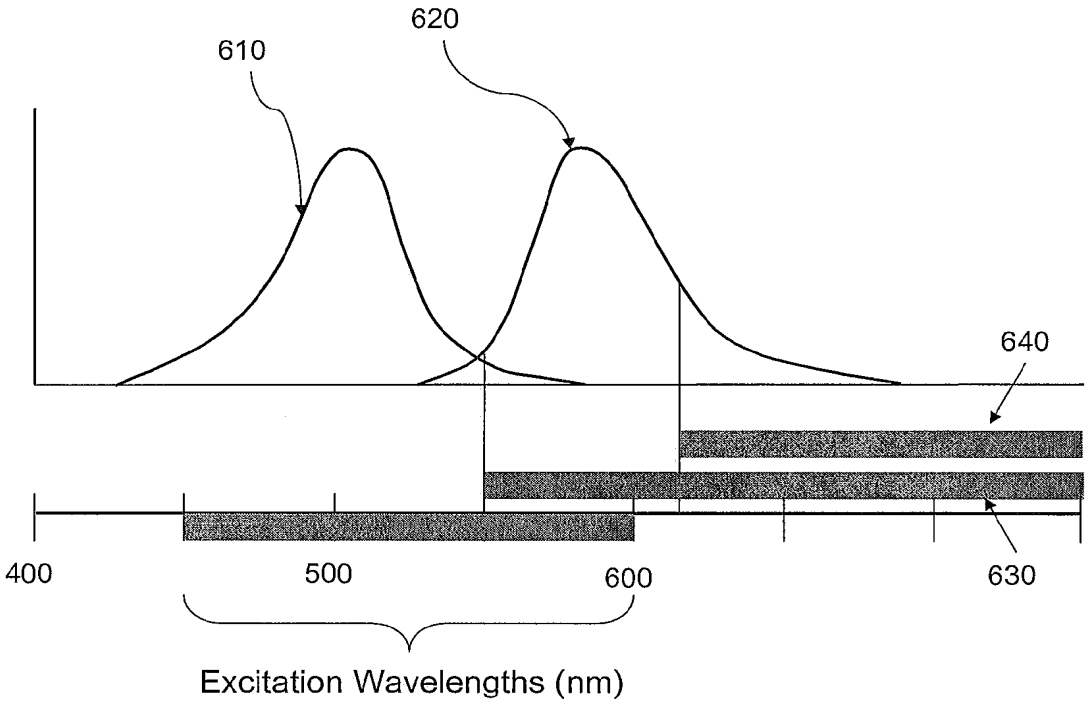
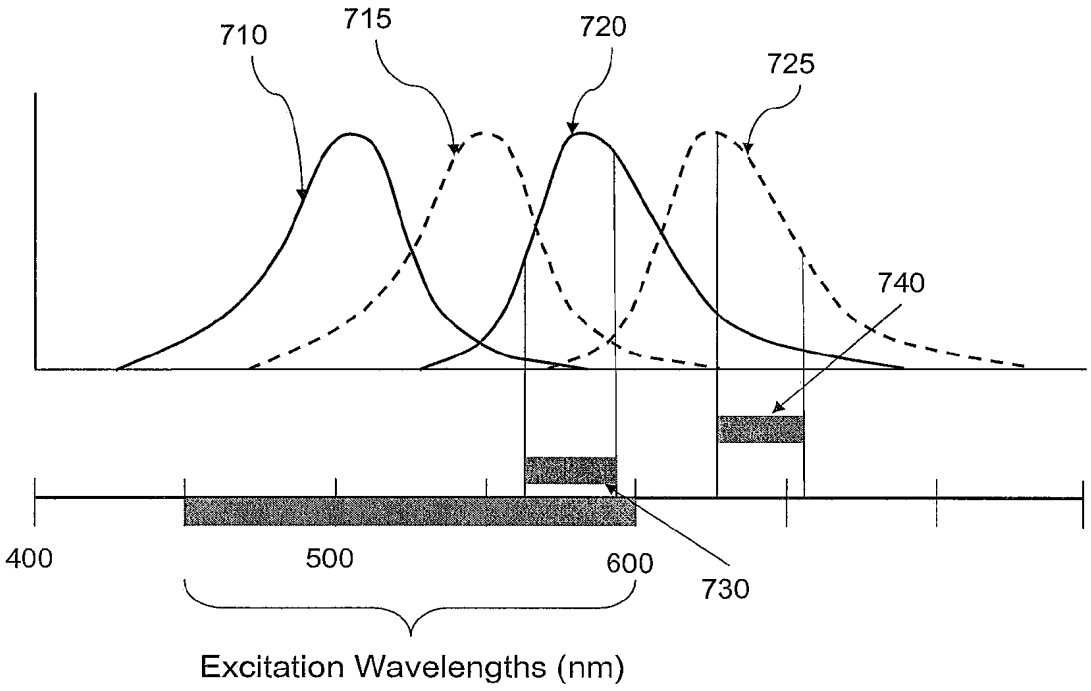

520
510
Detection Wavelengths (nm)
400
500
600
Excitation Wavelengths (nm)

Figure 6



12/12

Figure 7



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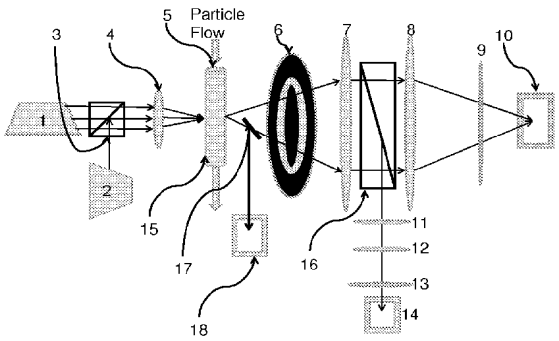
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(54) **Title**: INSTRUMENT AND METHOD FOR OPTICAL PARTICLE SENSING

ISADE Optical Path

Figure 1



(57) **Abstract**: Devices for detecting particle sizes and distributions using focused light scattering techniques, by passing a sample through a focused beam of light, are disclosed. In one embodiment, the devices include one or more lasers, whose light is focused into a narrow beam and into a flow cell, and dispersions are passed through the flow cell using hydrodynamic sample injection. In another embodiment, a plurality of lasers is used, optionally with hydrodynamic sample injection. Particles pass through and scatter the light. The scattered light is then detected using scatter and extinction detectors, and, optionally, fluorescence detectors, and the number and size of the particles is determined. Particles in the size range of 0.1 to 10 μm can be measured. Using the device, significantly smaller particles can be detected than if techniques such as EQELS, flow cytometry, and other conventional devices for measuring biological particles.

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Instrument and Method for Optical Particle Sensing

Field of the Invention

This application is generally in the area of single particle optical light scattering devices, and techniques for using them, particularly measure extremely small particles.

Background of the Invention

It is often essential to characterize biological particles by their size, surface condition, states of activation of any surface receptors, distribution, and the like. This information is useful in cell-based assays and other processes that rely upon those characteristics. Additionally, it is useful in certain diagnostic applications to detect known changes of the surface of a biological particle. Accordingly, it can be desirable to detect the surface and monitor changes to the surface in an efficient and accurate manner.

"Electrophoretic Quasi-Elastic Light Scattering" (EQELS) is one method for characterizing biological particles. This method uses electrophoresis that is dependent on the particle's surface charge density to identify and characterize suspended biological particles. EQELS uses cells placed in an electric field, where the surface charge of the particle will determine how that particle moves in the electric field. Monitoring the electrophoretic mobility of the cells provides information useful in distinguishing among different particles in the field. One can screen and optimize drug candidates which interact with the biological particles by comparing the spectra of the particles alone, or bound to the drug candidates.

Coulter counters can also be used to characterize biological particles. These devices are primarily used to count and size cells and other biological particles. The Coulter Counter works by drawing fluid containing the biological particle through a small opening located within a current between two electrodes. As the fluid is drawn through the opening, the biological particles flow through the current and measurably displace a portion of the current. The measurable displacement is translated to a pulse that is digitally processed by the Coulter Counter and translated to allow one to characterize the size and number of biological particles in the fluid.

Flow cytometry can also be used to characterize biological particles. Flow cytometry uses a beam of light, such as a laser, trained on a fluid to characterize, count and optionally sort particles in the fluid. The fluid is focused into a stream, and detectors at the intersection of the light and the fluid stream determine scatter – both forward and side. Additionally, a fluorescent detector may be present to detect fluorescent or fluorescently-tagged particles. One can determine various physical and chemical characteristics of each individual particle by analyzing the detected pattern.

These methods are useful in detecting and characterizing microparticles, including determining the number of particles, density within a fluid medium, size, and surface characteristics of the particle, confirming binding, or lack thereof, and the like. The microparticles are generally in the size of between 0.1 μm and 100 μm. However, developments in technology demand the characterization of smaller biological particles, including, but not limited to, nanoparticles.

The size of biological particles that can be analyzed using currently available technology is limited. Accordingly, there is a need for devices and processes for characterizing biological particles that can detect biological particles of varying sizes, including particles smaller than microparticles, and which can characterize the detected particles with accuracy, quantify the particles and/or monitor the particles. The present invention provides such devices and processes.

Summary of the Invention

The present invention relates to devices useful for, and methods of detecting sizes and distributions of particles using focused light scattering techniques. The devices use focused light scattering techniques, and can be used, for example, to diagnose disease, identify therapeutic agents, and obtain other useful information about biological particles and/or therapeutic agents in a sample medium. Representative particle sizes that can be measured range from between about 0.1 μm to about 100 μm, more typically in the range of between about 0.1 and about 20 μm.

Briefly, focused light scattering techniques involve passing a sample media through a particular path, where a focused beam of light passes through the sample media. The focused beam is of a size such that a particle in the size range of 0.1 to 10

μm is sufficient to block all of the beam, or a significant enough part of the beam, so that the particle size can be measured.

When there are no particles passing through the pathway of the beam, the beam passes through the media and onto a detector. When a particle, or part of a particle, passes through the beam, the beam is deflected. A diminished amount of light, or no light at all, then reaches the detector, thus indicating that a particle (or part of a particle) has interacted with the beam. The amount of diminished light reaching the detector provides information about the size of the particle. This is repeated as particles in the sample medium pass through the beam, for example, until the sample medium has entirely passed through the beam. Appropriate algorithms then take the information, and the output is a spectrum showing the particle size and particle distribution.

The device includes light from at least one, and, ideally, at least two laser light sources. Where two or more laser light sources are used, they each ideally provide light at different wavelengths. Where two or more light sources are used, they can be passed through a beam splitter, which combines the light beams. The combined light beams pass through a focusing lens, which focuses the light into a narrow beam. The beam is then passed through a flow cell.

Dispersions including particles which are to be counted and sized are passed through a flow cell, where they pass through the focused beam of light. In one embodiment, the solutions are introduced into the flow cell using a hydrodynamic sample injector. When the focused light beam comes into contact with a particle, the light is scattered.

The scattered light is then passed through a spatial filter, such as a circular spatial filter. The filter allows light reflected at certain angles, indicative of light hitting particles of a certain size, to pass through. Ideally, the filter does not permit light to pass through when it has not interacted with a particle. However, a portion of this light can be reflected off of a mirror, which can be a movable mirror, onto a detector (ideally an extinction detector).

The light passing through the spatial filter is then passed through a collimating lens, which focuses the beam from the reflected angles into a straight line. Where one

laser is used, the light can then pass through a focusing lens, which then focuses the light into a narrower beam, which is then passed on to a detector. Where two lasers are used, the light can then pass through a second beam splitter, which splits the beam into two beams.

Ideally, the splitter does not change the orientation of one of the beams. That is, one beam proceeds directly through the splitter, and the other is diverted, for example, at a 90 degree angle. In this manner, rather than using a combination of a collimating lens and focusing lens, one of the beams need only be passed through a focusing lens. The other beam may be spread out when it passes through the splitter, in which case it is advantageous to use both a collimating lens and a focusing lens.

The focused beams of light are passed through chromatic filters, which filters out light of a wavelength produced by one of the lasers, and passes light from the wavelength produced by the other of the lasers onto a detector. This way, it is possible to detect light from each of the lasers at separate detectors. Where some of the particles may be labeled, and the label is detectable, for example, by fluorescence, one can thus obtain information about all particles, and about a subset of particles that fluoresces. Those particles which fluoresce are typically those which form a complex with a fluorescently-labeled material, such as a fluorescently-labeled antibody. In one embodiment, a scattered light detector is used to detect light reflected from all particles, and a fluorescence detector is used to detect light reflected from particles that either are fluorescent, or are complexed with fluorescently-labeled materials, such as fluorescently-labeled antibodies.

The device also includes a dynamic monitoring system and method employed to monitor the size and/or number of particles, and, optionally, additional information on a subset of the particles, such as their number and/or size, which fluoresce when complexed to a particular fluorescent molecule, in a single particle optical sizing device as described herein. In one embodiment, the dynamic monitoring system is a computer either physically connected to the device, or connected remotely, for example, using Bluetooth, infrared, or other such technology, and capable of receiving information from the various detectors, as well as software which handles the digital interface with the device, wherein the computer and software are capable of counting particles, sizing particles, and storing information on the number and size of

the particles in a given sample based on the information received from the detectors and algorithms that use this information to determine particle size and/or number of particles.

Using focused light scattering techniques, significantly smaller particles can be detected than if techniques such as EQELS, flow cytometry, and other conventional methods of measuring biological particles are used. Mathematical algorithms described herein can enable one to not only detect small particles, but also to determine a range of particle sizes, relative quantities of such particles, and shapes of the particles.

Cells are one type of biological particle that can be detected. The method can be used to determine the presence or absence of a specific type of cell in a given solution. For example, a sample of blood, urine, spinal fluid, and the like can be evaluated for the presence or absence of bacteria, fungi, viruses, and the like. The particle size, and, optionally, particle shape, can also provide information about the specific type of bacteria, fungi or virus.

In one embodiment, suitable information on the particles can be obtained simply by obtaining a spectra using focused light scattering of a sample medium, wherein the particle size and distribution provides sufficient information about the presence or absence of certain biological particles present in the sample medium. For example, specific bacteria, fungi, or viruses can be identified solely on the basis of their size, and liposomal suspensions can be evaluated for agglomeration solely on the basis of the size of the agglomerated particles.

In other embodiments, where there is an interest in determining whether a particular agent forms a complex with a particular type of biological particle, additional information may be required. That is, one can determine the presence or absence of a particular cell type, or an ejected particle from a type of cell, by forming a complex between a) the cell or ejected particle and b) an active agent conjugated to a microparticle or nanoparticle ("conjugate"). The complex has a larger particle size than the cell, the ejected particle, or the conjugate, so the focused light scattering technique can determine whether a complex was formed on the basis of particle size.

The complex can be formed with a labeled material, such as a fluorescently-labeled antibody. In one embodiment, the device includes one or more additional lasers that shine a beam of light at a frequency in which the fluorescently-labeled antibody/antibodies absorb light. Thus, one source of light can be used to count and size the number of particles, and another can be used to determine the number of particles that formed a complex with a desired fluorescently-labeled material.

In some aspects of this embodiment, the biological particle is a cell that expresses a specific receptor, and the techniques permit high throughput screening of putative therapeutic agents that bind to the receptor.

In other aspects of this embodiment, the biological particle comprises cells from a patient, for example, blood cells or cancer cells, and these cells are incubated with putative therapeutic agents. Agents that bind to the cells can potentially be useful as therapeutic agents for the patient. Accordingly, this embodiment provides personalized medicine approaches.

In some of these embodiments, two spectra are taken. The first is taken on the sample media before complex formation, and the second is taken after complex formation, so one can look for the difference in particle size and distribution. However, in other embodiments, where the complex has a known particle size, and all that is required is to show that the complex formed, one can simply incubate the biological particle and the substance which may or may not form a complex with the biological particle, and use focused light scattering techniques to determine whether the complex was formed.

If the sample medium, with the biological particle and the conjugate both present, is passed repeatedly through the focused light scattering detector over a period of time, the kinetics of complex formation can be observed.

If the sample medium is scanned with the biological particle and the conjugate both present, but with different scans taken with differing concentrations of the biological particle and/or conjugate, one can determine additional information, relative to binding affinity, minimum inhibitory concentration, and the like.

If the sample medium includes cells of different sizes, expressing different receptors, then information on the selectivity of a putative therapeutic agent for one receptor over the other can be obtained.

If the agent binding to the cells results in cell rupture, then the efficacy of the active agent can be represented by a decrease in particle (cell) density in the sample medium over time.

Thus, complex formation provides useful information about the biological particle, or the agent bound to the microparticle or nanoparticle. For example, where the cell is a known cell, one can screen putative therapeutic agents for their ability to bind to the cell. Where the therapeutic agent is a known therapeutic agent, one can determine whether a particular cell binds to the therapeutic agent. This information can be useful in identifying personalized medical approaches for a patient.

For example, it is critical to determine in a timely manner whether a cancer patient will respond to a particular therapy. That is, the tumors can grow and metastasize before the physician determines that the patient does not respond to the therapy.

In one embodiment, the microparticles have a particle size in the range of between about 0.1 and 10 μm, and ideally have a relatively consistent amount of active agent bound to them. One way to produce particles with a relatively consistent amount of active agent bound to them is to use dendrimers, where the dendrimers include a known quantity of the active agent. Another way is to produce polymer particles with a) a relatively narrow size distribution, and b) a relatively consistent amount of protected functional groups, so that after the polymers are produced, the protecting groups can be removed, and the functional groups used to conjugate the polymer particles to an active agent.

The active agent can be conjugated with the particle in such a way that the portion of the active agent that is known to be active (i.e., binds a receptor) is not significantly sterically hindered by its conjugation with the particle. In some embodiments, this will involve preparing an analogue of the active agent which includes a further functional group which can be attached to the particle.

In one embodiment, metallic particles, such as gold particles, are used. Because these particles scatter a significant amount of light, they can be conjugated with a specific active agent, and used to identify even small molecules that bind to the agent. That is, the amount of light that the particle scatters is sufficiently large that the binding of the agent to the molecule of interest can be measured, even though the molecule is not within the size range of biological particles that can be measured. Means for conjugating active agents to metallic particles are known to those of skill in the art.

The present invention will be better understood with reference to the following detailed description.

Brief Description of the Drawings

Figure 1 is a schematic illustration of a single particle optical sizing device, referred to herein as a "surface antigen detection enumerator" light scattering device capable of measuring particles as small as around 0.01 μm in diameter.

Figure 2 is a schematic illustration of a device used for focused light scattering, using a single light source and a single detector.

Figure 3 is a schematic illustration of a hydrodynamic flow injector.

Figure 4 is a schematic illustration of a device to apply precise mechanical shear to a sample.

Figure 5 is a flow chart showing the path of a fluid sample from a pump controlled by robotics, through a shearing device, to a scattering cell, where dynamic light scattering or electrophoretic light scattering can be measured, to where the sample leaves the device as effluent.

Figure 6 is a chart showing the measurement of particles in a sample composed of 6 differently-sized polystyrene beads, assessed using the surface antigen detection enumerator light scattering device in terms of particle count (number) by particle size (μm). The chart shows the remarkable resolution of very small particle sizes. Current flow cytometers are not capable of resolution to this degree.

Figure 7 is a chart showing similar data from the data shown in Figure 6. The data in Figure 7 is presented as points rather than as histograms, and is reflected in particles per 10 ml sample, versus particle size (μm). Also, Figure 7 separates the size distribution into 3 different windows. In this embodiment, each window has a

separate detector that has been adjusted to detect particles in a specific size range. The smallest particles are assessed from scatted light focused onto a high-gain detector, the middle window from scattered light focused onto a low gain detector, and the window with the largest particles by a light extinction method.

Detailed Description

The present invention relates to devices for measuring particle size using focused light scattering techniques, and methods for using focused light scattering techniques in biological applications. Focused light scattering techniques provide one with the ability to analyze a fluid and determine the size and number of particles in a given sample and to, optionally, further characterize the particles in the sample. Where the particle is a biological particle, this information can be used to diagnose disease, to conduct high throughput bioassays, and to obtain information for personalized medical treatment.

The methods described herein provide numerous advantages over the previous methods in the art, including the ability to identify and characterize smaller particles, identify particles and determine particle size, number or other characteristics without using fluorescent antibodies or expensive flow cytometry, improving the identification of the initial onset of the change in voltage due, which would improve resolution of the generated spectra, control of particle shearing, and improved information regarding particle shape.

The methods also provide numerous characteristics of the particles being evaluated, including, but not limited to: identifying biological particles and distinguishing them from various cells, quantifying particles, identifying surface epitopes, identifying particle shape, and correlating this information with platelet activation, thrombin production, disease states, and the efficacy of putative therapeutic agents.

The present invention will be better understood with reference to the following definitions.

Definitions:

The term "cell" as used herein refers to any type of cell, including human cells, animal cells (such as swine cells, rodent cells, canine cells, bovine cells, ovine cells and/or equestrian cells) cloned cells, plant cells, or the like. The cells may be blood cells, cultured cells, biopsied cells, or cells that are fixed with a preservative. The cells can be nucleated, such as white blood cells or suspended endothelial cells, or non-nucleated, such as platelets or red blood cells.

The term "focused light scattering" refers to a method for sensing single particles, suspended in a solution, when the solution is passed through a focused beam. When the beam passes through the solution without being scattered by a particle, the beam passes on to a photodetector and the intensity is measured. When the beam is scattered, in whole or in part, by a particle, the intensity of the beam hitting the photodetector is altered. The particle size and concentration can be calculated, for example, using light-extinction, light-scattering detection, or both.

A "focused light scattering device" is a multi-particle optical sensor, which has high sensitivity and responds to relatively concentrated suspensions, uses a relatively narrow light beam to illuminate an optical sensing zone non-uniformly.

As used herein "particles" are small fragments or completely intact biological cells, and related to a living organism when referred to as "biological particles." Intact cells may range in size from about 1 micron to 20 microns. Aggregates of intact cells or fragments of cells may range in size from 2 microns to 100 microns. "Microparticles" are fragments of biological cells or particles that generally range in size from about 0.1 μm to about 0.8 μm, generally 0.1 - 20 μm. Examples include, but are not limited to blood cells, platelets (1-3 micron), cancer cells (5 -15 micron), red blood cells (~7μm), white blood cells (~5-10μm), bacteria (~0.5-1 μm), tumors, granulocytes, monocytes, neutrophils, lymphocytes, endothelial cells, stem cells, viruses, and fungi.

"Light extinction" as used herein is a measurement of the absorption and/or scattering of light in an electromagnetic field by particles as they pass through the field. As a particle passes through a field, there is a momentary reduction in the transmitted light intensity due to the light refraction, absorption and/or scattering. Measurement of light extinction by the particles provides additional information

regarding the characteristics of the particles. A light extinction spectrum can be generated for each particle.

"Light scattering" occurs when there is a momentary change in the intensity of the incident light caused by the interaction of the incident photons with the particle. In the case on focused scattering device, the intensity of the scattered light reaching the detector is proportional to the size of the particle. Thus, when the particles being characterized are biological particles, the method of light scattering will involve measuring voltage at the detector this will be proportional to the particle size. An exemplary focused light scattering system for detecting biological particles is shown in Figure 1.

"Nanoparticles" as used herein are particles or biological particles that are generally smaller than 0.1 μm in size. Because of their small size, nanoparticles have a very high surface area to volume ratio. Accordingly, nanoparticles often possess unique physical characteristics. The present invention provides a way to both quantify and monitor nanoparticles, in particular, cellular nanoparticles, which are often believed to be responsible for initiating further biochemical processes in living organisms.

The types of devices that can be used to carry out these diagnostic assays, and methods for performing these assays, are described in more detail below.

I. Focused Light Scattering Devices and Algorithms for Measuring Particle Size and Shape

The focused light scattering device includes the following components:

a) one or more lasers which produce beams of laser light,

b) a first focusing lens positioned in the path of the beams of laser light, which focusing lens focuses the beams of light to a size wherein a particle with a diameter of about 0.1 μm is sufficient to block all or substantially all of the light,

c) a flow cell positioned in the path of the focused light source, wherein the flow cell is capable of receiving a sample medium comprising a dispersion of particles, and passing the sample medium through the focused light source, where

light that interacts with one or more of the particles is scattered, and light that does not interact with one or more particles is not scattered,

d) a spatial filter, such as a circular spatial filter, positioned in the path of the light, which allows scattered light to pass through, and does not allow light that is not scattered to pass through,

e) a collimating lens positioned in the path of light passing through the circular spatial filter, through which scattered light is collimated,

f) a second focusing lens positioned in the path of the collimating lens, focusing the light passing through the collimating lens,

g) a scatter detector positioned in the path of the focused light passing through the focusing lens,

h) a mirror positioned between the flow cell and the circular spatial filter, which mirror reflects a portion of the scattered light, and

i) an extinction detector positioned in the path of the reflected scattered light.

Where more than two laser light sources are used, additional focusing lenses, collimating lenses, chromatic filters, and detectors can be used so that each source of laser light can be separated from the others. In one embodiment, the beam splitter used in these embodiments is one that is capable of splitting the beam into multiple beams, rather than just two beams. In another embodiment, a series of beam splitters is used, each of which splits a beam into two beams, so that multiple beams can be obtained.

The device can also include a hydrodynamic flow injector for introducing the sample medium into the flow cell. In one embodiment, the device includes a hydrodynamic flow injector, but only includes one source of laser light. In another embodiment, the device includes two or more sources of laser light, but does not include a hydrodynamic flow injector. In a third embodiment, the device includes two or more sources of laser light and a hydrodynamic flow injector.

In some embodiments, the device includes two or more lasers. In these embodiments, the device further includes a beam splitter that combines the light from the two lasers, and passes the combined light beams through the first focusing lens. In these embodiments, the device further includes a second beam splitter positioned between the collimating lens and the second focusing lens, splitting the beam into two beams, wherein one of the beams is not diverted from its original path toward the second focusing lens, and the other of the beams is diverted from its original path toward the second focusing lens toward a second path.

In order to distinguish between light from the different laser, so that light from only one laser passes through to a given detector, chromatic filters are used to block out light from one or the other of the lasers before the light impinges on a detector. For example, a first chromatic filter can be positioned between the second focusing lens and the scatter detector, which chromatic filter permits light from one of the laser beams to pass through, and which does not permit light from the other of the laser beams to pass through, to the scatter detector. A second collimating lens can be positioned along the second path, which lens collimates the diverted light beam. A focusing lens can be positioned in the path of light passing through the second collimating lens. A second chromatic filter can be positioned in the path of light passing through the focusing lens, which second chromatic filter permits light from the laser beam that did not pass through the first chromatic filter to pass through. A detector, for example, a fluorescence detector, can be positioned in the path of the light passing through the second chromatic filter.

The device also includes a dynamic monitoring system and method employed to monitor the size and/or number of particles, and, optionally, additional information on a subset of the particles, such as their number and/or size, which fluoresce when complexed to a particular fluorescent molecule, in a single particle optical sizing device as described herein. In one embodiment, the dynamic monitoring system is a computer either physically connected to the device, or connected remotely, for example, using Bluetooth, infrared, or other such technology, and capable of receiving information from the various detectors, as well as software which handles the digital interface with the device, wherein the computer and software are capable of counting particles, sizing particles, and storing information on the number and size of

the particles in a given sample based on the information received from the detectors and algorithms that use this information to determine particle size and/or number of particles.

The monitoring system includes a data acquisition module operatively coupled to the one or more detectors, and (iii) a processing and display unit operatively coupled to the data acquisition module for determining the size and/or number of particles in a given sample and responsively outputting a graphical representation of the size and/or number of the particles in the sample. The data acquisition module uses the data obtained from the detectors in the single particle optical sensing device described herein, and an algorithm which correlates the data to the size and/or number of particles in the sample medium.

The processing and display unit that is coupled to the data acquisition module may utilize any suitable processing means, e.g., a general purpose programmable digital computer or central processing unit (CPU) including memory and processor components. The processor may be arranged to communicate with the memory by means of an address/data bus, and can be constituted by a commercially available or custom microprocessor. The memory can include, without limitation, devices of varied type, such as cache, ROM, PROM, EPROM, EEPROM, flash memory, SRAM, and DRAM.

The memory may include several categories of software and data used in the data processing system: the operating system; the application programs; the input/output (I/O) device drivers and the data. The data may include a database of known profiles of particle sizes, for example, a reference library of the size of platelets, bacteria, viruses, fungi, cancer cells, stem cells, and complexes of the cells with various molecules, including fluorescently-labeled molecules, such as fluorescently-labeled antibodies, and the like.

It will be appreciated that the operating system in the processing and display unit can be of any suitable type for use with a data processing system. Illustrative examples of operating systems that can be usefully employed include, without limitation, OS/2, AIX, OS/390 or System390 (International Business Machines Corporation, Armonk, NY), Windows CE, Windows NT, Windows95, Windows98, Windows2000, or WindowsXP (Microsoft Corporation, Redmond, WA), Unix or

Linux or FreeBSD, Palm OS from Palm, Inc., Mac OS (Apple Computer, Inc.), LabView or proprietary operating systems.

The I/O device drivers typically include software routines accessed through the operating system by the application programs to communicate with devices such as I/O data port(s), data storage and certain components of the memory.

The application programs are illustrative of the programs that implement the various features of the system and can suitably include one or more applications that support analysis of the data. The data represent the static and dynamic data used by the application programs, the operating system, the I/O device drivers, and other software programs that may reside in the memory.

Any configuration of the processor capable of carrying out the operations for the methodology of the invention can be advantageously employed.

The I/O data port of the processing and display unit can be used to transfer information between the processing and display unit and another computer system or a network (e.g., the Internet) or to other devices controllable by the processor.

The processing and display unit optionally, but ideally, includes a display for graphically outputting information on the size and/or number of particles in a sample, in the form of a representation of the sample being assayed and the size and/or number of particles in the sample. This representation may be a graphic depiction, in which the size and/or number of particles are schematically depicted in a graphical output, as a two-dimensional column listing the size and/or number of particles, and the like. Such type of depictions can provide an intuitive and readily visually perceptible indication of the size and/or number of particles in the sample.

Where the dynamic monitoring system is a computer, which is either physically connected to the device, or capable of receiving information remotely, for example, via Bluetooth or infrared, the computer is capable of receiving information from the various detectors. The computer also includes software which handles the digital interface with the device. The computer and software are capable of counting particles, sizing particles, and storing information on the number and size of the particles in a given sample based on the information received from the detectors. The

types of algorithms used to relate information from the various detectors to particle size and/or particle number are described in more detail below.

An exemplary apparatus useful for performing the methods described herein is disclosed in U.S. Patent Application Publication No. 20040011975, the contents of which are hereby incorporated by reference in its entirety. The apparatus is described therein is useful in performing particle analysis using focused light scattering techniques. However, as described herein, other similar apparatus can be employed, including detectors for focused light scattering and/or light extinction.

The principal defining characteristic of the focused light scattering method described in U.S. Patent Publication No. 20070010974, the contents of which are hereby incorporated by reference, is not simply a significant reduction in the size of the illuminated area, A_0, resulting in a significant reduction in V_{OSZ} and improvement in sensitivity. Rather, it concerns the nature of the illuminating beam and the resulting OSZ thereby defined.

The term "focused light scattering" refers to a method for sensing single particles, suspended in a solution, when the solution is passed through a focused beam. When the beam passes through the solution without being scattered by a particle, the beam passes on to a photodetector and the intensity is measured. When the beam is scattered, in whole or in part, by a particle, the intensity of the beam hitting the photodetector is altered. The particle size and concentration can be calculated, for example, using light-extinction, light-scattering detection, or both.

In one embodiment, the beam is produced by a laser. The laser beam interacts with the particles, and produces scattered light when the laser beam interacts with a particle. In one aspect of this embodiment, the apparatus includes two or more different lasers, which can give off light at two or more different wavelengths, and/or which can interact with the particles at different angles. The use of light at different wavelengths can enable one to identify specific epitopes. Particles can interact with specific molecules, including fluorescently-labeled molecules, and the fluorescence can be detected using a laser with light at a predetermined wavelength that interacts with the fluorescent label. The use of more than two lasers can enable the use of two or more fluorescent labels, which labels fluoresce at different wavelengths. This technique is described in more detail below.

A beam of light (usually laser light) of a single wavelength is directed onto a hydrodynamically-focused stream of fluid that includes the platelets. A number of detectors are aimed at the point where the stream passes through the light beam. In one aspect of this embodiment, one detector is in line with the light beam (Forward Scatter or FSC) and one or more detectors are perpendicular to it, including Side Scatter or SSC detectors and one or more fluorescent detectors. Each suspended platelet passing through the beam scatters the ray, and fluorescent chemicals either present within the platelet or attached to the platelet are excited into emitting light at a longer wavelength than the light source. This combination of scattered and fluorescent light is picked up by the detectors, and, by analyzing fluctuations in brightness at each detector (one for each fluorescent emission peak), it is then possible to derive various types of information about the physical and chemical structure of each individual platelet.

Thus, in some embodiments of the apparatus described herein, there are three or more detectors. For example, one can include one detector for extinction [used to measure particles with a size > 0.7 microns], one for scattered light [used to measure particles with a size in the range of 0.15 to 0.7 microns] and one or more for fluorescence [used for phenotyping].

The sources of light can include lamps (i.e., mercury, xenon); high-power water-cooled lasers (i.e., argon, krypton, dye laser); low-power air-cooled lasers (i.e., argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (i.e., blue, green, red, violet). The detectors can convert fluorescence signals from light into electrical signals that can be processed by a computer.

The process of collecting data from samples is termed "acquisition." Acquisition is typically mediated by a computer physically connected to the apparatus, and the software which handles the digital interface with the apparatus. The software is capable of adjusting parameters (i.e. voltage, compensation, etc.) for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to insure that parameters are set correctly. An interactive database can allow the apparatus to be used in applications for both clinical and research purposes. A wide variety of analysis software and fluorescently-labeled antibodies has been developed, and are well known to those of skill in the art.

The apparatus can include multiple lasers (between 2 and 5, typically between two and four) and fluorescence detectors (typically between 2 and 18, more typically

between 2 and 10). Increasing the number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers.

Gating

The data generated by the apparatus can be plotted in a single dimension, in two dimensions, or even in three dimensions. The regions on these plots can be sequentially separated, based on fluorescence intensity, by creating a series of subset extractions, termed "gates." Specific gating protocols exist for diagnostic and clinical purposes especially in relation to hematology.

The plots are often made on logarithmic scales. Because different fluorescent dyes' emission spectra overlap, signals at the detectors have to be compensated electronically as well as computationally. Data accumulated using the apparatus can be analyzed using software, e.g., Flowjo, FCS Express, VenturiOne or CellQuest Pro. Data analysis can be performed on a separate computer, if desired.

Computational analysis

Automated population identification using computational methods can be used as an alternative to traditional gating strategies. Automated identification systems can potentially help find rare and/or hidden populations. Representative automated methods include FLOCK in Immunology Database and Analysis Portal (ImmPort), FLAME in GenePattern and flowClust, in Bioconductor.

Fluorescent labels

A wide range of fluorophores can be used as labels in flow cytometry. Fluorophores, or simply "fluors", are typically attached to an antibody that recognizes a target feature, epitope, on or in the cell; they may also be attached to a chemical entity with affinity for the cell membrane or another cellular structure. Each fluorophore has a characteristic peak excitation and emission wavelength, and the emission spectra of different labels often overlap. Consequently, the combination of labels which can be used depends on the wavelength of the lamp(s) or laser(s) used to excite the fluorochromes and on the detectors available (Loken MR (1990). Immunofluorescence Techniques in Flow Cytometry and Sorting (2nd ed.). Wiley. pp. 341–53). The maximum number of distinguishable fluorescent labels is thought to be

17 or 18, and this level of plexy necessitates laborious optimization to limit artifacts, as well as complex deconvolution algorithms to separate overlapping spectra (Ornatsky, O.; Bandura, D.; Baranov, V.; Nitz, M.; Winnik, M. A.; Tanner, S. (2010). "Highly multiparametric analysis by mass cytometry". Journal of Immunological Methods 361 (1–2): 1–20)

Quantum dots are sometimes used in place of traditional fluorophores because of their narrower emission peaks.

The fluorescent labels can be used, for example, to determine the degree of protein expression and localization, the existence of any protein modifications or intracellular antigens (various cytokines, secondary mediators, etc.), membrane fluidity, platelet viability, and platelet adherence.

Representative fluorescent labels are provided below:

Probe	Ex (nm)	Em (nm)
Hydroxycoumarin	325	386
Aminocoumarin	350	445
Methoxycoumarin	360	410
Cascade Blue	(375);401	423
Pacific Blue	403	455
Pacific Orange	403	551
Lucifer yellow	425	528
NBD	466	539
R-Phycoerythrin (PE)	480;565	578
PE-Cy5 conjugates	480;565;650	670
PE-Cy7 conjugates	480;565;743	767
Red 613	480;565	613
PerCP	490	675
TruRed	490,675	695
FluorX	494	520
Fluorescein	495	519
BODIPY-FL	503	512
TRITC	547	572
X-Rhodamine	570	576
Lissamine Rhodamine B	570	590
Texas Red	589	615
Allophycocyanin (APC)	650	660
APC-Cy7 conjugates	650;755	767
Alexa Fluor 350	343	442
Alexa Fluor 405	401	421
Alexa Fluor 430	434	540
Alexa Fluor 488	499	519
Alexa Fluor 500	503	525
Alexa Fluor 514	517	542
Alexa Fluor 532	530	555
Alexa Fluor 546	561	572

Alexa Fluor 555		553	568
Alexa Fluor 568		579	603
Alexa Fluor 594		591	618
Alexa Fluor 610		610	629
Alexa Fluor 633		632	648
Alexa Fluor 647		652	668
Alexa Fluor 660		663	691
Alexa Fluor 680		680	702
Alexa Fluor 700		696	719
Alexa Fluor 750		752	776
Alexa Fluor 790		782	804
Cy2		489	506
Cy3		(512);550	570;(615)
Cy3B		558	572;(620)
Cy3.5		581	594;(640)
Cy5		(625);650	670
Cy5.5		675	694
Cy7		743	767
DyLight 350	353	432	
DyLight 405	400	420	
DyLight 488	493	518	
DyLight 549	562	576	
DyLight 594	593	618	
DyLight 633	638	658	
DyLight 649	654	673	
DyLight 680	692	712	
DyLight 750	752	778	
DyLight 800	777	794	
Hoechst 33342		343	483
DAPI		345	455
Hoechst 33258		345	478
SYTOX Blue	431	480	
Chromomycin A3		445	575
Mithramycin	445	575	
YOYO-1		491	509
Ethidium Bromide		493	620
Acridine Orange		503	530/640
SYTOX Green		504	523
TOTO-1, TO-PRO-1	509	533	
Thiazole Orange		510	530
Propidium Iodide (PI)	536	617	
LDS 751		543;590	712;607
7-AAD	546	647	
SYTOX Orange		547	570
TOTO-3, TO-PRO-3	642	661	
DRAQ5		647	681,697
Indo-1	361/330	490/405	
Fluo-3	506	526	
DCFH	505	535	
DHR		505	534

SNARF	548/579	587/635
Y66H	360	442
Y66F	360	508
EBFP	380	440
EBFP2	383	448
Azurite	383	447
GFPuv	385	508
T-Sapphire	399	511
TagBFP	402	457
Cerulean	433	475
mCFP	433	475
ECFP	434	477
CyPet	435	477
Y66W	436	485
dKeima-Red	440	616
mKeima-Red	440	620
TagCFP	458	480
AmCyan1	458	489
mTFP1 (Teal)	462	492
S65A	471	504
Midoriishi-Cyan	472	495
Wild Type GFP	396,475	508
S65C	479	507
TurboGFP	482	502
TagGFP	482	505
TagGFP2	483	506
AcGFP1	484	510
S65L	484	510
Emerald	487	509
S65T	488	511
EGFP	488	507
Azami-Green	492	505
ZsGreen1	493	505
Dronpa-Green	503	518
TagYFP	508	524
EYFP	514	527
Topaz	514	527
Venus	515	528
mCitrine	516	529
YPet	517	530
TurboYFP	525	538
PhiYFP	525	537
PhiYFP-m	525	537
ZsYellow1	529	539
mBanana	540	553
Kusabira-Orange	548	559
mOrange	548	562
mOrange2	549	565
mKO	548	559
TurboRFP	553	574

tdTomato	554	581
DsRed-Express2	554	591
TagRFP	555	584
DsRed monomer	557	592
DsRed2 ("RFP")	563	582
mStrawberry	574	596
TurboFP602	574	602
AsRed2	576	592
mRFP1	584	607
J-Red	584	610
mCherry	587	610
HcRed1	588	618
mKate2	588	633
Katushka (TurboFP635)	588	635
mKate (TagFP635)	588	635
TurboFP635	588	635
mPlum	590	649
mRaspberry	598	625
mNeptune	600	650
E2-Crimson	611	646
Monochlorobimane	380	461
Calcein	496	517

In some applications, particularly clinical applications, it can be desirable to use microfluidics to introduce samples to the apparatus. The microfluidic device can be disposable (i.e., used once or perhaps a few times, followed by disposal and replacement) and/or composed of a polymeric material. The microfluidic device can be adapted to reduce the amount of sample used to determine whether a patient can benefit from a particular anti-thrombotic therapy. The microfluidic device preferably provides a tip adapted for delivering the biological sample including the platelets into the cell through which light passes, so that the platelets can then travel through the light beam(s). In some embodiments, the tip is adapted for sheath spraying. In other embodiments, the tip is adapted for non-sheath spraying. In any of the embodiments herein the apparatus may include a disposable inlet capillary.

The apparatus can also include an autodiluter, which can start with the most dilute sample, rather than the most concentrated sample, and can therefore use less sample. Autodiluters are well known to those of skill in the art. Representative autodiluters include the AutoDiluter-5.2, the CETAC ADX-500 Autodiluter, the ProLiquid AutoDiluter, and the DYNATECH Autodiluter III.

In high-throughput screening, it can be preferable to include robotics, which can introduce the samples to the apparatus. Ideally, the apparatus can then be cleaned

in between samples, for example, by flushing the various lines, and subsequent samples introduced, enabling the screening to be automated. Information on the screening results can be stored, for example, in a memory map, and the information correlated with the patient's identity.

A "focused light scattering device" is a single-particle optical sensor, which has high sensitivity and responds to relatively concentrated suspensions, uses a relatively narrow light beam to illuminate an optical sensing zone non-uniformly. It differs from conventional SPOS devices in that it can handle more concentrated solutions and smaller particle sizes.

In use, a solution including suspended platelets passes through a zone. The zone is smaller than the flow channel, so that the sensor responds to only a fraction of the total number of platelets flowing through the channel, detecting a statistically significant number of particles of any relevant diameter.

Because different particle trajectories flow through different parts of the zone illuminated at different intensities, it is necessary to deconvolute the result. Two methods of deconvolution can be used: modified matrix inversion or successive subtraction. Both methods use a few basis vectors measured empirically or computed from a theoretical model, and the remaining basis vectors are derived from these few. The sensor is compensated for turbidity.

The sensor apparatus for single-particle optical sizing of particles in a fluid suspension typically includes a means for establishing flow of the suspension through a physically well-defined measurement flow channel. There is also an illumination means for effectively directing a relatively narrow beam of light, having an axis, through the measurement flow channel to form an optical sensing zone within the measurement flow channel. The beam of light and the optical sensing zone are of such size relative to the size of the measurement flow channel that the sensor apparatus responds to only a fraction of the total number of particles flowing through the measurement flow channel. In this manner, the sensor apparatus responds effectively to a relatively concentrated fluid suspension.

The beam has a maximum intensity portion and a continuum of lesser intensities for positions spaced transverse to the axis from the maximum intensity portion. In this manner, some of the particles have trajectories through the maximum intensity portion, others of the particles have trajectories through the lesser intensity positions, and still others of the particles may have trajectories outside the zone.

Typically, the maximum intensity portion of the beam is in a central portion of the beam.

The device also includes a detector means for photo-detecting light from the zone to provide pulse height signals. These signals each respond to a particle flowing through the zone. The pulse height signals are functions of the sizes and trajectories of detected particles. Particles of a given size provide a maximum pulse height signal when flowing through the maximum intensity portion, and lesser pulse height signals when flowing through the lesser intensity positions of the zone. The pulse height signals, collectively, form a pulse height distribution PHD.

The device further includes a means for mathematically deconvoluting the pulse height distribution to extract a particle size distribution of the PSD particles in the fluid suspension. The sensor apparatus can detect a statistically significant number of particles of any given diameter or range of diameters that are relevant to the fluid suspension.

In one embodiment, the measurement flow channel has a thickness dimension axially of the beam of light, a width dimension transverse to the beam, and a flow direction substantially perpendicular to the thickness and width dimensions. The beam is narrower than the measurement flow channel in the width direction. The beam can be focused with a depth of field which is substantially larger than the thickness dimension, and the beam substantially has an effective width which does not vary substantially over the thickness dimension.

In another embodiment, the beam has an effective width between opposing positions transverse to the axis in the beam, at which the lesser intensities have fallen to a given fraction of the maximum intensity. The effective width is chosen so that the largest particles of interest can be effectively sized. The given fraction can be, for example, $1/e^2$ of the maximum intensity, where e is the base of the natural system of logarithms, and the effective width is substantially one half the size of the largest particle to be sized.

In yet another embodiment, the apparatus uses hydrodynamic sample injection, such as is described in Pelssers et al., Journal of Colloid and Interface Science, Volume 137, Issue 2, July 1990, Pages 350-361. Colloidal dispersions, such as platelets in serum or other media, can be hydrodynamically focused into a narrow stream, with widths ranging from about 3 to about 10, preferably about μm width. The use of a focused light scattering technique allows one to measure relatively small

particle sizes. However, where a focused beam hits particles in a relatively wide sample stream (as described above, where the beam is narrower than the measurement flow channel in the width direction), the detection method relies somewhat on statistics. That is, where the beam is substantially narrower than the sample stream, an assumption is made that there is an equal distribution of particles in the sample stream, so that one can extrapolate the results of the interaction of the light in the narrow beam with the particles in its path over the entire width of the sample stream. By hydrodynamically focusing the sample into a stream with a relatively narrow width, and using a focused light source, it is possible to count all or most of the particles in the sample stream, and rely to a lesser extent on statistics.

The light beam can have, for example, a Gaussian intensity profile, a circular cross-section, or an elliptical cross-section being wider in a direction transverse to particle flow.

The detector means can be include a light extinction-type detector, and can be a combination of detectors, for example, a light-extinction detector type and a light-scattering type detector. The light-scattering type detector means can include means for passing a portion of scattered light from the zone through a mask to select light scattered between a first and a second angle to the beam and a means for directing a portion of the light transmitted through the zone to a light-extinction type detector.

The detector means can include a mirror for deflecting a portion of the light from the optical-sensing zone to the light-extinction detector. The illuminating means can include a light source and optical fiber means for conveying light from the light source to the optical sensing zone, and projecting the light through the zone.

The detector means can also include an optical fiber means for conveying the light passing through the optical sensing zone to the light-extinction type detector. The detector means can also include means for passing a portion of the light scattered from the zone through a mask, to select light scattered between a first and second angle to the beam, and an optical fiber means for conveying the portion of the light to a light-scattering type detector. The detector means can also include a light-scattering detector.

In one embodiment, the illumination means provides two light beams directed through a pair of optical sensing zones positioned within the measuring flow channel,

and each beam has an effective width determined by a desired maximum particle size.

The detector means can include a light-scattering detector and a means for passing light scattered from the zone through a mask means. The mask means can include a plurality of masks and means for selecting one of the masks for passing the light scattered from the zone, each mask defining different angles between which the light is scattered. The masks can be located on a rotatable wheel, and a mask can be selected by rotating the wheel to a desired position.

The illuminating means can project a relatively wide collimated beam through the optical sensing zone, and can include an acceptance aperture to capture only those light rays that closely surround the axis of the beam. This reduces the effective width of the beam to a width in a direction transverse to the axis of the light beam which is substantially one-half the size of the largest particle to be sized. The illuminating means can also include a means for coupling the light rays to the detector means. This can be, for example, an optical fiber means.

In one aspect of the invention, a statistically significant number of particles of each relevant size flow through all portions and positions of the zone.

In another aspect of the invention, the fluid suspension is relatively concentrated and the apparatus further comprises means to compensate for turbidity of the suspension. In this aspect, the detector means can operate on a light extinction principle, and provide a signal having a baseline voltage level. The pulse height signals appear as downwardly extending pulses from the baseline voltage level, and the means for compensation for turbidity of the suspension can include means to sense the baseline voltage level and automatically increase the level to approximately the baseline voltage level present in the absence of turbidity in the suspension. The detector means can operate on a light extinction principle, and provide a signal having a baseline voltage level, wherein the means to compensate for turbidity can include a computer means for correcting the pulse height signals in response to the ratio of the baseline voltage level when the fluid suspension is not turbid, to the baseline voltage level for the turbid fluid suspension.

The detector means can also operate on a light extinction principle and provide a signal having a baseline voltage level, wherein the means to compensate for turbidity includes a means to adjust the intensity of the beam of light by increasing the amount of light produced by the illuminating means in response to the ratio of the

baseline voltage level when the fluid suspension is not turbid, to the baseline voltage level for the turbid fluid suspension.

The particle trajectories can be substantially uniformly distributed across the width of the measurement flow channel.

The means for deconvoluting the pulse height distribution can include basis vectors, each corresponding to a particular particle size, and a source vector representing a measured pulse height distribution for a fluid suspension as detected by the detector means. There can also be a means using a deconvolution algorithm to derive the particle size distribution from the pulse height distribution. At least some of the basis vectors can have values based upon measurements of particles of known size. Some of the basis vectors can also have values based upon measurements of particles of known size and others of the basis vectors can be computed from the sum of the basis vectors by interpolation and/or extrapolation.

The basis vectors can be computed, and the basis vectors can be column basis vectors of a matrix, where the means using a deconvolution algorithm performs matrix inversion and vector multiplication, or the means using a deconvolution algorithm can perform successive subtraction.

The means using a deconvolution algorithm can provide a deconvoluted pulse height distribution dPHD, and the apparatus further comprises means providing a calibration curve of the relationship of pulse height and diameter, and means using the calibration curve to transform each deconvoluted pulse height value in the dPHD into a unique particle diameter associated with this pulse height value. This can yield a "raw" particle size distribution PSD. There can also be a means for converting the raw PSD into a final PSD by renormalizing the raw PSD by multiplying by the value $1/PHI_d$, where PHI_d is the fraction of particles actually detected by the device for particles of each size.

The particle trajectories can be distributed non-uniformly across the width of the measurement flow channel, and the basis vectors can be based upon the response of particles of known size flowing through the measurement flow channel with the same non-uniform distribution of particle trajectories as the fluid suspension.

The sensor apparatus may respond only to a fraction of the total number of particles flowing through the measurement flow channel.

One can prepare a matrix for deconvoluting pulse height distributions derived from particles of unknown size flowing along different trajectories through a non-

uniform light field of a single-particle optical sizing device. This can enable one to size particles in a fluid suspension. To do this, one can determine the value of at least one empirical basis vector for the matrix by measuring the response of particles of known size flowing through the single-particle optical sizing device. Then, one can compute other basis vectors for the matrix corresponding to particles of other sizes, by interpolating and/or extrapolating the other basis vectors from the empirical basis vector.

One can also determine the value of additional empirical basis vectors for the matrix by measuring the response of particles of known sizes flowing through the single-particle optical sizing device, and computing the other basis vectors for the matrix corresponding to particles of other sizes from the at least one empirical basis vector and the additional empirical basis vectors.

Another way to prepare a matrix for deconvoluting pulse height distributions derived from particles of unknown size flowing along different trajectories through a non-uniform light field of a single-particle optical sizing device for sizing particles in a fluid suspension involves determining the value of at least one computed basis vector corresponding to particles of at least one size for the matrix. One can compute other basis vectors for the matrix corresponding to particles of other sizes from computed basis vectors.

Also disclosed is a method of deconvoluting a pulse height distribution derived from particles of unknown size flowing along different trajectories through a non-uniform light field of a single-particle optical sizing device for sizing particles in a fluid suspension. The method involves setting up a matrix having a plurality of columns, each containing a basis vector comprising a pulse height distribution of particles of a known size corresponding to the response of a photo-detector of the device to the particles of known size. Each successive column contains a basis vector for particles of successively larger sizes. The matrix also has a like plurality of rows, each corresponding to a successive pulse height channel, each channel including a range of pulse heights, with successive rows corresponding to successively larger pulse heights, and with each column having a maximum count pulse height value at a location for a row which relates to a pulse height corresponding to the particle of known size associated with the column. The maximum count pulse height values for successive columns are arranged on a diagonal of the matrix. The matrix is modified by setting all terms corresponding to pulse height values greater than the maximum

count pulse height value in a column to zero. A deconvolution algorithm is used to perform matrix inversion and vector multiplication of the pulse height distribution and the matrix as modified.

Before the modifying step, one can renormalize the values of the basis vectors in the columns by setting the maximum count pulse height value to equal 1.0 and all other count pulse height values in the column to a value maintaining the same proportionate value to 1.0 that the other count pulse height values had to the maximum count pulse height value of the column.

The response of the photo-detection to the particles of known size is developed empirically for some of the basis vectors by sending particles of the substantially known size through the device and providing a response by the device to the particles of known size. The response to the photo-detector for the remaining basis vectors can be computed by interpolating and/or extrapolating the response for the remaining basis vectors from the some of basis vectors.

The response of the photo-detector to the particles of known size can be computed for some of the basis vectors and the response to the photo-detector for the remaining basis vectors can be computed by interpolating and/or extrapolating the response from the some basis vectors.

A pulse height distribution ("PHD") can be derived from particles of unknown size flowing along different trajectories through a non-uniform light field of a single-particle optical sizing device for sizing particles in a fluid suspension can be deconvoluted by setting up a matrix having a plurality of columns. Each column includes a basis vector comprising a pulse height distribution of particles of a substantially known size corresponding to the response of a photo-detector of the device to the particles of known size, and each successive column contains a basis vector for particles of successively larger sizes. The matrix can also include a like plurality of rows, each corresponding to a successive pulse height channel, each channel including a range of pulse heights, successive rows corresponding to successively larger pulse heights, each column having a maximum count pulse height value at a location for a row which relates to a pulse height corresponding to the particle of known size associates with the column. The maximum count pulse height values for successive columns can be arranged on a diagonal of the matrix. A successive subtraction algorithm can be implemented, by starting with the basis vector with its maximum count value in the largest row number; scaling a column basis

vector by a factor corresponding to the value of the row in the PHD that matches the column number; subtracting the scaled basis vector from the PHD to form an element of a deconvoluted PHD (dPHD), leaving an intermediate PHD vector with a smaller number of total particles; and repeating this process using the remaining basis vectors until the entire PHD has been consumed and all the elements of the deconvoluted dPHD have been formed.

A single-particle optical sizing sensor for sizing particles in a relatively concentrated fluid suspension sample for turbidity of the suspension sample can be compensated using a sensor operating on a light extinction principle whereby a photo-detector produces signal $V_{LE}(t)$ having a baseline voltage level and a response to blockage of light by a particle as a downwardly extending pulse from the baseline voltage level. The compensation method involves passing a non-turbid suspension through the sensor; measuring a baseline voltage level V_0 produced in response to the non-turbid suspension; passing the relatively concentrated suspension sample through the sensor; measuring a baseline voltage V_0^T produced in response to the relatively concentrated suspension sample, calculating the ratio $V_oV_0^T$; and adjusting the sensor in response to G to compensate for the turbidity when the relatively concentrated suspension sample passes through the sensor.

The baseline voltage V_0^T can effectively be subtracted from the signal $V_{LE}(t)$, the remaining signal can be inverted to produce a pulse height signal 2 $V_{LE}^T(t)$, and an adjustable gain amplifying means can be used to amplify the pulse height signal 3 $V_{LE}^T(t)$. The adjustable gain amplifying means can be controlled by the ratio G to provide a compensated pulse height signal $\Delta V_{LE(t)}$.

The signal $V_{LE}(t)$ produced by the sensor in response to the relatively concentrated suspension sample can be amplified by adjustable gain amplifier means, the gain of which is controlled by the ratio G to provide a compensated signal $V_{LE}(t)$ having a compensated baseline voltage V_0, subtracting the baseline voltage V_0 from the compensated signal $V_{LE}(t)$, and inverting the remaining signal to produce compensated pulse height signal $\Delta V_{LE}(t)$.

In one embodiment, the single-particle optical sizing sensor comprises a light source producing a light beam of adjustable intensity, wherein the intensity is increased in response to the ratio G to compensate for the turbidity.

Particles in a fluid suspension can also be optically sized by establishing a flow of the suspension through a physically well-defined measurement flow channel

of a single-particle optical sizing sensor apparatus wherein a beam of light, having an axis, is directed through the measurement flow channel to form an optical sensing zone within the measurement flow channel. The beam of light and the optical sensing zone are ideally of such size relative to the size of the measurement flow channel that the sensor apparatus responds to only a fraction of the total number of particles flowing through the measurement flow channel. The sensor apparatus can respond effectively to a relatively concentrated fluid suspension. The beam can have a maximum intensity portion in the beam and a continuum of lesser intensities for positions in the beam spaced transversely from the axis, whereby some of the particles have a trajectory through the maximum intensity portion, others of the particles have trajectories through the lesser intensity positions, and still others of the particles may have trajectories outside the zone. Light from the zone can be detected to provide pulse height signals, each responsive to a particle flowing through the zone. The pulse height signals are functions of the sizes and trajectories of detected particles, and the pulse height signals collectively form a pulse height distribution PHD. The PDH can be mathematically deconvoluted and processed to extract from the PHD a particle size distribution PSD of the particles in the fluid suspension.

The step of mathematically deconvoluting the PHD can involve determining the value of at least one empirical basis vector by measuring the response to particles of known size flowing through the single-particle optical sizing device. Other basis vectors corresponding to particles of other sizes can be computed by interpolating and/or extrapolating the other basis vectors from the empirical basis vector.

The value of additional empirical basis vectors for particles of known sizes flowing through the single-particle optical sizing device can be determined; and the other basis vectors for the matrix corresponding to particles of other sizes can be calculated by interpolating and/or extrapolating the other basis vectors from at least one empirical basis vector and the additional empirical basis vectors. The method can further involve determining the value of at least one computed basis vector corresponding to particles of at least one size. Other basis vectors corresponding to particles of other sizes can also be computed by interpolating and/or extrapolating the other basis vectors from computed basis vectors.

The step of deconvoluting and processing the PHD can involve setting up a matrix having a plurality of columns, each containing a basis vector comprising a pulse height distribution of particles of a known size corresponding to the response of

a photo-detector of the device to the particles of known size, each successive column containing a basis vector for particles of successively larger sizes. The matrix can also have a like plurality of rows, each corresponding to a successive pulse height channel, each channel including a range of pulse heights, successive rows corresponding to successively larger pulse heights, each column having a maximum count pulse height value at a location for a row which relates to pulse heights corresponding to the particle of known size associated with the column. The maximum count pulse height values for successive columns can be arranged on a diagonal of the matrix. The matrix can be modified by setting all terms corresponding to pulse height values greater than the maximum count pulse height value in a column to zero. A deconvolution algorithm can be used to perform matrix inversion and vector multiplication of the pulse height distribution and the inverted matrix as modified. The response of the photo-detector to the particles of known size can be developed empirically for some of the basis vectors by directing a flow of particles of the known size through the device and providing a response by the device to the particles of known size. The response to the photo-detector for the remaining basis vectors can be calculated by interpolating and/or extrapolating the response for the remaining basis vectors from the some of basis vectors.

The step of mathematically deconvoluting the PHD can also involve using a deconvolution algorithm to provide a deconvoluted pulse height distribution dPHD. The method can further involve providing a calibration curve of the relationship of pulse height and diameter, and using the calibration curve to translate each deconvoluted pulse height value in the dPHD into a unique particle diameter associated with this pulse height value yielding a "raw" particle size distribution in PSD. The raw PSD can be converted into a final PSD by renormalizing the raw PSD by multiplying by the value $1/PHI_d$, where PHI_d is the fraction of particles actually detected by the device for particles of each size.

In use, a focused laser light beam passes through a chamber through which fluid flows, and the laser light scatters as the particles pass through the focused laser beam. An extinction detector determines when particles have passed through the beam. In the absence of a particle interfering with the beam of light, the light would pass, uninterrupted, to the extinction detector. When a particle blocks the passage of light, the resulting loss of light hitting the extinction detector signals that a particle

has passed through the beam. The light hitting the particles is reflected, and passed through a scatter collimating lens, which re-focuses the light, which then passes through a scatter focus lens, which sends a single beam through to a scatter detector.

A representative focused light scattering device is shown in Figure 1. A first laser (1) emits light at a first wavelength, and a second laser (2) emits light at a second wavelength. Both beams of light pass through a first beam splitter (3) and through a first focusing lens (4) before they enter into a flow cell (15). The flow cell includes a site (5) for hydrodynamic injection of the sample. As the platelets in the flow cell pass through the beams of light, the light is scattered as it hits the platelets. The scattered light passes through a circular spatial filter (6) and then through a first collimating lens (7). The light beam passes through a second beam splitter (16), which splits the light into two beams. A first beam passes through a second focusing lens (8) and through a first chromatic filter (9) that passes scattered light from the first laser (1) through a first detector (10). The second beam passes through a second collimating lens (11), a third focusing lens (12) and a second chromatic filter (13) that passes scattered light from the second laser (2) to a second detector (14).

The two photodetectors (10 and 14) each are able to detect light at a certain frequency, so that light transmitted at different frequencies (as a result of the two lasers hitting particles, and which may interact with fluorescent tags on the particles) can be separately determined.

A third detector (an extinction detector) (18) receives a portion of the light passing through the flow cell. A portion of the light passing through the flow cell is reflected off of a movable mirror (17) and onto the third detector.

As is shown in Figure 2, there are two important features inherent in the optical design. First, the incident beam alone (in conjunction with the front and back windows 36 and 37 of the measurement flow channel 35) defines the OSZ. The side walls 38 and 39 that confine the fluid-particle suspension along the x-axis are no longer of any consequence with respect to definition of the OSZ. Second, the physical volume associated with the OSZ can no longer be described by a single value; rather, it now depends on the size of the particles being measured.

The approach shown schematically in Figure 2 involves illuminating measurement flow channel 35 with a light beam 41 from a laser light source 40 which is focused by a lens 42 to form a beam 44 of relatively narrow cross section--i.e., smaller than. a typical illuminated width, a, of the flow cell in a conventional LE-type sensor. The resulting OSZ is therefore defined approximately by a "pencil" beam of light 46, together with the front and back windows of the flow cell, separated by dimension "b." The schematic diagram in Figure 2 provides a simplified picture of the OSZ defined by focused light beam 46. First, the region of illumination that comprises the OSZ is not sharply defined, as implied by the approximately cylindrical zone indicated in Figure. 1. Rather, the outer boundary of the OSZ is "fuzzy," extending well beyond the zone indicated, as discussed below. Second, the beam passing through the flow channel 10, assuming that it has been focused, typically is not uniform in width. Rather, in the case of a focused beam, its width varies over the depth of the measurement flow cell 35. The extent to which the beam waist varies over the depth of the channel depends on the depth of field of the focused beam, defined as the distance (y-axis) between the points at which the beam waist grows to 2 times its minimum value. Ideally, the depth of field is significantly greater than the channel thickness, b, resulting in a relatively uniform beam width throughout the flow channel.

Consequently, focused light scattering devices may include a fundamentally different sensor. In the conventional design, the physical width of the flow channel 10 and the effective width (x-axis) of the OSZ are one and the same, equal to dimension "a." By contrast, the physical width of the flow channel in a sensor used for focused light scattering devices (also defined as "a") is typically much larger than the nominal width, 2w, of the incident light beam and therefore has no significant influence on the OSZ. Hence, the spacers (or shims) 38 and 39 that separate the front and back windows 36 and 37, determining the depth, b, of the flow cell (and OSZ), no longer need to be opaque or smooth on an optical scale to avoid scattering by the edges. This is a significant advantage, making fabrication of the flow cell easier and less expensive.

It is usually convenient and effective to employ a "circularized" light beam, in which the incident intensity ideally depends only on the radial distance, r, from the beam axis (coincident with the y-axis, with x=z=0, as seen in FIG. 1). Typically, one employs a "Gaussian" light beam--i.e. one having a Gaussian intensity profile,

described in the focal plane (minimum beam waist), at y=b/2, by $I(r)=I_0\exp(-2r^2/w^2)$ (7) where $r^2=x^2+z^2$ for the assumed circular beam.

Quantity 2w is the diameter of an imaginary cylinder containing most of the incident light flux. The intensity on its surface equals $1/e^2$, where e is the base for natural logarithms, or 0.135 times its value, I^0, at the center of the beam (r=0). Essentially 100% (apart from losses due to reflections at optical interfaces and extinction by particles in the beam) of the light flux contained in the incident beam traverses the fluid-particle mixture in the flow channel and impinges on the distant detector D_{LE}. This causes detector D_{LE} to provide a light extinction signal V_{LE} in the form of a downwardly extending pulse.

This behavior is in sharp contrast to the illumination scheme employed in a conventional LE-type sensor. There, the starting light beam is expanded greatly along the lateral (x) axis of the flow cell, so that its width ($1/e^2$ intensity) is much larger than the width, a, of the front window (and OSZ). As a result, there is relatively little variation in the incident intensity along the x-axis (i.e. for y=z=0) where the beam enters the flow cell, because the light is captured at the top of the x-expanded Gaussian beam. Therefore, a particle passing through the OSZ will experience substantially the same maximum beam intensity (i.e. at z=0), regardless of its trajectory. The specific values of x and y defining the trajectory ideally have no influence on the resulting sensor response, i.e. the pulse height.

There is a sharp contrast between the conventional optical design and the scheme employed in the sensor used for focused light scattering devices. There is a large variation in the incident intensity as a function of position (x-axis) across the width of the flow channel. In the case in which the incident light beam has a symmetric (circular) Gaussian profile, the intensity variation is given by Equation 7, with r=x. The maximum intensity, I_0, is achieved at the center of the beam (x==0), where for simplicity x=0 represents the midpoint of the channel (with the side walls at x= ±a/2). As noted, the intensity occurring at x= ±w, z=0 is reduced substantially, to 0.135 I_0. The intensity drops steeply with increasing distance from the beam, falling, for example, to 0.018 I_0 at x= ± 2w, z=0 and 0.00033 I_0 at x=±4 w, z=0.

The consequences for the light-extinction signal thus generated by the passage of particles through the new OSZ are far-reaching. First, as for a conventional LE-type sensor, the pulse height, ΔV_{LE}, generated by passage of a particle through the OSZ in general increases with increasing particle size, all other factors being equal. In

general, the larger the particle, the larger the fraction of light "removed" from the incident beam, thus unable to reach the detector D_{LE}. However, with the new sensor the fraction of light removed from the beam now depends on the precise trajectory of the particle--specifically, the minimum distance, |x|, of the particle to the center of the beam, x=0. (To first approximation, the response of the sensor will not vary significantly with changes in the y-axis value of the trajectory, assuming that the beam width is approximately constant over the depth of the flow channel, given an appropriately large depth of field, as discussed above.)

For a particle of given size and composition (hereinafter assumed to be spherical and homogeneous, for simplicity), the maximum "signal," or pulse height, is achieved when the particle passes through the center of the beam, x=0. A particle of given effective cross-sectional area, ΔA, blocks the largest amount of incident light at the center of the beam, where the intensity is greatest. Particles of identical size that pass through the flow channel along different trajectories, with different minimum distances, |x|, from the beam axis, are exposed to varying, but smaller, maximum levels of illumination. The greater the distance from the beam axis, the lower the integrated intensity incident on a particle and, hence, the less light flux removed from the beam, and the smaller the resulting pulse height. The response therefore consists of a continuous "spectrum" of pulse heights, ranging from a maximum value, for trajectories that pass through the center of the beam, to essentially zero (i.e. indistinguishable from noise fluctuations), for trajectories located very far from the incident beam ($|x|>>w$). The maximum pulse height depends on the beam waist, 2w, and the size of the particles, as well as in some cases the refractive indices of the particles and surrounding fluid. (This depends on the extent to which light scattering is significant relative to refraction and reflection in contributing to the overall light extinction signal.) A crucial assumption is that the particle trajectories are distributed randomly (i.e. occur with equal frequency) within the flow channel. This assumption is usually valid, given the typical dimensions of the flow channel and the relatively low flow rates utilized. It is also assumed that the number of particles passing through the sensor is sufficiently large that the statistical fluctuations in the number of particles having trajectories with any given x-axis value (i.e. over any (narrow) range of x values) can be ignored.

The relationship between particle size and pulse height for the sensor in a focused light scattering device is therefore radically different from that obtained for a

sensor of conventional design. In the latter case, irrespective of their trajectories, particles of a given size (and composition) give rise to pulses of nearly uniform height. This behavior is important for sensor design for the conventional SPOS method. The typically small variations in pulse height that occur, for example, when measuring polystyrene latex "standard" particles of essentially uniform size are caused by variations in the incident beam intensity within the OSZ along the x- and y-axes, for a given z-axis value. These variations ultimately determine the resolution of the sensor. The resulting width of the PSD is therefore mostly a consequence of residual non-uniformity of illumination across the OSZ, rather than an actual range of particle diameters.

By contrast, there is an obvious deterioration in the particle size resolution for sensor design for focused light scattering devices. When a single particle passes through the sensor, it gives rise to a light-extinction pulse with a height, ΔV_{LE} that can vary between a given maximum value and essentially zero. Conversely, given a single detected pulse, it is impossible to determine the size of the particle that has produced it, solely from knowledge of the pulse height. For example, a particle that is relatively small, but which passes directly through the beam axis, yields the maximum pulse height possible for a particle of that size (and composition). Alternatively, a particle that is much larger but which passes relatively far from the beam axis yields a pulse height that could actually be the same, depending on its size and trajectory. Even though the large particle is able to intercept a much larger area of incident illumination than the small one, the average intensity incident on it is smaller than the intensity incident on the small particle. Hence, the resulting pulse height could turn out to be the same as that produced by the small particle. Obviously, there are an infinite number of pairs, $\{d, |x|\}$, of particle diameters and minimum beam-trajectory distances that can give rise to the same pulse height. The particle diameter, d, and the resulting pulse height, ΔV_{LE}, are effectively "decoupled" from each other. This is the problem of "trajectory ambiguity", which for more than twenty years has motivated the search for new light-scattering based schemes for particle size determination using Gaussian beams.

The effects of trajectory ambiguity described above might present a difficulty in measuring the size of a single particle, or a relatively small number of particles. However, the apparently poor size resolution associated with the sensor used for focused light scattering devices can be restored to a very acceptable level by means of

appropriate mathematical deconvolution of the pulse-height data. The resulting dramatic improvement in the effective sensor resolution is possible by virtue of the fact that the sensor in a focused light scattering device is intended to be exposed to a large, statistically significant number of particles of every relevant diameter, or range of diameters, contained in the sample of interest. This is the circumstance that renders the new sensing method very useful for particle size analysis, in sharp contrast to the situation that holds for "contamination" applications. There, the sensor is exposed to relatively small numbers of particles of any given size, for which statistical significance is often not achieved.

The "raw" response of the sensor used in a focused-beam device, like its conventional SPOS predecessor, consists of the pulse height distribution (PHD)--a histogram of particle "counts" vs pulse height, $\Delta V_{I,E}$. The pulse-height scale is typically divided into a relatively large number (e.g. 32, 64 or 128) of "channels," or "bins," each of which encompasses an appropriately narrow range of pulse height voltages, thus defining the voltage resolution of the PH). It is usually convenient to establish channels that are evenly spaced on a logarithmic voltage scale. Measurement of a new pulse causes the number of particle counts stored in the appropriate pulse height channel in the histogram to be incremented by one. Data are ideally collected from the particle suspension of interest for a sufficiently long time that the resulting PHD becomes statistically reliable, and thus smooth and reproducible. This means that the number, N_I, of particle counts collected in the I-th pulse-height channel is statistically significant, dominating the fluctuations due to statistical "noise," for all I, e.g. for $1 \leq I \leq 128$, in the case of 128 channels. Assuming Poisson statistics, this means that $N_I >> N_I$, for all I.

Relatively high levels of particle concentration are possible because the sensor responds to only a small fraction of the total number of particles passing through it. For example, concentrations in the range of hundreds of thousands of particles/ml, in sample sizes of tens of mls, can be measured. That is, millions of particles can be present, a portion of which is passed through the beam of light and counted. The fraction of particles that are actually counted, relative to the number of particles present in the sample (Np), is known as phi_d, or "sensor efficiency," and is calculated by taking the ratio of the particles actually detected over the number of particles in the sample. The number of particles detected over the number of particles in the sample typically ranges from about 0.5 to about 5%.

The fact that the sensor efficiency is so relatively small is not surprising. In the case of a tightly focused beam, the width, a, of the flow channel is invariably much larger than the nominal width, 2w, of the focused beam. Therefore, most of the particles passing through the sensor are exposed to negligible levels of light intensity, because their trajectories are located so relatively far from the beam axis--i.e. $|x|>>w$. Consequently, only a small fraction of the total number of particles is able to "block" enough light to give rise to detectable pulses, relative to the prevailing noise level. The great majority of particles pass undetected through the sensor.

While this limitation may appear to be serious, in practice it is of little concern, for two reasons. First, the fraction, phi_d, of particles that produce detectable, measurable pulses will be fixed for a given sensor width, a, even though the value changes with particle diameter, d. Second, the new sensing method is intended for use in determining the particle size distribution (PSD) for samples that, by definition, are highly concentrated to begin with. Even following dilution, if required, the concentration of particles of any given size (i.e. within any (narrow) size range) is, by definition, relatively high. Assuming a suitable flow rate and data collection time, the resulting PHD will possess an acceptable signal/noise ratio, with a low level of statistical fluctuations. Hence, even though only a small fraction of the available particles will contribute to the raw data, the resulting PHD will be representative of the much larger number of particles in the sample that are ignored. Therefore, a reliable and accurate PSD, representative of.the entire sample, can be obtained from the "inefficient" sensor used in the focused light scattering devices described herein.

Several additional features of the PHD that can be obtained are noteworthy. First, as a consequence of the fact that the particle trajectories span a large range of $|x|$ values, passage of uniform particles through the sensor indeed results in a PHD containing a wide range of pulse heights. In this case, these range from the threshold of individual pulse detection (dictated by the prevailing r.m.s. noise level), roughly 5 millivolts (mV), to a maximum of approximately 326 mV for the nominal "end" of the distribution. (This excludes a small number of "outlier" pulses, due to agglomerates and over-size primaries that extend to 500 mV). Given the uniformity of the particles, this 65-fold range of pulse heights can only be ascribed to differences in particle trajectory. (To a first approximation, one can neglect variations in the beam width over the depth of the flow channel, as discussed earlier.)

Second, as expected, the PHD is highly asymmetric, skewed greatly in the direction of smaller pulse heights. Clearly, there are many particle trajectories that sample a large range of |x| values (and, hence, beam intensities), but only relatively few that probe the central portion of the Gaussian profile, where the intensity is substantially uniform. The PHD exhibits a broad, smooth upswing in the number of particles with increasing pulse height, accelerating to a relatively sharp peak, followed by a dramatic decline to the baseline, representing zero pulse events. This sharp "cut-off" at the upper end of the distribution defines the maximum pulse height, referred to hereafter as $^{M}\Delta V_{LE}$. The counts collected at this maximum value represent particles that passed through, or very close to, the center of the beam--i.e. trajectories with x approximately equal to 0--where the fraction of total incident light flux "blocked" by the particles is the largest value possible. The counts collected in smaller pulse height channels represent particles that passed further from the beam axis; the greater parameter |x|, the smaller the resulting pulse heights.

There is a relationship between the particle trajectory and the resulting pulse height. Trajectory "A" gives rise to extinction pulses having the maximum pulse height, $^{M}\Delta V_{LE}$, immediately preceding the upper cut-off of the PHD. Trajectories "B," "C," "D" and "E" located progressively further from the beam axis, give rise to pulses with correspondingly lower pulse heights and progressively lower numbers of particle counts. Eventually, the number of particle counts per channel approaches zero, as the pulse height reaches the detection limit (approximately equal to 5 mV).

The reproducibility of the PHD depends only on the degree to which the number of counts contained in the various channels is large compared to statistical fluctuations. Therefore, the "reliability" (i.e. the smoothness and reproducibility) of the PHD should depend on the total number of particles counted during a measurement. For a given particle size there will obviously exist a minimum number of pulses that should be counted and analyzed, below which the PHD should be expected to exhibit significant, irreproducible "structure" from one measurement to the next, due to statistical noise. Again, the PHDs produced by the new sensor have meaning only to the extent that relatively large, statistically meaningful numbers of particles of the same size are detected during the data collection period. Only if this is true can one expect to obtain optimal, reproducible PHD results, and correspondingly accurate, representative particle size distribution (PSD) results derived from the latter using the methods discussed below.

To confirm that the data measured is significant, one can overlay two or more PHDs taken from measuring the same sample in multiple runs.

Exposing the sensor to larger particles will yield a PHD that is shifted to larger pulse heights. Specifically, the maximum pulse height, ${}^{M}\Delta V_{LE}$, corresponding to particle trajectories passing through, or very close to, the beam axis, increases.

An LS-type sensor can be used in place of, or in addition to, an LE sensor. The LS-type sensor uses a light collection means--typically one or more lenses--in order to gather scattered light rays originating from individual particles passing through the OSZ, created by the incident light beam.

The lens system is designed to collect scattered light over a particular, optimal range of angles, typically encompassing relatively small angles of scattering. In the scheme shown in Figure 2, a mask 50 has been placed in front of the first collection lens. Mask 50 comprises an outer opaque ring 52 and an inner opaque area 54, which form a transparent ring 56. Mask 50 allows only light rays with scattering angles, theta, located within an imaginary annular cone defined by angles $theta_1$ and $theta_2$ (i.e. $theta_1 \leq theta_2$) to impinge on the first collection lens 62. Typically, this lens is centered on the axis of the incident beam, at an appropriate distance (i.e. its focal length) from the center of the flow channel, causing a portion of the diverging scattered light rays from the OSZ to be captured by the lens and become approximately collimated. A second lens 64 can then be used to focus the resulting parallel scattered rays onto a suitable (small-area) detector DLs. The resulting signal is "conditioned" by one or more electronic circuits, typically including the functions of current-to-voltage conversion and amplification.

There is a crucial difference between the signal, V_{LS}, created by this optical scheme and the signal, V_{LE}, produced by the LE-type sensor. Unlike the latter, the LS-type sensor, by design, prevents the incident light beam emerging from the back window of the flow cell from reaching the detector, D_{LS}. Instead, the incident beam is either "trapped" by means of a suitable small opaque beam "stop" (e.g., the inner opaque area 54) or deflected by a small mirror away from the lens that is used to collect the scattered light rays originating from the OSZ. Consequently, the relatively large "baseline" level, V.sub.0, necessarily present in the overall signal, V_{LE}, produced by the LE-type sensor is now absent from the LS signal, V_{LS}. Ideally, the new "baseline" signal level is zero--i.e. there should be no scattered light generated from sources within the OSZ in the absence of a particle. In practice, of course, there

will be some amount of background light caused by light scattered from the surfaces of the front and/or back windows of the flow channel, due to imperfections on, or contaminants attached to, the latter surfaces. In addition, there may be fluctuating background light due to scattering from small contaminant particles suspended in the diluent fluid. Also, for some samples there may be fluctuations in background light produced by a "sea" of ultra-fine particles which comprise a major fraction of the overall particle population, but which are too small to be detected individually.

When a particle of sufficient size passes through the OSZ, defined by the incident Gaussian light beam and front and back windows of flow channel 35, a momentary pulse occurs in the output signal produced by the detector, D_{LS}, and associated signal-conditioning circuit. In general, one might naively expect that the larger the particle, the greater the amount of light scattered by it, assuming the same trajectory, and therefore the greater the height of the signal pulse.

In practice, the actual pulse height depends not only on the size of the particle, but also its composition--specifically, its index of refraction (and that of the surrounding fluid) and absorbance, if any, at the incident wavelength. The pulse height also depends on the wavelength of the beam and the orientation of the particle as it passes through the OSZ, if it is not spherical and homogeneous. Finally, for particles comparable in size to, or larger than, the wavelength, the scattering intensity varies significantly with the scattering angle. Consequently, in this case the pulse height depends on the range of angles over which the scattered light is collected and measured.

The relationship between the scattered light "radiation pattern" (i.e. intensity vs angle) and all of these variables is described by classical Mie scattering theory, which takes into account the mutual interference of the scattered light waves within the particle. In general, the larger the particle, the more complex (i.e. non-isotropic) the angular dependence of the scattered intensity resulting from intra-particle interference. In order to optimize the response and performance of the LS-type sensor, one must confine the collection of scattered light to a range of angles, theta, for which the net integrated response, ΔV_{LE}, increases monotonically with the diameter, d, of particles of a given composition (i.e. refractive index) over the largest possible, or expected, size range. This requirement can usually be satisfied by choosing a range of relatively small angles, $theta_1<theta<theta_2$, close to the forward direction. In this way, one avoids "reversals" in the integrated scattering intensity with increasing particle

size due to variations of the intensity with changes in angle, especially significant at larger angles as a consequence of Mie intra-particle interference.

There are two properties of the signal, V_{LS}, produced by the new LS-type sensor that are qualitatively different from the properties of the signal, V_{LE}, produced by the corresponding LE-type sensor. First, the signal pulse caused by passage of a particle through the OSZ and the "overall" signal, V_{LS}, are essentially the same in the case of the LS-type sensor. The relatively high background signal level that accompanies the pulse of interest in the LE-type sensor is absent: (The same situation clearly holds for a conventional LS-type sensor).

Therefore, in the case of relatively small particles that give rise to pulses of low magnitude, the signal/noise ratio achieved in practice using the LS method should be significantly better than that realized using the LE method. This advantage becomes more important the smaller the particle and the weaker the resulting pulse, as the latter approaches the prevailing noise fluctuations. Another way of appreciating the inherent advantage of the LS method over its LE counterpart is to realize that the former is based on "detection at null." That is, quantitative detection of a pulse ideally is carried out in the presence of zero background signal. From a signal/noise perspective, this is in sharp contrast to the situation that obtains for the LE method, which requires high "common-mode rejection." The "common-mode" signal, V_0, is always present in the raw signal, V_{LE}, and must be subtracted, or otherwise suppressed, in order to extract the (often small) signal pulse of interest.

There is a second important and distinguishing property of the LS signal, V_{LS}. The signal/noise ratio associated with the measurement of ΔV_{LS} can in principle be improved by increasing the power of the incident light beam, so as to increase the light intensity incident on a particle at all points within the OSZ. Therefore, in principle one can reduce the lower size detection limit for the new LS sensor by increasing the power of the light source, as for a conventional LS sensor. Eventually, a lowest size limit will be reached, based on noise fluctuations associated with the suspending fluid and/or the light source and detection system. Of course, as discussed above, the lower particle size limit can also be improved for the new LS-type sensor by reducing the width, 2w, of the incident beam, assuming no change in the power of the latter. This action will obviously increase the maximum intensity incident on the particles that pass through the beam axis (x=0), and therefore the height of the largest resulting pulse for a particle of given size, as well. However, this method of

improving the sensitivity eventually reaches a point of diminishing return, due to limitations imposed by diffraction theory (establishing a minimum beam width) and excessive variation of the focused beam width over the depth, b, of the flow cell due to excessively-long depth of field.

By contrast, an increase in the power of the light source has relatively little effect on the lowest particle size that can be measured using the LE method. For example, a doubling of the power of the light source will result in a doubling of the baseline signal level (FIG. 2), to $2V_0$. The height of the pulse, ΔV_{LE}, produced by a particle of the same size and trajectory will also be doubled, assuming no change in the beam width. However, the root-mean-square magnitude of the noise fluctuations associated with the relatively high baseline signal level will typically also be approximately doubled, because these fluctuations are usually associated with the light source and therefore scale with the output power. Hence, one expects little or no improvement in the signal/noise level for the LE-type sensor. Consequently, there should be little or no reduction in the lower size detection limit achievable by the LE method as a consequence of increasing the power of the light source. An improvement will be realized only if the signal/noise ratio associated with the light source improves with increased power.

When uniform size particles flow through the new LS-type sensor, depending on their trajectories they are individually exposed to different values of maximum incident intensity, given by Equation 7, with r=x, z=0. (For simplicity, it can be assumed that the particles are much smaller than the beam width, so that every point in a given particle is exposed to the same intensity at any given time.) Therefore, as with the new LE-type sensor, the height, ΔV_{LS}, of the resulting pulse generated by a particle of given size depends on the distance, |x|, of closest approach (z=0) to the axis of the incident beam. The smaller the distance |x|, the larger the value of ΔV_{LS}. Hence, like its LE counterpart, the LS-type sensor generates a distribution of widely varying pulse heights, ΔV_{LS}, when a suspension of uniform particles passes through it at an appropriate flow rate. The shape of the resulting PHD bears a strong qualitative resemblance to the highly asymmetric shape of the PHDs obtained using the new LE method, exemplified in FIGS. 4, 6 and 7. That is, the number of pulse counts (y-axis) is relatively small at the smallest measurable pulse height just above the noise fluctuations) and rises with increasing pulse height, ΔV_{LS}. The pulse count value culminates in a peak value at a maximum pulse height, referred to as ${}^{M}\Delta V_{LS}$,

corresponding to particle trajectories for which |x|.apprxeq.0. Above ΔAV_{LS} the number of pulse counts ideally falls to zero, assuming that the particle concentration is below the coincidence concentration (discussed earlier) for particles of that size, so that at most one particle effectively occupies the OSZ at any given time. Of course, a PHD obtained using the new LS method usually pertains to particles that are smaller--often significantly so--than those used to generate a typical PHD using the new LE method.

As noted above, the shape of the PHD--number of pulse counts vs ΔV_{LS}--generated for uniform particles using the new LS method is qualitatively similar to the shape of the PHD obtained for uniform (typically larger) particles using the new LE method. Both kinds of PHDs share the distinguishing characteristic of a sharp "cut-off" following their respective peak number of pulse counts, coinciding with their maximum pulse height values, $^{M}\Delta V_{LS}$ and $^{M}\Delta V_{LE}$. However, it should be appreciated that there are quantitative differences in the shapes of the two kinds of d=1, notwithstanding their qualitative similarities, even for the same particle size--e.g. d=1 μm. The "front end" design of the new LS-type sensor--i.e. the focused light beam and relatively thin flow cell--is essentially the same as that utilized for the new LE-type sensor. Therefore, what distinguishes one type of sensor from the other concerns the means and manner of light detection and the type and magnitude of the response pulses generated by each method, even in the case of particles of the same size. For the new LS method, the response is due only to light scattering, and its magnitude, ΔV_{LS}, is proportional to the intensity of the light incident on the particle, all other relevant variables being the same.

By contrast, for the new LE method the magnitude of the response, ΔV_{LE}, is a more complex function of the intensity incident on the particle. First, the response is due to a combination of physical effects--refraction (and reflection) plus light scattering. However, the scattering phenomenon asserts itself in an "inverse" sense. That is, a small fraction of the incident light flux is removed from the beam before it reaches the detector. Second, over the typical size range for which the new LE method is applicable, there is a substantial variation in the incident intensity across the particle. Therefore, it should not be surprising that the fractional change of pulse height due to a given change in |x|, dependent on both particle size and trajectory, is generally different for the two methods. Similarly, the fractional change in pulse

height with particle diameter, dependent on both particle size and trajectory, is also generally different for the two methods.

The task of converting the "raw" data--the PHD--obtained from a sample of suspended particles into the object ultimately desired--the particle size distribution, or PSD, is described in detail below.

It is useful to compare this task conceptually with the operation required in the case of a conventional LE- or LS-type sensor. There, the height of the pulse due to passage of a particle through the OSZ is nearly independent of its trajectory, because the intensity of the incident beam is designed to be approximately constant across the flow channel (i.e. along the x-axis) for a given z-axis value (e.g. z=0). Consequently, particles of a given size ideally give rise to pulses of substantially the same height, and the resulting PHD is therefore, in effect, equivalent to the final desired PSD. There is a one-to-one correspondence between a given, measured pulse height, ΔV_{LE} (or ΔV_{LS}), and the particle diameter, d. If particles of a larger or smaller size pass through the sensor, the resulting pulse heights are larger or smaller, respectively. A "calibration curve," consisting of pulse height vs particle diameter, is all that is needed to obtain, by simple interpolation, the PSD from the PHD. Obtaining the raw PHD data using the conventional SPOS method is equivalent to determining the final, desired PSD.

By contrast, as discussed earlier, the response of the LE- (or LS-) type sensor is much more "convoluted." Even in the simplest case of particles of a single size, the resulting PHD consists of a broad spectrum of pulse heights, from the smallest values just above the prevailing noise fluctuations, to the maximum value, ${}^{M}\Delta V_{LE}$ (or ${}^{M}\Delta V_{LS}$), associated with that size. Therefore, in the typical case of particles of widely varying size, the resulting PHD consists of an even wider assortment of pulse heights. No longer is there a simple correspondence between pulse height and particle size. It is therefore no longer a simple, straightforward procedure to transform the set of particle counts vs pulse-height values contained in the PHD into the desired size distribution--particle counts vs particle diameter.

It typically involves three procedures to convert the PHD to the desired PSD. First, the raw PHD must be inverted, or deconvoluted, using a specialized mathematical algorithm. Its purpose is to convert the "wide-spectrum" PHD produced by the new LE- (or LS-) type sensor into a "sharp", idealized PHD, equivalent, in effect, to what would have been obtained using a conventional LE- (or LS-) type

sensor. Such an idealized, deconvoluted PHD--hereinafter referred to as the dPHD--has the property that all pulses of a given height, ΔV_{LE} (or ΔV_{LS}), belong exclusively to particles of a given size (assuming, always, particles of a given composition). The dPHD is equivalent to what would have been obtained if all of the particles contributing to the original PHD had passed through the center (axis) of the incident beam.

A second straightforward procedure is then carried out. A preliminary, or "raw", PSD is obtained from the dPHD by simple interpolation of the calibration curve that applies to the specific new LE- (or LS-) type sensor utilized--e.g. the curve shown in FIG. 8A. This procedure permits a one-to-one translation of each deconvoluted pulse height value in the dPHD into a unique particle diameter associated with this value, thus yielding the raw PSD. A third procedure is then needed to convert the raw PSD thus obtained into a final PSD that is quantitatively accurate. The number of particle counts in each diameter channel of the raw PSD is the number of this size that actually contributed to the measured PHD. As discussed above, this is typically only a small fraction of the total number of particles of the same size (i.e. within the size range defined by the diameter channel) residing in the volume of sample suspension that passed through the sensor during data collection. This fraction, phi_d, of particles actually detected by the new LE- (or LS-) type sensor varies significantly with the particle diameter, d.

The third procedure involves multiplying the number of particles contained in each diameter channel of the raw PSD by the value of $1/phi_1$ that applies for that channel. This operation yields the final, desired PSD, describing the number of particles of each size estimated to reside in the quantity of sample suspension that passed through the sensor during data acquisition. Values of $1/phi_d$ for each value of diameter, d, can be obtained from the sensor efficiency curve, phi_d vs d, by interpolation.

There are two independent algorithms presented herein for deconvoluting a measured PHD, to obtain the dPHD, hereinafter referred to as "matrix inversion" and "successive subtraction." Implementation of either procedure is based on the property that the response of the new LE- (or LS-) type sensor--like its conventional SPOS counterpart--is additive. Because the particles passing through the sensor give rise to signal pulses one at a time, the resulting PHD can be considered to be composed of a linear combination, or weighted sum, of individual PHDs corresponding to uniform

particles of various sizes, referred to as "basis vectors." (This term is well known in linear algebra.) Each of these basis vectors represents the response of the system to a statistically significant number of particles of a single, given size.

In one embodiment, the focused light scattering device described herein incorporates both the new LE- and LS-type SPOS sensors in a single sensor, having two independent output signals, V_{LE} and V_{LS}. The resulting dual "LE+LS" design offers increased capability and flexibility, providing single-particle counting and sizing over a relatively large range of particle sizes. The LS-type sensor subsystem can be used to extend the size range below the lower detection limit provided by the new LE-type sensor subsystem. The extent to which the lower particle size limit can be extended depends on a variety of parameters. These include: the width, 2w, of the narrow (typically focused) beam within the measurement flow cell; the power of the light source; the range of angles over which scattered light is collected for implementation of the new LS-type sensing function; and the physical properties, including the refractive index, of both the particles and the suspending fluid.

The dual LE+LS sensor includes a light source, preferably consisting of a laser diode module, typically having an output wavelength in the range of 600 to 1100 nanometers (nm). The beam produced by the light source means preferably is collimated (parallel) and "circularized"--i.e. the intensity is a function only of the distance, r, from the central axis. Furthermore, the beam preferably has a Gaussian intensity profile, along any axis normal to the axis of propagation of the beam. The new LE+LS sensor also includes a focusing means, typically a single- or multi-element lens, capable of focusing the starting collimated light beam to the desired beam width, 2w, at the center of the measurement flow channel in the OSZ, consistent with the desired particle size range. It is assumed that the focusing means has an appropriate focal length, thus yielding acceptable values for both the width and depth of field of the focused beam. The latter is preferably significantly longer than the thickness, b, of the flow channel, in order to optimize the resolution of the resulting PSD.

A measurement flow cell is typically fabricated from a suitable transparent material, such as glass, quartz or sapphire, or alternative semi-transparent material, such as PTFE (e.g. Teflon™, manufactured by DuPont) or other suitable plastic that is sufficiently transparent at the operating wavelength and compatible with the fluid-particle mixture. A suitable fluidics system, including a flow pump means and

optional means for automatic dilution of the starting sample suspension (if needed), are typically required to facilitate the steady flow of the particle-fluid suspension through the flow cell. The flow rate, F, is usually chosen to be the same as, or close to, the value used to generate the calibration curve for the LE- or LS-type sensor.

The thickness, b, of the flow channel should be small enough to achieve a high coincidence concentration limit and as uniform a beam width as possible (ideally with b<<depth of field), resulting in improved resolution for the final PSD. However, it must be large enough to prevent frequent clogging by over-size particles (e.g. agglomerated primaries and contaminants in the fluid/diluent). The width, a, of the flow channel is also chosen to strike a compromise between two competing effects. A relatively large value reduces the impedance to the flowing fluid-particle mixture and lowers the velocity (and increases the pulse width) for a given flow rate, F. However, the larger parameter a, the smaller the sensor efficiency, phi_d, for any given particle diameter, d. This results in a smaller fraction of particles in the sample actually contributing to the measured PHD and final PSD, which, if too small, may be undesirable.

The new LE+LS sensor contains two separate light collection and detection subsystems, used independently to extract the desired LE- and LS-type signals. The LE-type signal can be captured using a small light reflecting means M (e.g. mirror), positioned so as to intercept the narrow beam of incident light after it passes through the flow cell and fluid-particle mixture. The resulting transmitted beam, thus deflected away from the optical axis of the combined sensor, is caused to impinge on a nearby light detection means D_{LE}. The latter typically consists of a small-area, solid-state (silicon) detector, operating in a linear region and having a spectral response that is matched to the wavelength of the light source, thus providing an output signal with an acceptable signal/noise (S/N) ratio. The output of the detector means is typically a current (the "photocurrent"), which can be conditioned by a current-to-voltage converter ("transimpedance" amplifier), yielding an output signal in the generally desired form of a time-varying voltage, $V_{LE}(t)$.

Alternatively, a small detector element can be placed directly in the path of the light beam after it emerges from the flow cell, thus eliminating the need for the intermediate light reflecting means discussed above. Regardless of whether a mirror or detector element is used to "capture" the transmitted light beam, there are two requirements. First, the means used must function as an effective beam "stop." That is,

it must be able to prevent any significant fraction of the arriving light flux from being reflected back toward the flow cell, thus becoming a source of "stray" light. Through unintended internal reflections from the various optical surfaces, a portion of the stray light can find its way to the scattering detection means D_{LS}, thus corrupting the resulting LS signal, by contributing a portion of the incident intensity to the latter. Second, the means used to capture the LE signal must be small enough not to intercept, and therefore block, scattered light rays at any angles that are intended to be captured and redirected to the light detection means D_{LS}, as discussed below.

Separately, scattered light originating from particles passing through the OSZ is collected over a range of scattering angles, theta, with $theta_1<theta<theta_2$, where angles $theta_1$ and $theta_2$ are defined by a suitable aperture means, such as an annular mask fabricated from a photographic negative with an outer opaque portion, a transparent intermediate portion, and an inner opaque portion. The scattered rays selected by the mask are allowed to impinge on a collecting lens of appropriate focal length and location, which converts the diverging scattered rays into an approximately parallel beam. A second lens is then typically used to refocus the rays onto a relatively small light detection means D_{LS}. As in the case of the LE subsystem, the output signal of D_{LS} is typically a current, which can be optionally conditioned, typically by means of a transimpedance amplifier, so that the final output is in the form of a time-varying voltage, $V_{LS}(t)$.

The signals $V_{LE}(t)$ and $V_{LS}(t)$ can be organized into respective pulse height distributions PHD by pulse height analyzers. The PHDs are then respectively deconvoluted in computer deconvolution means, which ultimately compute a pair of respective particle size distributions ("PSD").

This embodiment can be implemented as an LE-type or LS-type sensor only, simply by removing (or not installing in the first place) the optical elements, detection means and signal conditioning circuitry associated with the unwanted subsystem. In this case, it may be useful to adjust the width, 2w, of the focused beam within the measurement flow channel, in order to optimize the resulting performance of the LE- or LS-type sensor. This parameter will impact the usable particle size range, coincidence concentration limit and minimum detectable particle size differently for the two sensing modes, as discussed earlier.

Hydrodynamic Sample Injection

In one embodiment, the apparatus uses hydrodynamic sample injection, such as is described in Pelssers et al., Journal of Colloid and Interface Science, Volume 137, Issue 2, July 1990, Pages 350-361. Colloidal dispersions, such as platelets in serum or other media, can be hydrodynamically focused laser. into a narrow stream, with widths ranging from about 3 to about 10, preferably about μm width. The use of a focused light scattering technique allows one to measure relatively small particle sizes. However, where a focused beam hits particles in a relatively wide sample stream (as described above, where the beam is narrower than the measurement flow channel in the width direction), the detection method relies somewhat on statistics. That is, where the beam is substantially narrower than the sample stream, an assumption is made that there is an equal distribution of particles in the sample stream, so that one can extrapolate the results of the interaction of the light in the narrow beam with the particles in its path over the entire width of the sample stream. By hydrodynamically focusing the sample into a stream with a relatively narrow width, and using a focused light source, it is possible to count all or most of the particles in the sample stream, and rely to a lesser extent on statistics.

A representative injector for carrying out hydrodynamic injection is shown in more detail in Figure 3. As shown in Figure 3, As shown in Figure 3, a sample passes through an inlet port (100), through a fluid sheath (110), and outward through the bottom of the injector into a flow cell (120). The sample travels through the sample injection tube, with fluid from a fluid sheath surrounding the stream, and hydrodynamic focusing within the flow cell forcing particles into a single-particle-file stream where laser light intercepts the stream at a sample interrogation point. The design of the flow cell, when a hydrodynamic injector is used, permits particles to flow through the center of the flow cell. Increasing the sample pressure increases the core diameter and the flow rate.

The device described herein is similar to the device disclosed in U.S. Patent Application Publication No. 20040011975, the contents of which are hereby incorporated by reference in its entirety. The main differences between the device disclosed in U.S. Patent Application Publication No. 20040011975 and the instant

device are that the instant device further includes a hydrodynamic injector, or additional lasers and detectors, such as detectors that can detect fluorescent labels.

The apparatus is described therein is useful in performing particle analysis using focused light scattering techniques. However, as described herein, other similar apparatus can be employed, including detectors for focused light scattering and/or light extinction.

The principal defining characteristic of the focused light scattering method described herein is not simply a significant reduction in the size of the illuminated area, A_0, resulting in a significant reduction in V_{OSZ} and improvement in sensitivity. Rather, it concerns the nature of the illuminating beam and the resulting OSZ thereby defined.

The term "focused light scattering" refers to a method for sensing single particles, suspended in a solution, when the solution is passed through a focused beam. When the beam passes through the solution without being scattered by a particle, the beam passes on to a photodetector and the intensity is measured. When the beam is scattered, in whole or in part, by a particle, the intensity of the beam hitting the photodetector is altered. The particle size and concentration can be calculated, for example, using light-extinction, light-scattering detection, or both.

In one embodiment, the beam is produced by a laser. The laser beam interacts with the particles, and produces scattered light when the laser beam interacts with a particle. In one aspect of this embodiment, the apparatus includes two or more different lasers, which can give off light at two or more different wavelengths, and/or which can interact with the particles at different angles. The use of light at different wavelengths can enable one to identify specific epitopes. Particles can interact with specific molecules, including fluorescently-labeled molecules, and the fluorescence can be detected using a laser with light at a predetermined wavelength that interacts with the fluorescent label. The use of more than two lasers can enable the use of two or more fluorescent labels, which labels fluoresce at different wavelengths. This technique is described in more detail below.

A beam of light (usually laser light) of a single wavelength is directed onto a stream of a fluid, in one embodiment, a hydrodynamically-focused stream of a fluid, which includes particles whose size and number is to be determined.

A number of detectors are aimed at the point where the stream passes through the light beam. In one aspect of this embodiment, one detector is in line with the light beam (Forward Scatter or FSC) and one or more detectors are perpendicular to it, including Side Scatter or SSC detectors and one or more fluorescent detectors. Each suspended particle passing through the beam scatters the ray, and fluorescent chemicals either present within the particle or attached to the particle are excited into emitting light at a longer wavelength than the light source. This combination of scattered and fluorescent light is picked up by the detectors, and, by analyzing fluctuations in brightness at each detector (one for each fluorescent emission peak), it is then possible to derive various types of information about the physical and chemical structure of each individual particle.

Thus, in some embodiments of the apparatus described herein, there are three or more detectors. For example, one can include one detector for extinction [useful for measuring particles with a size > 0.7 microns], one for scattered light [useful for measuring particles with a size between 0.15 and 0.7 microns] and one or more for determining fluorescence [used for phenotyping].

xaxv

Light Sources

The sources of light can include lamps (i.e., mercury, xenon); high-power water-cooled lasers (i.e., argon, krypton, dye laser); low-power air-cooled lasers (i.e., argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (i.e., blue, green, red, violet). The detectors can convert fluorescence signals from light into electrical signals that can be processed by a computer.

Data Acquisition

The process of collecting data from samples is termed "acquisition." Acquisition is typically mediated by a computer physically connected to the apparatus, and the software which handles the digital interface with the apparatus. The software is capable of adjusting parameters (i.e. voltage, compensation, etc.) for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to insure that parameters are set correctly. An interactive

database can allow the apparatus to be used in applications for both clinical and research purposes. A wide variety of analysis software and fluorescently-labeled antibodies has been developed, and are well known to those of skill in the art.

The apparatus can include multiple lasers (between 2 and 5, typically between two and four) and fluorescence detectors (typically between 2 and 18, more typically between 2 and 10). Increasing the number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers.

Gating

The data generated by the apparatus can be plotted in a single dimension, in two dimensions, or even in three dimensions. The regions on these plots can be sequentially separated, based on fluorescence intensity, by creating a series of subset extractions, termed "gates." Specific gating protocols exist for diagnostic and clinical purposes especially in relation to hematology.

The plots are often made on logarithmic scales. Because different fluorescent dyes' emission spectra overlap, signals at the detectors have to be compensated electronically as well as computationally. Data accumulated using the apparatus can be analyzed using software, e.g., Flowjo, FCS Express, VenturiOne or CellQuest Pro. Data analysis can be performed on a separate computer, if desired.

Computational analysis

Automated population identification using computational methods can be used as an alternative to traditional gating strategies. Automated identification systems can potentially help find rare and/or hidden populations. Representative automated methods include FLOCK in Immunology Database and Analysis Portal (ImmPort), FLAME in GenePattern and flowClust, in Bioconductor.

Microfluidics

In some applications, particularly clinical applications, it can be desirable to use microfluidics to introduce samples to the apparatus. The microfluidic device can be disposable (i.e., used once or perhaps a few times, followed by disposal and replacement) and/or composed of a polymeric material. The microfluidic device can be adapted to reduce the amount of sample used to determine whether a patient can

benefit from a particular therapy. The microfluidic device preferably provides a tip adapted for delivering the biological sample including the platelets into the cell through which light passes, so that the platelets can then travel through the light beam(s). In some embodiments, the tip is adapted for sheath spraying. In other embodiments, the tip is adapted for non-sheath spraying. In any of the embodiments herein the apparatus may include a disposable inlet capillary.

Auto-Diluters

The apparatus can also include an autodiluter, which can start with the most dilute sample, rather than the most concentrated sample, and can therefore use less sample. Autodiluters are well known to those of skill in the art. Representative autodiluters include the AutoDiluter-5.2, the CETAC ADX-500 Autodiluter, the ProLiquid AutoDiluter, and the DYNATECH Autodiluter III.

Mechanical Shearing Devices (For EQELS and/or ISADE Devices)

Certain biological and non-biological species require mechanical shear for activation. Species may be a cell, a protein, ribo- or deoxyribonucleic acid, polysaccharides, aggregated cells or molecules or the like. Representative examples of cells include, but are not limited to, endothelial cells and platelets, and representative examples of molecules include von Willebrand factor and DNA. Both biological and non-biological species may aggregate to exhibit an effect on the mechanical properties of a fluid. Examples would be thixotropy (shear thinning) and rheopexy (shear thickening). Biological cells, molecules like fibrin, vWF, tubulin, myosin, and the like, and non-biological materials, like paints or inks, are examples.

The provision of mechanical shear can be accomplished by integrating a mechanical shearing device into the apparatus described herein. Although there are many means for introducing mechanical shear, any one of which can be used, in one embodiment, a suitable device is shown in Figure 4. In this device, a sample is placed in a suitable container. The sample in the container may then be extricated from the container manually, or by a robotic and then introduced into a pump. In one aspect of this embodiment, the pump is a precision pump that can produce a precise and continuous flow rate of the sample within the shearing device (200). The shearing device (200) is composed of a hollow fiber that may be linear or coiled, though in the embodiment shown in Figure 4, it is coiled. In one aspect of this embodiment, the

hollow fiber is composed of materials that minimize interaction with the sample or under certain other cases or circumstances may interact with the sample in a specific manner. For example, the inner surface of the hollow fiber may be coated with collagen that interacts with vWF or with platelets. The length of the hollow fiber and the inter diameter of the hollow fiber can be precisely known. Further, the pressure drop across the fiber can be precisely known, from the flow rates, volume flux, and/or from pressure sensors located at each end of the hollow fiber. From these data, the mechanical shear rate experienced by the sample can be calculated. The effluent sample can then be passed on to the scattering chamber of EQELS or the hydrodynamic injector of the ISADE device described herein by means of a linking hollow fiber. In this manner, the entire system is closed, and the sample can pass in a continuous manner from the original sample container though the shearing device and into the respective scattering chambers or hydrodynamic injector, then into an effluent chamber via an exit port (210).

In use, the sample follows a path from a storage device, via a pump (310) controlled by robotics (300) through a shearing device (320), into a cell where scattered light can be detected (330), and then to an effluent container (340). Although a scattering chamber of an EQELS device is shown in Figure 5, when the shearing device is used in connection with the hydrodynamic injector of an ISADE device as described herein, the hydrodynamic injector can substitute for the scattering chamber shown in Figure 5.

In an alternative embodiment, shear can be provided using ultrasound; vibration; radiowaves; cone-plate, parallel plate or coutte shearing surfaces; a coaxial plunger device and the like).

Robotics

In high-throughput screening, it can be preferable to include robotics, which can introduce the samples to the apparatus. Ideally, the apparatus can then be cleaned in between samples, for example, by flushing the various lines, and subsequent samples introduced, enabling the screening to be automated. Information on the screening results can be stored, for example, in a memory map, and the information correlated with the patient's identity.

A "focused light scattering device" is a single-particle optical sensor, which has high sensitivity and responds to relatively concentrated suspensions, uses a relatively narrow light beam to illuminate an optical sensing zone non-uniformly. It differs from conventional single particle optical sizing (SPOS) devices in that it can handle more concentrated solutions and smaller particle sizes.

In use, a solution including suspended particles passes through a zone. The zone is smaller than the flow channel, so that the sensor responds to only a fraction of the total number of particles flowing through the channel, detecting a statistically significant number of particles of any relevant diameter.

Because different particle trajectories flow through different parts of the zone illuminated at different intensities, it is necessary to deconvolute the result. Two methods of deconvolution can be used: modified matrix inversion or successive subtraction. Both methods use a few basis vectors measured empirically or computed from a theoretical model, and the remaining basis vectors are derived from these few. The sensor is compensated for turbidity.

The sensor apparatus for single-particle optical sizing of particles in a fluid suspension typically includes a means for establishing flow of the suspension through a physically well-defined measurement flow channel. There is also an illumination means for effectively directing a relatively narrow beam of light, having an axis, through the measurement flow channel to form an optical sensing zone within the measurement flow channel. The beam of light and the optical sensing zone are of such size relative to the size of the measurement flow channel that the sensor apparatus responds to only a fraction of the total number of particles flowing through the measurement flow channel. In this manner, the sensor apparatus responds effectively to a relatively concentrated fluid suspension.

The beam has a maximum intensity portion and a continuum of lesser intensities for positions spaced transverse to the axis from the maximum intensity portion. In this manner, some of the particles have trajectories through the maximum intensity portion, others of the particles have trajectories through the lesser intensity positions, and still others of the particles may have trajectories outside the zone. Typically, the maximum intensity portion of the beam is in a central portion of the beam.

The device also includes a detector means for photo-detecting light from the zone to provide pulse height signals. These signals each respond to a particle flowing

through the zone. The pulse height signals are functions of the sizes and trajectories of detected particles. Particles of a given size provide a maximum pulse height signal when flowing through the maximum intensity portion, and lesser pulse height signals when flowing through the lesser intensity positions of the zone. The pulse height signals, collectively, form a pulse height distribution PHD.

The device further includes a means for mathematically deconvoluting the pulse height distribution to extract a particle size distribution of the PSD particles in the fluid suspension. The sensor apparatus can detect a statistically significant number of particles of any given diameter or range of diameters that are relevant to the fluid suspension.

In one embodiment, the measurement flow channel has a thickness dimension axially of the beam of light, a width dimension transverse to the beam, and a flow direction substantially perpendicular to the thickness and width dimensions. The beam is narrower than the measurement flow channel in the width direction. The beam can be focused with a depth of field which is substantially larger than the thickness dimension, and the beam substantially has an effective width which does not vary substantially over the thickness dimension.

In another embodiment, the beam has an effective width between opposing positions transverse to the axis in the beam, at which the lesser intensities have fallen to a given fraction of the maximum intensity. The effective width is chosen so that the largest particles of interest can be effectively sized. The given fraction can be, for example, $1/e^2$ of the maximum intensity, where e is the base of the natural system of logarithms, and the effective width is substantially one half the size of the largest particle to be sized.

The light beam can have, for example, a Gaussian intensity profile, a circular cross-section, or an elliptical cross-section being wider in a direction transverse to particle flow.

The detector means can be include a light extinction-type detector, and can be a combination of detectors, for example, a light-extinction detector type and a light-scattering type detector. The light-scattering type detector means can include means for passing a portion of scattered light from the zone through a mask to select light scattered between a first and a second angle to the beam and a means for directing a portion of the light transmitted through the zone to a light-extinction type detector.

The detector means can include a mirror for deflecting a portion of the light from the optical-sensing zone to the light-extinction detector. The illuminating means can include a light source and optical fiber means for conveying light from the light source to the optical sensing zone, and projecting the light through the zone.

The detector means can also include an optical fiber means for conveying the light passing through the optical sensing zone to the light-extinction type detector. The detector means can also include means for passing a portion of the light scattered from the zone through a mask, to select light scattered between a first and second angle to the beam, and an optical fiber means for conveying the portion of the light to a light-scattering type detector. The detector means can also include a light-scattering detector.

In one embodiment, the illumination means provides two light beams directed through a pair of optical sensing zones positioned within the measuring flow channel, and each beam has an effective width determined by a desired maximum particle size.

The detector means can include a light-scattering detector and a means for passing light scattered from the zone through a mask means. The mask means can include a plurality of masks and means for selecting one of the masks for passing the light scattered from the zone, each mask defining different angles between which the light is scattered. The masks can be located on a rotatable wheel, and a mask can be selected by rotating the wheel to a desired position.

The illuminating means can project a relatively wide collimated beam through the optical sensing zone, and can include an acceptance aperture to capture only those light rays that closely surround the axis of the beam. This reduces the effective width of the beam to a width in a direction transverse to the axis of the light beam which is substantially one-half the size of the largest particle to be sized. The illuminating means can also include a means for coupling the light rays to the detector means. This can be, for example, an optical fiber means.

In one aspect of the invention, a statistically significant number of particles of each relevant size flow through all portions and positions of the zone.

In another aspect of the invention, the fluid suspension is relatively concentrated and the apparatus further comprises means to compensate for turbidity of the suspension. In this aspect, the detector means can operate on a light extinction principle, and provide a signal having a baseline voltage level. The pulse height

signals appear as downwardly extending pulses from the baseline voltage level, and the means for compensation for turbidity of the suspension can include means to sense the baseline voltage level and automatically increase the level to approximately the baseline voltage level present in the absence of turbidity in the suspension. The detector means can operate on a light extinction principle, and provide a signal having a baseline voltage level, wherein the means to compensate for turbidity can include a computer means for correcting the pulse height signals in response to the ratio of the baseline voltage level when the fluid suspension is not turbid, to the baseline voltage level for the turbid fluid suspension.

The detector means can also operate on a light extinction principle and provide a signal having a baseline voltage level, wherein the means to compensate for turbidity includes a means to adjust the intensity of the beam of light by increasing the amount of light produced by the illuminating means in response to the ratio of the baseline voltage level when the fluid suspension is not turbid, to the baseline voltage level for the turbid fluid suspension.

The particle trajectories can be substantially uniformly distributed across the width of the measurement flow channel.

Deconvoluting the Pulse Height Distribution

The means for deconvoluting the pulse height distribution can include basis vectors, each corresponding to a particular particle size, and a source vector representing a measured pulse height distribution for a fluid suspension as detected by the detector means. There can also be a means using a deconvolution algorithm to derive the particle size distribution from the pulse height distribution. At least some of the basis vectors can have values based upon measurements of particles of known size. Some of the basis vectors can also have values based upon measurements of particles of known size and others of the basis vectors can be computed from the sum of the basis vectors by interpolation and/or extrapolation.

The basis vectors can be computed, and the basis vectors can be column basis vectors of a matrix, where the means using a deconvolution algorithm performs matrix inversion and vector multiplication, or the means using a deconvolution algorithm can perform successive subtraction.

The means using a deconvolution algorithm can provide a deconvoluted pulse height distribution dPHD, and the apparatus further comprises means providing a

calibration curve of the relationship of pulse height and diameter, and means using the calibration curve to transform each deconvoluted pulse height value in the dPHD into a unique particle diameter associated with this pulse height value. This can yield a "raw" particle size distribution PSD. There can also be a means for converting the raw PSD into a final PSD by renormalizing the raw PSD by multiplying by the value $1/PHI_d$, where PHI_d is the fraction of particles actually detected by the device for particles of each size.

The particle trajectories can be distributed non-uniformly across the width of the measurement flow channel, and the basis vectors can be based upon the response of particles of known size flowing through the measurement flow channel with the same non-uniform distribution of particle trajectories as the fluid suspension.

The sensor apparatus may respond only to a fraction of the total number of particles flowing through the measurement flow channel.

One can prepare a matrix for deconvoluting pulse height distributions derived from particles of unknown size flowing along different trajectories through a non-uniform light field of a single-particle optical sizing device. This can enable one to size particles in a fluid suspension. To do this, one can determine the value of at least one empirical basis vector for the matrix by measuring the response of particles of known size flowing through the single-particle optical sizing device. Then, one can compute other basis vectors for the matrix corresponding to particles of other sizes, by interpolating and/or extrapolating the other basis vectors from the empirical basis vector.

One can also determine the value of additional empirical basis vectors for the matrix by measuring the response of particles of known sizes flowing through the single-particle optical sizing device, and computing the other basis vectors for the matrix corresponding to particles of other sizes from the at least one empirical basis vector and the additional empirical basis vectors.

Another way to prepare a matrix for deconvoluting pulse height distributions derived from particles of unknown size flowing along different trajectories through a non-uniform light field of a single-particle optical sizing device for sizing particles in a fluid suspension involves determining the value of at least one computed basis vector corresponding to particles of at least one size for the matrix. One can compute other basis vectors for the matrix corresponding to particles of other sizes from computed basis vectors.

Also disclosed is a method of deconvoluting a pulse height distribution derived from particles of unknown size flowing along different trajectories through a non-uniform light field of a single-particle optical sizing device for sizing particles in a fluid suspension. The method involves setting up a matrix having a plurality of columns, each containing a basis vector comprising a pulse height distribution of particles of a known size corresponding to the response of a photo-detector of the device to the particles of known size. Each successive column contains a basis vector for particles of successively larger sizes. The matrix also has a like plurality of rows, each corresponding to a successive pulse height channel, each channel including a range of pulse heights, with successive rows corresponding to successively larger pulse heights, and with each column having a maximum count pulse height value at a location for a row which relates to a pulse height corresponding to the particle of known size associated with the column. The maximum count pulse height values for successive columns are arranged on a diagonal of the matrix. The matrix is modified by setting all terms corresponding to pulse height values greater than the maximum count pulse height value in a column to zero. A deconvolution algorithm is used to perform matrix inversion and vector multiplication of the pulse height distribution and the matrix as modified.

Before the modifying step, one can renormalize the values of the basis vectors in the columns by setting the maximum count pulse height value to equal 1.0 and all other count pulse height values in the column to a value maintaining the same proportionate value to 1.0 that the other count pulse height values had to the maximum count pulse height value of the column.

The response of the photo-detection to the particles of known size is developed empirically for some of the basis vectors by sending particles of the substantially known size through the device and providing a response by the device to the particles of known size. The response to the photo-detector for the remaining basis vectors can be computed by interpolating and/or extrapolating the response for the remaining basis vectors from the some of basis vectors.

The response of the photo-detector to the particles of known size can be computed for some of the basis vectors and the response to the photo-detector for the remaining basis vectors can be computed by interpolating and/or extrapolating the response from the some basis vectors.

A pulse height distribution ("PHD") can be derived from particles of unknown size flowing along different trajectories through a non-uniform light field of a single-particle optical sizing device for sizing particles in a fluid suspension can be deconvoluted by setting up a matrix having a plurality of columns. Each column includes a basis vector comprising a pulse height distribution of particles of a substantially known size corresponding to the response of a photo-detector of the device to the particles of known size, and each successive column contains a basis vector for particles of successively larger sizes. The matrix can also include a like plurality of rows, each corresponding to a successive pulse height channel, each channel including a range of pulse heights, successive rows corresponding to successively larger pulse heights, each column having a maximum count pulse height value at a location for a row which relates to a pulse height corresponding to the particle of known size associates with the column. The maximum count pulse height values for successive columns can be arranged on a diagonal of the matrix. A successive subtraction algorithm can be implemented, by starting with the basis vector with its maximum count value in the largest row number; scaling a column basis vector by a factor corresponding to the value of the row in the PHD that matches the column number; subtracting the scaled basis vector from the PHD to form an element of a deconvoluted PHD (dPHD), leaving an intermediate PHD vector with a smaller number of total particles; and repeating this process using the remaining basis vectors until the entire PHD has been consumed and all the elements of the deconvoluted dPHD have been formed.

A single-particle optical sizing sensor for sizing particles in a relatively concentrated fluid suspension sample for turbidity of the suspension sample can be compensated using a sensor operating on a light extinction principle whereby a photo-detector produces signal $V_{LE}(t)$ having a baseline voltage level and a response to blockage of light by a particle as a downwardly extending pulse from the baseline voltage level. The compensation method involves passing a non-turbid suspension through the sensor; measuring a baseline voltage level V_0 produced in response to the non-turbid suspension; passing the relatively concentrated suspension sample through the sensor; measuring a baseline voltage V_0^T produced in response to the relatively concentrated suspension sample, calculating the ratio $V_oV_0^T$; and adjusting the sensor in response to G to compensate for the turbidity when the relatively concentrated suspension sample passes through the sensor.

The baseline voltage V_0^T can effectively be subtracted from the signal $V_{LE}(t)$, the remaining signal can be inverted to produce a pulse height signal 2 $V_{LE}^T(t)$, and an adjustable gain amplifying means can be used to amplify the pulse height signal 3 $V_{LE}^T(t)$. The adjustable gain amplifying means can be controlled by the ratio G to provide a compensated pulse height signal $\Delta V_{LE(t)}$.

The signal $V_{LE}(t)$ produced by the sensor in response to the relatively concentrated suspension sample can be amplified by adjustable gain amplifier means, the gain of which is controlled by the ratio G to provide a compensated signal $V_{LE}(t)$ having a compensated baseline voltage V_0, subtracting the baseline voltage V_0 from the compensated signal $V_{LE}(t)$, and inverting the remaining signal to produce compensated pulse height signal $\Delta V_{LE}(t)$.

In one embodiment, the single-particle optical sizing sensor comprises a light source producing a light beam of adjustable intensity, wherein the intensity is increased in response to the ratio G to compensate for the turbidity.

Particles in a fluid suspension can also be optically sized by establishing a flow of the suspension through a physically well-defined measurement flow channel of a single-particle optical sizing sensor apparatus wherein a beam of light, having an axis, is directed through the measurement flow channel to form an optical sensing zone within the measurement flow channel. The beam of light and the optical sensing zone are ideally of such size relative to the size of the measurement flow channel that the sensor apparatus responds to only a fraction of the total number of particles flowing through the measurement flow channel. The sensor apparatus can respond effectively to a relatively concentrated fluid suspension. The beam can have a maximum intensity portion in the beam and a continuum of lesser intensities for positions in the beam spaced transversely from the axis, whereby some of the particles have a trajectory through the maximum intensity portion, others of the particles have trajectories through the lesser intensity positions, and still others of the particles may have trajectories outside the zone. Light from the zone can be detected to provide pulse height signals, each responsive to a particle flowing through the zone. The pulse height signals are functions of the sizes and trajectories of detected particles, and the pulse height signals collectively form a pulse height distribution PHD. The PDH can be mathematically deconvoluted and processed to extract from the PHD a particle size distribution PSD of the particles in the fluid suspension.

The step of mathematically deconvoluting the PHD can involve determining the value of at least one empirical basis vector by measuring the response to particles of known size flowing through the single-particle optical sizing device. Other basis vectors corresponding to particles of other sizes can be computed by interpolating and/or extrapolating the other basis vectors from the empirical basis vector.

The value of additional empirical basis vectors for particles of known sizes flowing through the single-particle optical sizing device can be determined; and the other basis vectors for the matrix corresponding to particles of other sizes can be calculated by interpolating and/or extrapolating the other basis vectors from at least one empirical basis vector and the additional empirical basis vectors. The method can further involve determining the value of at least one computed basis vector corresponding to particles of at least one size. Other basis vectors corresponding to particles of other sizes can also be computed by interpolating and/or extrapolating the other basis vectors from computed basis vectors.

The step of deconvoluting and processing the PHD can involve setting up a matrix having a plurality of columns, each containing a basis vector comprising a pulse height distribution of particles of a known size corresponding to the response of a photo-detector of the device to the particles of known size, each successive column containing a basis vector for particles of successively larger sizes. The matrix can also have a like plurality of rows, each corresponding to a successive pulse height channel, each channel including a range of pulse heights, successive rows corresponding to successively larger pulse heights, each column having a maximum count pulse height value at a location for a row which relates to pulse heights corresponding to the particle of known size associated with the column. The maximum count pulse height values for successive columns can be arranged on a diagonal of the matrix. The matrix can be modified by setting all terms corresponding to pulse height values greater than the maximum count pulse height value in a column to zero. A deconvolution algorithm can be used to perform matrix inversion and vector multiplication of the pulse height distribution and the inverted matrix as modified. The response of the photo-detector to the particles of known size can be developed empirically for some of the basis vectors by directing a flow of particles of the known size through the device and providing a response by the device to the particles of known size. The response to the photo-detector for the remaining basis

vectors can be calculated by interpolating and/or extrapolating the response for the remaining basis vectors from the some of basis vectors.

The step of mathematically deconvoluting the PHD can also involve using a deconvolution algorithm to provide a deconvoluted pulse height distribution dPHD. The method can further involve providing a calibration curve of the relationship of pulse height and diameter, and using the calibration curve to translate each deconvoluted pulse height value in the dPHD into a unique particle diameter associated with this pulse height value yielding a "raw" particle size distribution in PSD. The raw PSD can be converted into a final PSD by renormalizing the raw PSD by multiplying by the value $1/PHI_d$, where PHI_d is the fraction of particles actually detected by the device for particles of each size.

II. Particles that can be Detected

Using the techniques described herein, various biological particles can be detected. Cells are one type of biological particle that can be detected. The method can be used to determine the presence or absence of a specific type of cell in a given solution. For example, a sample of blood, urine, spinal fluid, and the like can be evaluated for the presence or absence of bacteria, fungi, viruses, and the like. The particle size, and, optionally, particle shape, can also provide information about the specific type of bacteria, fungi or virus.

III. Microparticles and Nanoparticles Suitable for Use in Focused Light Scattering

In some embodiments, where the complex between an active agent and a biological particle does not result in a change in particle size (i.e., no particle agglomeration or cell rupture), it may be necessary to conjugate the active agent with a microparticle (such as a nanoparticle, polystyrene bead, gold particle, and the like). Thus, when the agent forms a complex with the biological particle, the complex increases the size of the biological particle by the size of the microparticle, and this increased particle size is measurable using the techniques described herein.

In one embodiment, the particles have a particle size in the range of between about 0.1 and 10 μm, and ideally have a relatively consistent amount of active agent bound to them. That is, if all that is important is to determine that the biological

particles bound to an active agent of interest, then one can simply incubate the biological particle with the conjugate, and look for the decrease in peak corresponding to the biological particle. This will confirm that a complex of the biological particle and the active agent was formed.

If there is an interest in quantifying how much of the biological particle was present, then it may be important to use particles with a nearly uniform particle size, defined as having 90% or more of the particles within 5% of the mean particle size, more preferably around 99% uniformity or better. In addition to a uniform particle size, in some embodiments, it may be desirable to have uniform substitution on the particles themselves. That is, rather than having particles of a relatively uniform particle size form complexes of different particle sizes with the biological particle of interest, it may be desirable to form complexes with a relatively uniform particle size, to ease their quantification.

One way to produce particles with a relatively constant particle size, and with a relatively consistent amount of active agent conjugated to the particles, is to use dendrimers. The dendrimers can include a known quantity of the active agent, by virtue of the active functional groups at the terminus on the dendrimers.

Another way is to produce polymer particles with: a) a relatively narrow size distribution, and b) a relatively consistent amount of protected functional groups, so that after the polymers are produced, the protecting groups can be removed, and the functional groups used to conjugate the polymer particles to an active agent.

The active agent can be conjugated with the particle in such a way that the portion of the active agent that is known to be active (i.e., binds a receptor) is not significantly sterically hindered by its conjugation with the particle. In some embodiments, this will involve preparing an analogue of the active agent which includes a further functional group which can be attached to the particle.

In one embodiment, metallic particles, such as gold particles, are used. Because these particles scatter a significant amount of light, they can be conjugated with a specific active agent, and used to identify even small molecules that bind to the agent. That is, the amount of light that the particle scatters is sufficiently large that the binding of the agent to the molecule of interest can be measured, even though the

molecule is not within the size range of biological particles that can be measured. Means for conjugating active agents to metallic particles are known to those of skill in the art.

Metallic particles, such as gold particles, can also be used. These can be conjugated with active agents using known methodology to form particles capable of forming a complex with a biological particle, including particles too small to detect using focused light scattering techniques. Because the particles are highly dense, they produce enough light scattering to be detected, despite their small size. Binding of even a small molecule to the particles can be detected because of the intense scattering from the metallic bead so that enough light is scattered for the complex formation to be measured.

IV. Methods for Detecting the Presence or Absence of Specific Particles in a Solution

A sample medium can be evaluated for the presence or absence of specific particles. For example, a sample of blood, urine, spinal fluid, amniotic fluid, pleural fluid, peritoneal fluid and the like, can be evaluated for the presence or absence of specific microbes (cells (lymphocytes, B-cells, T-cells, neutrophils, monocytes, and the like), bacteria, fungi, viruses, and the like), and/or for the presence of relatively high concentrations of white blood cells or other biological particles indicative of a disease state. The methods also permit one to determine presence or absence of shed particles.

The particle (intact cell, microparticle, bacteria, virus, fungus, and the like) can be further identified and characterized by addition of probe particles that are coated with a specific agent that recognizes and binds to the specific surface epitope on the unknown particle surface. If the recognition and binding of the probe particle occurs, a new particle size will be created and appear, thus identifying the unknown particle. For example if the unknown particle was a stem cell and the probe particle was labled with an anti-CD34 antibody, binding of the probe particle with the stem cell would occur and a new sized particle would appear that would identify the presence of CD34 positive stem cells.

Once the particles are identified by size, one can confirm the identity of the particles, for example, by using a reference library correlating the particle size to a given biological species. Thus, in one embodiment, the method involves comparing information obtained on a biological particle using focused light scattering techniques, with a library of data on biological particles obtained using similar focused light scattering techniques. The library can include information on two or more biological particles, preferably, ten or more particles, more preferably, one hundred or more particles, and, most preferably, more than a thousand particles.

After a preliminary identification has been made on the type of biological particle, other biological techniques can optionally be used to confirm the identity of the particle. For example, an EQELS spectra of the particle can be taken, and compared to a library of EQELS spectra of known particles, to confirm the identity of the particle.

Antibodies or other molecules specific for the specific biological particle can be added to the solution, and if the particles bind to the antibodies, the method will detect the absence of the particles. Ideally, the molecules will be conjugated with a microparticle or nanoparticle, such as a latex particle. As the conjugate interacts with a biological particle, peaks representing the particle and the conjugate will decrease, and peaks corresponding to the complex of the particle and the conjugate will increase.

In one embodiment, the biological particle is a microbe. The technique can be used to identify the type of microbe (*i.e.*, bacteria, fungi, or virus), and, ideally, the specific class of microbe (*i.e.*, Pneumonia, Clostridia, and the like). In this embodiment, once a preliminary identification of the microbe is made, a confirmation assay can be conducted by first taking an EQELS spectra of the microbe in solution, subjecting the microbe to an antibody specific for the microbe, and taking a second EQELS spectra. If the EQELS spectra are different, this provides confirmation that the microbe was properly identified and was bound by the antibody. Further, once a microbe has been identified, a putative antimicrobial compound can be put in solution/suspension along with the microbe, to determine whether it is able to kill the microbe.

In one embodiment, the methods can be used to identify a potential therapeutic agent capable of interacting with a known cell, such as a cancer cell, bacteria, fungi, or virus. In this embodiment, one first uses focused light scattering techniques to generate a spectrum showing the particle size and distribution for the known cancer cell, bacteria, fungi, or virus in a sample medium. Then, a putative therapeutic agent conjugated to a microparticle or nanoparticle is added to the sample medium and allowed to incubate with the known cell, microbe or virus. A second spectrum is generated using focused light scattering techniques. If peaks corresponding to a complex of the microparticle-conjugated therapeutic agent and the known cell or microbe are observed, then the therapeutic agent has bound to the cell. This is indicative of the potential utility of the putative therapeutic agent against the known cell, microbe or virus.

In one aspect of this embodiment, spectra of a microbe in a sample medium are compared with a reference database of spectra of known microbes, thus providing a rapid means for identifying a particular microbe. The spectra can also provide an initial determination of particle size and/or particle density in the medium.

Bacterial detection

In one example of identifying microbes, one can determine whether the microbes are mono-dispersed or poly-dispersed by their number and size. Since *E. coli* tend to mono-disperse and *Streptococcus* tend to poly-disperse, this embodiment can be used to observe particle size in a sample, where observation of clumping identifies presence bacteria known to clump (*i.e. Streptococcus*).

Detection of Particle Shedding:

In another embodiment, the method is used to detect particle shedding. Representative biological particles which shed smaller particles include tumors, red blood cells, white blood cells, granulocytes, platelets, monocytes, neutrophils, lymphocytes, endothelial cells, cancer cells, stem cells, bacteria, viruses, and fungi. Particle shedding may result from cell interactions, a change of cellular state such as activation or deactivation, as a result of expression, cell death, etc. The methods described herein can be used to identify such particle shedding.

In one embodiment, a drug or ligand is added to a vessel with a cell. Where the drug or ligand reacts with the cell, the cell may shed membrane particles or other particles. As described in detail herein, the method can be used to detect the size, number and/or type of particles shed by the reaction. Therefore, where the cell is known, this technique may be used to detect efficacy of unknown drug agent; and where the drug is known, the technique may be used to identify the presence of a specific cell type.

The ejected particles can be observed using the methods described herein. The ejected particles can similarly be characterized by comparing the size and/or shape with a library of data collected using focused light scattering techniques on known ejected particles, and/or by binding some or all of the ejected particles to an antibody or other such molecule.

V. Detection of Microparticles Ejected from Cells

In another embodiment, the ISADe device can be used to detect particle shedding. Representative biological particles which shed smaller particles include tumors, red blood cells, white blood cells, granulocytes, platelets, monocytes, neutrophils, lymphocytes, endothelial cells, cancer cells, stem cells, bacteria, viruses, and fungi. Particle shedding may result from cell interactions, a change of cellular state such as activation or deactivation, as a result of expression, cell death, etc. The methods described herein can be used to identify such particle shedding.

In one embodiment, a drug or ligand is added to a vessel with a cell. Where the drug or ligand reacts with the cell, the cell may shed membrane particles or other particles. As described in detail herein, the method can be used to detect the size, number and/or type of particles shed by the reaction. Therefore, where the cell is known, this technique may be used to detect efficacy of unknown drug agent; and where the drug is known, the technique may be used to identify the presence of a specific cell type.

The ejected particles can be observed using the devices and methods described herein. The ejected particles can similarly be characterized by comparing the size and/or shape with a library of data collected using focused light scattering techniques

on known ejected particles, and/or by binding some or all of the ejected particles to an antibody or other such molecule.

The ISADe device described above can be used to identify particles in the given size range (i.e., the size of the ejected (shed) microparticles and the size of the platelets). As shown in Figure 6, a sample composed of 6 differently-sized polystyrene beads was introduced to the device, and assessed using the device in terms of particle count (number) by particle size (μm). The chart shows the remarkable resolution of very small particle sizes. Current flow cytometers are not capable of resolution to this degree.

Figure 7 is a chart showing similar data from the data shown in Figure 6. The data in Figure 7 is presented as points rather than as histograms, and is reflected in particles per 10 ml sample, versus particle size (μm). Also, Figure 7 separates the size distribution is separated into 3 different windows. In this embodiment, each window has a separate detector that has been adjusted to detect particles in a specific size range. The smallest particles are assessed from scatted light focused onto a high-gain detector, the middle window from scattered light focused onto a low gain detector, and the window with the largest particles by a light extinction method.

Detecting Individual Molecules

In one embodiment, the methods permit one to detect the presence of specific molecules, where the molecules are of a size below the threshold limit of detection for focused light scattering techniques. In this embodiment, highly reflective metallic particles, such as gold particles, are covalently linked to a ligand that binds to the molecules of interest. Because the metallic particles are dense and highly efficient light scattering particles, the amount of light scattering when the ligand binds to a molecule of interest can be measured using the focused light scattering technique, thus confirming the presence or absence of a molecule of interest in the solution. In another embodiment, microparticles conjugated to an active agent known to bind in a specific manner to a particular secies of shed particles are used, rather than metallic particles. The complex of the shed particle and the microparticle is then measured. Representative agents include antibodies and small molecules known to bind to the particular shed particles.

Detection of Particle Aggregation

In another embodiment, the method is used to determine particle aggregation and types of aggregation. Such aggregation may include, but is not limited to aggregation of a particle with other particles, or aggregation of a particle with a drug.

Particularly with respect to liposomal therapy, agglomeration of particles (such as liposomes) can result in significant mortality or morbidity when the agglomerated particles are administered. Accordingly, the method can be used to evaluate a sample of liposomes before administration to ensure that the particles have not agglomerated before a patient is treated.

VI. Methods for Detecting Binding of a Particle to a Known Molecule

In some embodiments, one knows the identity of a known molecule, for example, a drug molecule that is known to interact with the receptor on a biological particle that may or may not be present in the sample medium. By incubating the sample medium with a conjugate of the drug molecule and a microparticle, one can look for complex formation indicative of the binding of the drug molecule with biological particles in the sample medium. The complex formation can be observed over time, or simply after a suitable incubation period.

The methods can be performed by using, as a starting material, either a drug or a biological microparticle in a vessel, and then adding a known material (either a known cell or microbe, for example) to test for interaction with drug; or a known drug to test for interaction with microparticle). Then, focused light scattering techniques can be performed to look for change in particle size from size of starting material, where an increase or decrease in particle size is indicative of interaction and binding.

In order to preserve the ability of the known molecule to bind to the particle of interest, in those embodiments where a molecule is conjugated to a microparticle, it is conjugated in a way that does not adversely impact its ability to bind to the particle of interest. This may involve developing a modified molecule, wherein the molecule is modified to include a functional group capable of being conjugated to the microparticle, such that the molecule still maintains its ability to form a complex with the particle of interest. Such modifications are routine in the art.

By using a biological particle known to form a complex with the active agent, one can evaluate such modified compounds for their ability to maintain their binding affinity for the particle of interest by incubating the modified compound, or the conjugate of the compound with the microparticle with the particle. Those compounds which maintain the ability to bind the particle of interest can be identified using focused light scattering techniques, because the particle size of the complex is larger than the particle size of the non-complexed particle and non-complexed conjugate.

Column-Based Approaches to Removing Biological Particles

Rather than forming a complex of the biological particle and a conjugate of an active agent and a microparticle, one can optionally prepare a column including microparticles conjugated to agents that bind to the biological particle of interest, and pass the sample medium through a column including the microparticles. The sample, minus any complexed biological particles, will elute from the column. By performing the focused light scattering method on the eluted material, one can identify changes in particle number/population density, as compared to the starting material. A decrease in particle number/population density is indicative of interaction and binding in the column, and, therefore, an indication that the biological particle of interest was present in the sample.

Magnetic Bead-Based Approaches to Remove Biological Particles

Rather than forming a complex of the biological particle and a conjugate of an active agent and a microparticle, and using focused light scattering methods to identify the presence of the complex between the biological particle and the conjugate, one can optionally use a magnetic microparticle conjugated to the active agent. Thus, one can first obtain a focused light scattering spectra using the methods described herein, then use magnetic particles to complex with the biological particle of interest. The complex can be removed from the sample media using a magnet. Then, one can obtain a second focused light scattering spectra, and identify whether the number of biological particles of interest has been reduced.

VII. Methods for Determining Binding of a Known Particle to an Unknown Compound

In other embodiments, it is desired to learn whether a putative therapeutic agent can bind to known biological particles. In these embodiments, putative therapeutic agents are conjugated to a microparticle or metallic nanoparticle, such as a gold nanoparticle, and incubated with the known biological particles. Thus, one can determine whether a compound forms a complex with the biological particles.

In some aspects of this embodiment, it is desirable to know the minimum inhibitory concentration, or binding affinity, of an active agent. The agent can be complexed with the biological particle at differing concentrations, and this information can be obtained. Some degree of extrapolation may be required, since the agent is conjugated to a microparticle, and therefore may behave differently than the native drug.

In other aspects of this embodiment, it is desired to know the selectivity of an active agent for one receptor over another. In this aspect, the agent can be complexed with a plurality of biological particles expressing differing receptors, and binding information can be obtained for each of the receptors. Thus, selectivity can be determined.

Determining Therapeutic Activity/Efficacy/Selectivity

In another embodiment, the method is used to identify therapeutic agents. While small molecules, proteins and peptides are not likely to be large enough to see, even with this technique, they can either be coupled to a microparticle, such as a latex particle or magnetic bead, which can be incubated with the molecules, or placed on a column.

In one aspect of this embodiment, a biological particle with a target for the therapeutic agent (i.e., a receptor) is placed in suspension, and microparticles conjugated to one or more putative therapeutic agents are added to the suspension.

If the therapeutic agent binds to the biological particle, the binding can be observed because the size of the complex of the biological particle and the conjugate is greater than that of the conjugate or the biological particle.

This technique can be used, for example, to determine the efficacy of specific antibacterial or other drug candidates for a particular infection, or to identify agents useful for treating specific types of cancer. In one aspect of this embodiment, the techniques are useful for personalized medicine, where a particular patient's bacterial infection, platelet or cancer cells, or erythrocytes, are analyzed for their ability to bind to and interact with specific therapeutic agents. In another aspect of this embodiment, one can generate a plurality of spectra and compare the results, to determine minimum inhibitory concentrations and, therefore, useful dosage ranges for a given drug (where the drug is an inhibitor).

VIII. Methods for Identifying Patients Likely to Benefit from Treatment

Certain patients respond to therapy due to an interaction of a cellular receptor with a drug molecule. However, certain other patients have genetic mutations which do not permit the patients cells to bind to the drug molecule, thus rendering the drug ineffective. To determine whether a patient will respond to a given therapy, a solution of the patient's cells can be combined with a drug molecule of interest, where the molecule is bound to a probe particle, which can be a microparticle or nanoparticle. If the drug molecule of interest binds to the patient's cells, then the concentration of the conjugate, and/or the patient's cells will be lower, and a new peak corresponding to the complex of the cells and the conjugate will be observed. The absence of a new peak corresponding to the complex of the cells and the conjugate is indicative that the patient will not respond to the particular drug therapy. Thus, the method can confirm that the patient will or will not achieve a benefit using the particular drug therapy. This enables a personalized medicine approach using rapid and inexpensive methods.

In one aspect of this embodiment, the cells are cancer cells from an individual patient, and sample media containing the cancer cells are incubated with a series of therapeutic agents bound to a microparticle or nanoparticle. Those therapeutic agents that form a complex with the cancer cells are potential candidates for personalized treatment of the patient, since they bind to and interact with the cancer cells. This technique can provide a relatively quick and inexpensive method for identifying patients who have certain mutations, such as HER2 positive patients, patients whose cancer cells have vitamin D receptors, patients with estrogen-responsive cancer cells, and the like.

In another aspect of this embodiment, the cells are blood cells from a patient suffering from atherosclerosis, and who is being evaluated to see if his blood cells will respond to treatment with clopridogrel bisulfate (Plavix®). The interaction with the blood cells and clopidogrel bisulfate is a surface interaction, but a small percentage of patients have a mutation in their blood cells that inhibits the surface interaction with this compound. For most of those patients, there is an alternative therapeutic agent, but it is important to identify those patients before the symptoms worsen, possibly leading to a heart attack. In this aspect, blood cells of a patient are incubated with microparticles conjugated to clopidogrel bisulfate, and a spectrum is obtained using focused light scattering techniques. For example interaction of the probe particle can be between the ADP receptor and Plavix target, P2Y12 or to the activation of the of VASP pathway. The presence of a complex between the clopidogrel bisulfate and the blood cells can be observed. Further, if the patient is responsive to Plavix, no activation will occur when ADP or other specific agonists are added to the patient's platelets. Thus, no activation will occur and specific activation epitopes, like CD62, glycoprotein alpha2, beta3, etc., will be detected by specific probe particles. Because the size of blood cells and the microparticle/clopidogrel bisulfate conjugate are known, only one spectrum needs to be obtained, and the only peak of interest is the peak corresponding to the complex of the conjugate with the blood cells.

IX. Methods for Performing High Throughput Bioassays

Any and all of these assays can be optimized for high throughput screening using suitable robotics. Liquid handlers can transfer samples to a multi-tube or multi-well plate, and a "memory map" can be used to correlate the samples to their location on the plate. Information on each sample can then be stored, and used to provide information about drug candidates, patient diagnoses, and proposed patient treatment options.

Robotics systems are known in the art, and can be used to move samples taken from individual patients to known positions in a multi-tube or multi-well plate. Once information on the sample is obtained using the focused light scattering techniques described herein, the information can be correlated to the individual patient via the stored information correlating the location of the tube and the patient identification.

Liquid handlers can take portions of the sample and evaluate a plurality (i.e., at least two) of different screening assays, for example, by incubating portions of the sample with different microparticles, bound to different active agents.

Automated processes using known robotics can be used to pull and place samples (like high throughput screening) with use of a "memory map". A user can then pick desired screens to be run and the robotic apparatus will implement desired processes.

In another aspect of the embodiments described herein, the methods can be automated using robotics to pull and place samples (analogous to conventional high throughput screening methods), optionally in conjunction with a "memory map". A user can then pick desired screens to be performed, and the robotic apparatus can implement the desired processes. In this embodiment, a laboratory can be set up to automatically screen numerous samples.

In a preferred embodiment, the personalized medicine processes described herein are automated, to provide relatively inexpensive, and relatively fast, high throughput screening methods to identify preferred therapies for patients suffering from disease.

X. Reference Libraries

A reference database of information gained by performing focused light scattering on known compounds can be used. One can compare the sample to the reference database in order to identify or characterize the particles in the tested sample. A reference database includes at least two, preferably more than ten, more preferably greater than a hundred, and most preferably, greater than a thousand bits of information on particle size that can be used to correlate the particle size measured using the techniques described herein with particle sizes for known biological particles that are stored in the reference database.

The present invention will be better understood with reference to the following non-limiting examples.

Example 1: Representative Focused Light Scattering Device

A representative focused light scattering device is shown in Figure 1. A first laser (1) emits light at a first wavelength, and a second laser (2) emits light at a second wavelength. Both beams of light pass through a first beam splitter (3) and through a first focusing lens (4) before they enter into a flow cell (15). The flow cell includes a site (5) for hydrodynamic injection of the sample. As the platelets in the flow cell pass through the beams of light, the light is scattered as it hits the platelets. The scattered light passes through a circular spatial filter (6) and then through a first collimating lens (7). The light beam passes through a second beam splitter (16), which splits the light into two beams. A first beam passes through a second focusing lens (8) and through a first chromatic filter (9) that passes scattered light from the first laser (1) through a first detector (10). The second beam passes through a second collimating lens (11), a third focusing lens (12) and a second chromatic filter (13) that passes scattered light from the second laser (2) to a second detector (14).

The two photodetectors (10 and 14) each are able to detect light at a certain frequency, so that light transmitted at different frequencies (as a result of the two lasers hitting particles, and which may interact with fluorescent tags on the particles) can be separately determined.

A third detector (an extinction detector) (18) receives a portion of the light passing through the flow cell. A portion of the light passing through the flow cell is reflected off of a movable mirror (17) and onto the third detector.

Example 2: Detection of Microparticle's (MP) present in a biological sample.

In this example, a specimen is subject to particle sizing and counting. After an appropriate dilution of the sample, the diluted specimen is introduced into the device for analysis. As counting proceeds, counts will accumulate in the size region less that 1 micron. The appearance of MP in this size region will indicate the presence of MPs.

Example 3: Microparticle Characterization

Once MPs are detected, as described in Example 2, it may be important to determine the source of the MP (from platelets neutrophils, tumor cell, etc.). This Example provides two different options for characterizing the MP.

The first option is to use MP sizing and counts. In this case, the specimen is incubated with a second particle with a specific ligand conjugated to its surface. The choice of the ligand will depend on the specific MP be characterized. For example, if the MP of interest has coagulation tissue factor (TF) on its surface, the conjugated particle can be conjugated with an antibody against TF-particle or with coagulation factor FVII. Either ligand will specifically bind to MPs with TF on their surfaces.

When the conjugated particles are incubated with the anti-TF conjugated particles, a new size corresponding to the TF-MP+anti-TF-particles will appear when the MP and the probe particle are counted. In a like manner, other MPs can be characterized by developing probe particles with a conjugated ligand specific for the MP of interest.

The second option is to use an EQELS device to analyze the MPs. The basic procedure is the same, except that differences in the particles electrophoretic mobility can be analyzed.

Specifically, a baseline electrophoretic mobility of the MP can be obtained. The MP will then be mixed with the probe particle that is conjugated with the specific ligand for the MP of interest, or just with the ligand without conjugation to a probe particle. When the probe particle binds to the MP of interest, a difference in the electrophoretic mobility is observed. These data will provide a specific identification of the MP. If the ligand alone was used to bind to the MP, the EQELS data, in addition to IP of the particle, will also provide binding constants for the ligand to the MP.

Example 4: Determination of Cellular Activation

In this Example, the cellular activation, such as platelet activation, is determined. A baseline resting platelet run on the sizing-counting device is determined. The platelet or cell is then activated using appropriate agonists. Once activation occurs, new surface epitopes appear on the surface. In the case of the platelet DC62, CD41, various integrins, and the like, begin to appear.

A probe particle with a ligand for the specific epitope of interest is then added to the activated platelets (or other cells). The ligand can be an antibody, or small molecule, that specifically binds to the activated epitope. When binding occurs, a particle is created (the activated cell + the probe particle) which has a larger size than

either the activated cell or the probe particle. The appearance of these new particles represents the appearance of the activated platelet + the bound probe particle.

Example 5: Microbe identification.

In this Example, microbes are identified using the techniques described herein. The microbe may be bacterial, viral, fungal, or protozoa. A sample containing a microbe is analyzed using the counting/sizing device described herein. The sample can be from a water supply, from a patient (human or animal), or other source. The size distribution will be determined, and compared to a database, to determine whether one or more of the particles in the sample fall into any of the sizes typical for known microbes. If so, a probe particle conjugated with a ligand that specifically binds to certain microbes can be added. The identity of the microbe is confirmed when a new size distribution is obtained when the sample is incubated with the probe particle.

Additional verification can be obtained if the specimen is examined by EQELS, where a swim rate is determined for flagellated organisms using the velocimetry mode of the EQELS device. The EQELS device can also determine the microbe's electrophoretic mobility. This information can be compared with data in an EQELS database for further confirmation of the identity of the particle.

If indicated, EQELS will then be used to help determine the appropriate antimicrobial (antibiotic, antiviral, etc) agent. A know concentration of antimicrobial agent will be added to the specimen. Binding of the agent to the microbe will be determined by a change in the microbe's electrophoretic mobility. Further, if the drug kills the microbe, its surface charge density changes resulting in a rather large change in the killed microbe's electrophoretic mobility.

Example 6: Drug Efficacy: Effectiveness of Cell Inhibition.

Specific example of Aspirin or/and P2Y12 inhibition of platelet activation

Platelet inhibition in arterial thrombophilic diseases is considered the standard of care. Unfortunately, in some patients, conventional drugs do not inhibit the patient's platelets. Approximately 25% of ASA-treated and 29% of Plavix-treated patients do not respond to treatment. Currently, there is no accepted assay to identify the resistant/refractory patients.

The assay described herein can use particle sizing, based on the following concept, to identify resistant/refractory patients. The goal of treatment with anti-platelet drugs is to inhibit activation. When the platelet is activated, new surface epitopes appear. So, in this embodiment, the patient's platelets are first obtained as platelet rich plasma (PRP). A baseline measurement of the particle size and/or distribution is then made. Next, an aliquot of the patient PRP can be mixed with a platelet activator (agonist). If the drug works, no activation occurs. If it does not work, the platelet will activate. The assay can then probe the platelet surface for the appearance of new epitopes like CD62, CD41 and the like. The surface can be probed with a ligand (antibody or otherwise) that is conjugated to a particle. If binding occurs, the probe particle will bind with the activated platelet, and a particle with a different (larger) particle size (i.e., the activated platelet+conjugated-probe particle) will appear. If the drug works, no change in the particle size distribution will occur.

Example 7: Lipid Droplets as Drug Delivery Vehicles.

Certain sized particles do not perfuse capillaries well. Since a typical capillary is approximately 2-3 microns in diameter, particles larger that that size must have a surface to volume ratio that will permit the distortion of the particle so that is can enter the capillary system; similar to a red blood cell. In this Example, particle sizing is used to identify particles which fall into a range that will not be perfused, and result in potential malaise or death to the patient. Further, the surface characteristics of some particles lead to instability, that may cause particle aggregation or fragmentation. Similar problems are well recognized in colloidal chemistry.

In this embodiment, drugs like Ambisome™, Daunosome™, Doxil™, or other liposomal drug delivery vehicles, can be screened for a safe particle size distribution prior to infusion. The screen can identify particles by particle size and distribution, so if relatively large particles, corresponding to agglomerated liposomal or other particles are observed, then the sample can be rejected before being infused. Alternatively, the sample can be subjected to conditions which de-agglomerate the particles (for example, ultrasound and the like), and the sample re-tested. These assays can also be used to optimize the solution, and particle surface optimization for identifying the best lead compound(s).

Example 8: Small Molecule Distribution Assays. Specific examples of vWF or serum plasma.

vWF is a polydispersed molecule with a molecular weight distribution range of 500,000 to 20 million or even higher. Thus, there is a substantial difference in the molecular size distribution. Current technology requires a minimum of several days to complete an analysis.

In this embodiment, gold beads are conjugated with an anti-vWF antibody in a manner that would bind one bead to one vWF multimer. Since the mass density of the gold bead results in more efficient light scattering, vWF molecules bound to the gold beads will be visible to the focused scattering sizing and counting device. Thus, the presence of, and in some embodiments, the amount of, vWF can be determined.

Accordingly, although the invention has been described herein with reference to various illustrative aspects, features and embodiments, it will be recognized that the invention is not thus limited, but rather extends to and encompasses other variations, modifications and alternative embodiments, such as will suggest themselves to those of ordinary skill in the art, based on the disclosure herein. The claims hereafter set forth therefore are intended to be broadly construed and interpreted as including all such variations, modifications and alternative embodiments within their spirit and scope.

Claims

1. A device for measuring the size and/or number of particles in a sample medium, comprising:

a) one or more lasers which produce beams of laser light,

b) a first focusing lens positioned in the path of the beams of laser light, which focusing lens focuses the beams of light such that the effective width in a direction transverse to the axis of the light beam is between about 0.05 and 0.5 μm,

c) a flow cell positioned in the path of the focused light source, wherein the flow cell is adapted to receive and pass a sample medium comprising a dispersion of particles, and passing the sample medium through the focused beams of light, so that beams of light that interact with one or more of the particles are scattered, and beams of light that do not interact with one or more particles are not scattered,

d) a hydrodynamic flow injector for introducing the sample medium into the flow cell,

e) a spatial filter positioned in the path of the beams of light, which allows scattered beams of light to pass through, and does not allow beams of light that are not scattered to pass through,

f) a collimating lens positioned in the path of beams of light passing through the circular spatial filter, to collimate scattered beams of light,

g) a second focusing lens positioned in the path of the collimating lens, for focusing the beams of light passing through the collimating lens,

h) a scatter detector positioned in the path of the focused beams of light passing through the focusing lens,

i) a mirror positioned between the flow cell and the spatial filter, to reflect a portion of the scattered beams of light, and

j) an extinction detector positioned in the path of the reflected beams of light.

2. The device of Claim 1, wherein the device comprises two lasers, further comprising a beam splitter that combines the beams of light from the two lasers, and passes the combined beams of light through the first focusing lens.

3. The device of Claim 2, further comprising a second beam splitter positioned between the collimating lens and the second focusing lens, to split the combined beams of light into two separate beams of light, wherein one of the separate beams of light is not diverted from its original path toward the second focusing lens, and the other of the separate beams of light is diverted from its original path toward the second focusing lens toward a second path.

4. The device of Claim 3, further comprising a chromatic filter between the second focusing lens and the scatter detector, for permitting beams of light from one of the laser beams to pass through, and not permitting beams of light from the other of the laser beams to pass through, to the scatter detector.

5. The device of Claim 3, further comprising a second collimating lens positioned along the second path, for collimating the diverted light beams.

6. The device of Claim 5, further comprising a focusing lens positioned in the path of light passing through the second collimating lens for focusing the diverted light beams.

7. The device of Claim 6, further comprising a second chromatic filter, positioned in the path of light passing through the focusing lens, to permit beams of light from the laser beam that did not pass through the first chromatic filter to pass through.

8. The device of Claim 1, further comprising a detector positioned in the path of the beams of light passing through the second chromatic filter.

9. The device of Claim 8, wherein the detector is a fluorescence detector.

10. The device of Claim 1, further comprising:

k) a processor adapted to receive information from the various detectors, and to responsively generate an output correlative of the size and/or number of particles in the sample medium.

11. The device of Claim 10, further comprising a memory map for storing information on the size and/or number of particles in the sample medium.

12. The device of Claim 10, further comprising a video display interface operatively coupled to the processor for outputting information on the size and/or number of particles in the sample medium.

13. The device of Claim 1, wherein the first focusing lens focuses the beams of light such that the effective width in a direction transverse to the axis of the light beam is between about 0.05 and 0.25 μm.

14. The device of Claim 1, wherein the first focusing lens focuses the beams of light such that the effective width in a direction transverse to the axis of the light beam is between about 0.05 and 0.15 μm.

15. A device useful for measuring the size and/or number of particles in a sample medium, comprising:

a) two lasers which produce beams of laser light,

b) a first focusing lens positioned in the path of the beams of laser light, which focusing lens focuses the beams of light such that the effective width in a direction transverse to the axis of the light beam is between about 0.05 and 0.5 μm,

c) a flow cell positioned in the path of the focused light source, wherein the flow cell is adapted to receive and pass a sample medium comprising a dispersion of particles, and passing the sample medium through the focused beams of light, so that beams of light that interact with one or more of the particles are scattered, and beams of light that do not interact with one or more particles are not scattered,

d) a spatial filter positioned in the path of the beams of light, to allow scattered beams of light to pass through, and to not allow beams of light that are not scattered to pass through,

e) a collimating lens positioned in the path of beams of light passing through the spatial filter, to collimate scattered beams of light,

f) a second focusing lens positioned in the path of the collimating lens, to focus the beams of light passing through the collimating lens,

g) a scatter detector positioned in the path of the focused beams of light passing through the focusing lens,

h) a mirror positioned between the flow cell and the circular spatial filter, to reflect a portion of the reflected beams of light, and

i) an extinction detector positioned in the path of the reflected beams of light.

16. The device of Claim 15, further comprising a hydrodynamic flow injector for introducing the sample medium into the flow cell.

17. The device of Claim 15, further comprising a beam splitter that combines the beams of light from the two or more lasers, and passes the combined beams of light through the first focusing lens.

18. The device of Claim 17, further comprising a second beam splitter positioned between the collimating lens and the second focusing lens, splitting the beams of light into two beams, wherein one of the beams is not diverted from its original path toward the second focusing lens, and the other of the beams is diverted from its original path toward the second focusing lens toward a second path.

19. The device of Claim 18, further comprising a chromatic filter between the second focusing lens and the scatter detector, for permitting beams of light from one of the laser beams to pass through, and not permitting beams of light from the other of the laser beams to pass through, to the scatter detector.

20. The device of Claim 18, further comprising a second collimating lens positioned along the second path, which lens collimates the diverted light beam.

21. The device of Claim 20, further comprising a focusing lens positioned in the path of beams of light passing through the second collimating lens.

22. The device of Claim 21, further comprising a second chromatic filter, positioned in the path of light passing through the focusing lens, for permitting beams of light from the laser beam that did not pass through the first chromatic filter to pass through.

23. The device of Claim 22, further comprising a detector positioned in the path of the beams of light passing through the second chromatic filter.

24. The device of Claim 23, wherein the detector is a fluorescence detector.

25. The device of Claim 15, further comprising one or more additional lasers.

26. The device of Claim 25, further comprising one additional detector for each additional laser.

27. The device of Claim 26, further comprising one additional beam splitter for each additional laser.

28. The device of Claim 26, further comprising one additional collimating lens for each additional laser.

29. The device of Claim 26, further comprising one additional focusing lens for each additional laser.

30. The device of Claim 15, wherein the first focusing lens focuses the beams of light such that the effective width in a direction transverse to the axis of the light beam is between about 0.05 and 0.25 μm.

31. The device of Claim 15, wherein the first focusing lens focuses the beams of light such that the effective width in a direction transverse to the axis of the light beam is between about 0.05 and 0.15 μm.

32. A method of identifying a biological particle of interest in a sample medium, said method comprising:

a) introducing a sample medium, which may or may not include a biological particle of interest, into the device of any of Claims 1-31,

b) using focused light scattering techniques to prepare a spectrum showing particle size distribution from within the sample medium, and

c) identifying the presence or absence of the biological particle of interest by comparing the sizes of the particles in the sample medium with the known size of the particle of interest.

33. The method of Claim 32, wherein the size of the particles in the sample medium is compared with a reference database comprising a library of particle sizes obtained using focused light scattering techniques.

34. The method of Claim 33, wherein the library comprises ten or more spectra.

35. The method of Claim 32, wherein, after an initial determination is made that a particle of interest is present in the sample medium, a confirmatory assay is performed.

36. The method of Claim 35, wherein the confirmatory assay involves taking an EQELS spectra of the sample medium, and verifying the existence of a biological particle by identifying a feature unique to the biological particle.

37. The method of Claim 35, wherein the confirmatory assay comprises incubating the sample medium with a compound known to bind to the biological particle of interest, wherein the compound is covalently linked to a microparticle or nanoparticle.

38. The method of Claim 37, wherein the confirmatory assay comprises detecting a conjugate of the biological particle of interest with the compound bound to the microparticle or nanoparticle.

39. The method of Claim 35, wherein the confirmatory assay comprises:

a) treating the sample medium with a compound known to kill a particular type of cell, wherein the biological particle of interest is the particular type of cell that is killed by the compound, and

b) performing a focused light scattering analysis on the treated sample medium to determine whether the concentration of the particle of interest is lowered.

40. The method of Claim 32, wherein the biological particle of interest is a lymphocytes, erythrocyte, B-cell, T-cell, neutrophil, monocyte, bacteria, fungi, viruses, or protozoa.

41. The method of claim 32, wherein the biological microparticle is selected from tumor cells, red blood cells, white blood cells, granulocytes, platelets, monocytes, neutrophils, lymphocytes, cancer cells, stem cells, bacteria, viruses, and fungi.

42. The method of claim 34, wherein the biological microparticle has a size ranging from about 0.1μm to about 20μm.

43. The method of claim 42, wherein the sample medium comprises one or more fluids from the group consisting of blood, blood products, water, cerebrospinal fluid, ascites, pleural fluid, and synovial fluid.

44. A method of determining the efficacy of a putative therapeutic agent, comprising:

a) obtaining a spectra showing particle size and distribution using focused light scattering techniques on a sample medium comprising a biological particle with a receptor to which a putative therapeutic agent will bind, using the device of any of Claims 1-31,

b) incubating the sample medium with a putative therapeutic agent,

b) obtaining a second spectra showing particle size and distribution on the incubated sample medium using focused light scattering techniques, and

c) determining whether the particle size and distribution has been altered by the incubation of the putative therapeutic agent, a change in the particle size and/or distribution is indicative of a complex formation of the putative therapeutic agent and the biological particle.

45. The method of claim 44, wherein the biological microparticle is selected from tumor cells, red blood cells, white blood cells, granulocytes, platelets, monocytes, neutrophils, lymphocytes, cancer cells, bacteria, viruses, and fungi.

46. The method of claim 44, wherein the biological microparticle is 0.1μm to 20μm.

47. The method of claim 44, wherein the sample medium comprises a fluid selected from the group consisting of blood, blood products, water, cerebrospinal fluid, ascites, pleural fluid, or synovial fluid.

48. The method of claim 44, wherein the putative therapeutic agent is conjugate to a microparticle, such that there is a measurable size difference between an uncomplexed biological particle and a particle complexed to the microparticle/therapeutic agent conjugate.

49. A method for determining whether a biological particle will form a complex with a known therapeutic agent, comprising:

a) obtaining a spectra showing particle size and distribution using focused light scattering techniques on a sample medium comprising a biological particle with a receptor to which a known therapeutic agent may or may not bind, using a device of any of Claims 1-31,

b) incubating the sample medium with a known therapeutic agent,

b) obtaining a second spectra showing particle size and distribution on the incubated sample medium using focused light scattering techniques, and

c) determining whether the particle size and distribution has been altered by the incubation of the known therapeutic agent, a change in the particle size and/or distribution is indicative of a complex formation of the known therapeutic agent and the biological particle.

50. The method of claim 49, wherein the biological microparticle is selected from tumor cells, red blood cells, white blood cells, granulocytes, platelets, monocytes, neutrophils, lymphocytes, cancer cells, bacteria, viruses, and fungi.

51. The method of claim 49, wherein the biological microparticle has a size in the range of from about 0.1μm to about 20μm.

52. The method of claim 50, wherein the sample medium comprises a biological fluid from the group consisting of blood, blood products, water, cerebrospinal fluid, ascites, pleural fluid, and synovial fluid.

53. A method for determining an effective dosage of a therapeutic agent against a known cell, microbe or virus comprising:

a) generating a first spectrum showing particle size and distribution using focused light scattering for a known cell, microbe or virus;

b) incubating a first concentration of a therapeutic agent with the known cell, microbe or virus;

c) generating a second spectrum showing particle size and distribution using focused light scattering of the combination of the therapeutic agent and the known cell, microbe or virus; and

d) comparing the first and second spectra, wherein a change in the particle size and/or distribution is indicative of binding of the therapeutic agent and the cell, microbe or virus, and wherein binding is indicative of inhibition of the known cell, microbe or virus;

e) repeating steps a-e with varying amounts of the therapeutic agent; and

f) comparing the spectra to determine the minimum amount of therapeutic agent required to effectively bind the known cell, microbe or virus.

54. The method of claim 53, wherein the therapeutic agent is an antibody.

55. The method of claim 53, wherein the known cell, microbe or virus is a cell selected from the group consisting of tumor cells, red blood cells, white blood cells, granulocytes, platelets, monocytes, neutrophils, lymphocytes, and cancer cells.

56. The method of claim 53, wherein the known cell is a cancer cell and the therapeutic agent is effective in treating cancer.

57. The method of claim 53, wherein the known cell, microbe or virus is a microbe selected from bacteria and fungi.

58. The method of claim 26, wherein the known microbe is a bacteria and the therapeutic agent is effective in treating bacterial infection.

59. The method of claim 58, wherein the known cell, microbe or virus is a virus.

60. A method of detecting particle shedding resulting from a cell interaction comprising:

a) generating a first spectrum showing particle size and distribution, using focused light scattering techniques, for a known cell in a sample medium; using the device of any of Claims 1-31,

b) incubating a potential therapeutic agent with the known cell in the sample medium, wherein the potential therapeutic agent is a microparticle;

c) generating a second spectrum showing particle size and distribution, using focused light scattering techniques, for the incubated sample medium;

d) comparing the first and second spectra, wherein the presence of, or increase in particle density of, particles of a size less than those of the original known cell is indicative of particle shedding.

61. A method of identifying particle aggregation in a sample medium comprising:

a) generating a first spectrum showing particle size and distribution, using focused light scattering techniques, for a sample medium comprising a biological particle; using a device of any of Claims 1-31,

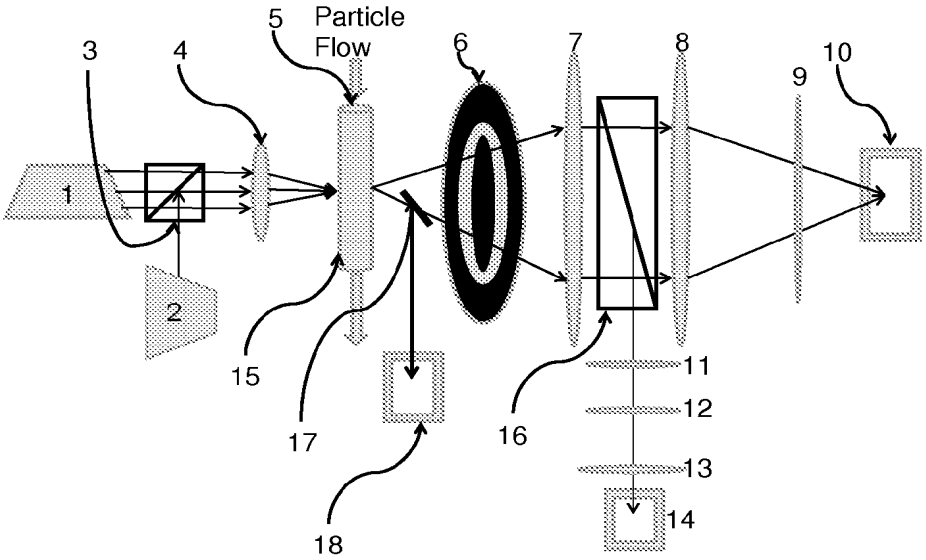
wherein the presence of a peak corresponding to the particle size of aggregated particles is indicative of the presence of an aggregate.

62. The method of Claim 61, further comprising incubating the sample medium is incubated with an active agent which promotes or inhibits particle aggregation in the absence of a specific mutation in the biological particle,

wherein the development of, or lack of development of, particle aggregation provides information on the activity, or lack of activity, of the active agent against this particular biological particle.

ISADE Optical Path

Figure 1



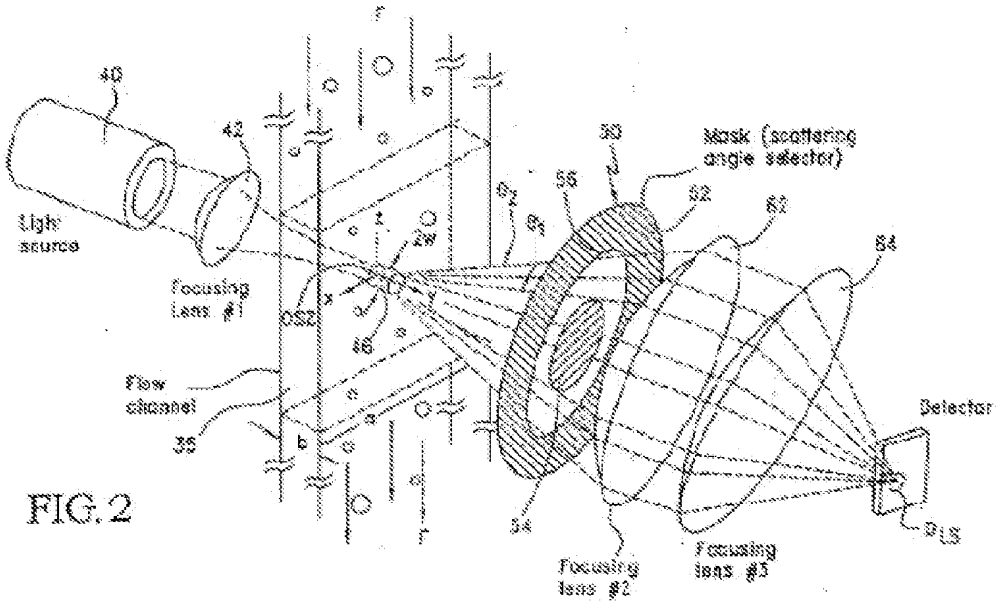

40
42
Light source
Focusing lens #1
Flow channel
35
46
50
Mask (scattering angle selector)
55
52
62
64
Detector
Focusing lens #2
Focusing lens #3

FIG. 2

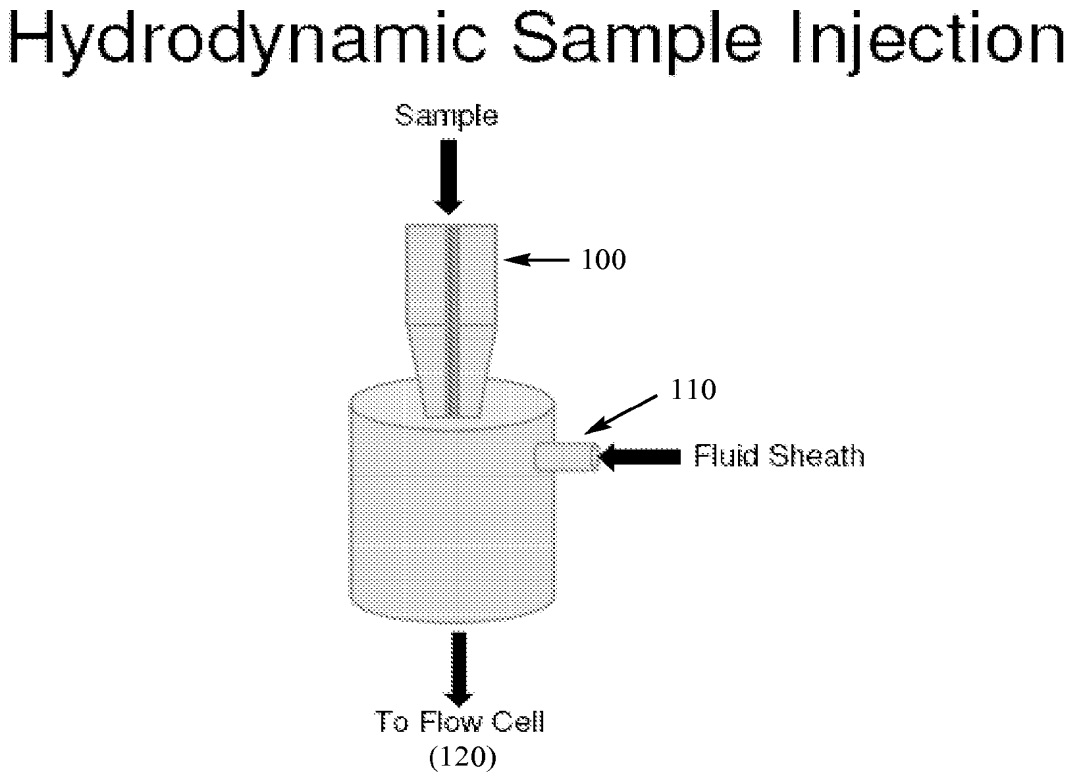

Hydrodynamic Sample Injection
Sample
100
110
Fluid Sheath
To Flow Cell
(120)

Figure 3

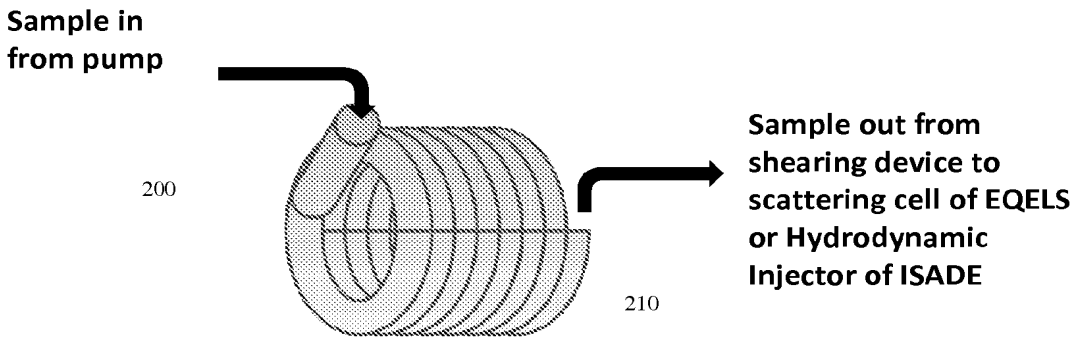

Sample in from pump
200
210
Sample out from shearing device to scattering cell of EQELS or Hydrodynamic Injector of ISADE

Figure 4

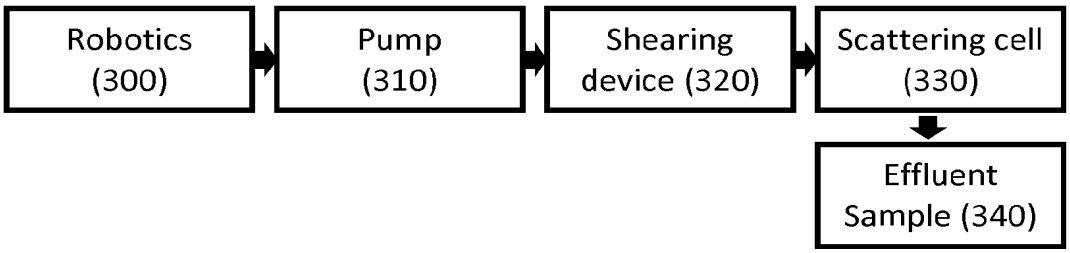

Robotics (300)
Pump (310)
Shearing device (320)
Scattering cell (330)
Effluent Sample (340)

Figure 5

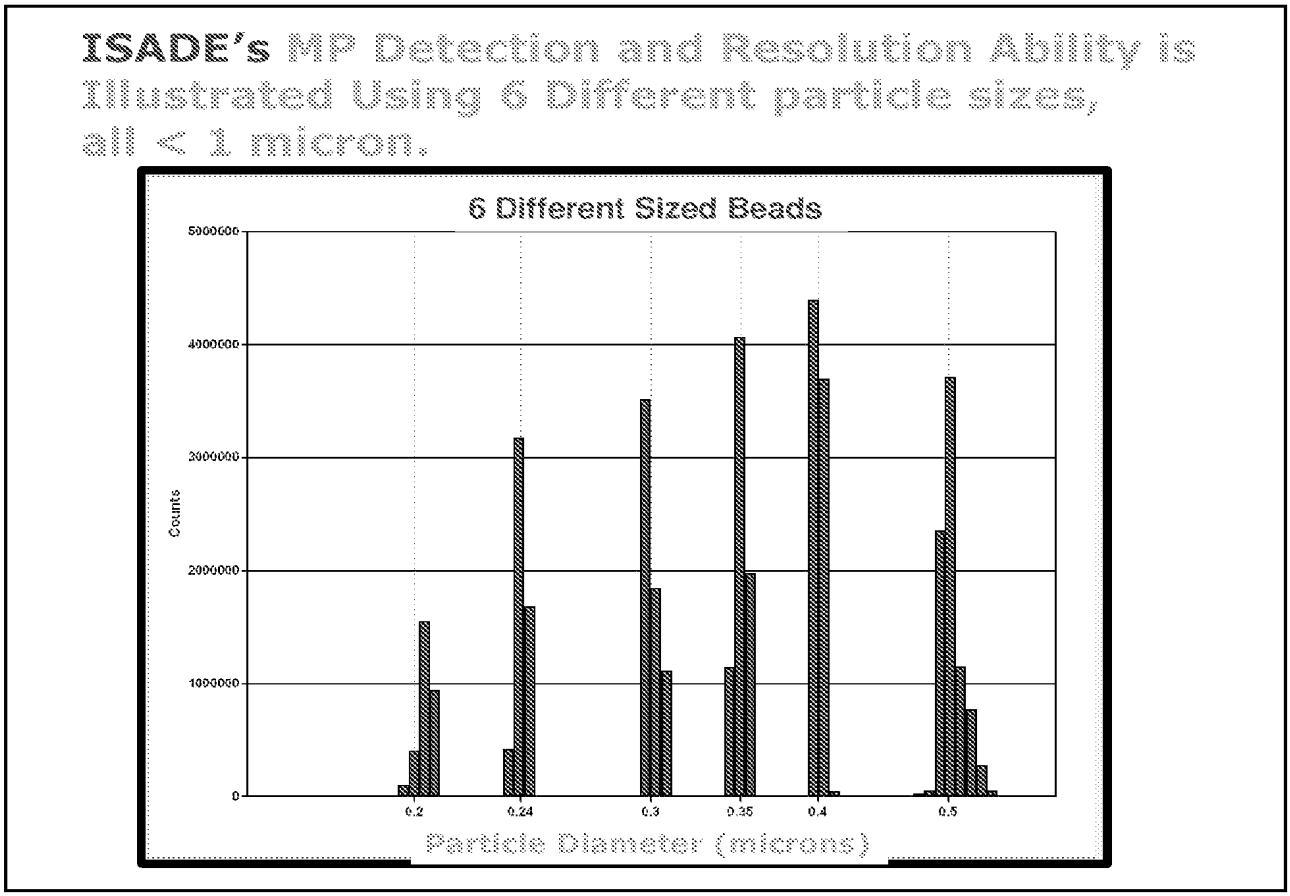

ISADE's MP Detection and Resolution Ability is Illustrated Using 6 Different particle sizes, all < 1 micron.
6 Different Sized Beads
5000000
4000000
3000000
2000000
1000000
0
Counts
0.2
0.24
0.3
0.35
0.4
0.5
Particle Diameter (microns)

Figure 6

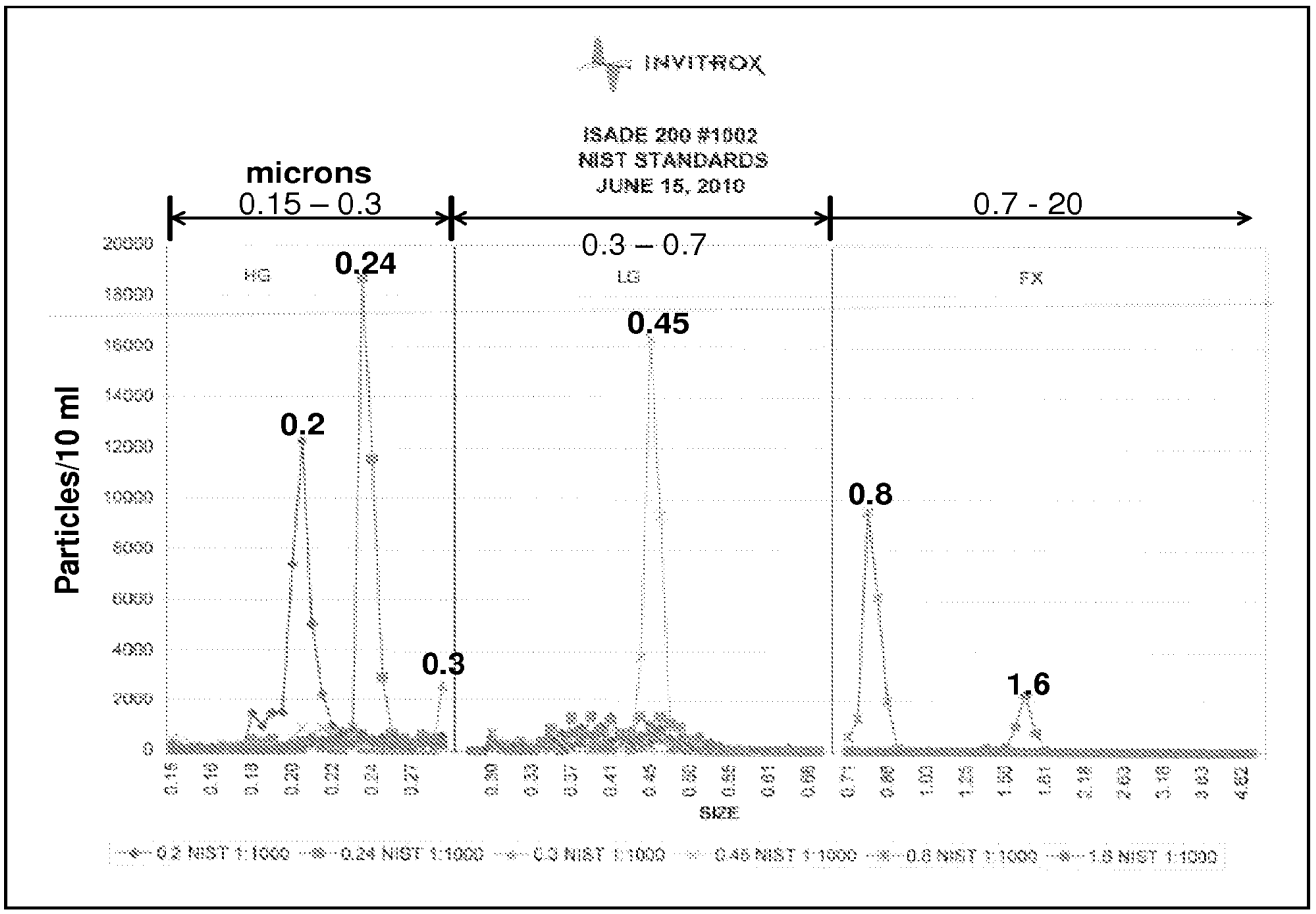

INVITROX
ISADE 200 #1002
NIST STANDARDS
JUNE 15, 2010
microns
0.15 – 0.3
0.3 – 0.7
0.7 - 20
HG
LG
FX
0.24
0.45
0.2
0.8
0.3
1.6
Particles/10 ml
SIZE
0.2 NIST 1:1000
0.24 NIST 1:1000
0.3 NIST 1:1000
0.45 NIST 1:1000
0.8 NIST 1:1000
1.6 NIST 1:1000

Figure 7

INTERNATIONAL SEARCH REPORT

International application No.
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSearch (RUPTO internal), Esp@cenet, PAJ, USPTO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0035235 A1 (INVITROX, INC.) 11.02.2010, [0011], [0053], [0130]-[0138], claims, fig. 3	1, 10-14, 32-62
Y		2-9, 15-31
Y	US 2009/0323061 A1 (LUKAS NOVOTNY et al.) 31.12.2009, col. 50, line 50-col. 54, line 16, fig. 29, 30	2-9, 15-31
A	US 7127356 B2 (PARTICLE SIZING SYSTEMS, INC.) 24.10.2006	1-62
A	WO 2010/063455 A1 (GRUNDFOS MANAGEMENT et al.) 10.06.2010	1-62

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2012 (18.10.2012)	15 November 2012 (15.11.2012)
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer E. Andreichenko Telephone No. 499-240-25-91

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(54) **Title**: SYSTEM AND METHOD FOR HIGH RESOLUTION, INSTANTANEOUS WIDE DYNAMIC RANGE, MULTI-COLOUR LUMINESCENCE DETECTION OF BIOLOGICAL SAMPLES IN A MICROFLUIDIC SYSTEM

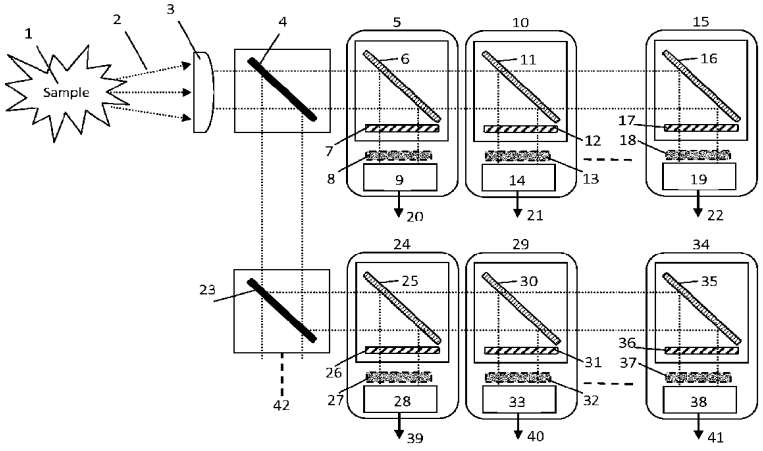


Figure 1

(57) **Abstract**: The invention provides a high resolution, wide dynamic range, multi-colour detection platform for microfluidic analysers/instruments and methods. The detection platform uses multiple high gain semiconductor optical sensors for the detection of luminescence from cellular or biological samples. The digitized outputs from these sensors are combined and weighted in a signal processing unit, using pre-determined algorithms for each colour, which optimise the resolution in each of these high gain semiconductor optical sensors while extending the dynamic range of the detection platform.

WO 2013/093035 A1

Title

System and Method for High resolution, instantaneous wide dynamic range, multi-colour luminescence detection of biological samples in a microfluidic system

Field of the Invention

This invention relates generally to a microfluidic platform comprising a high resolution detection system and method for the detection of luminescence from cellular or biological samples over a wide dynamic range.

Background to the Invention

Light emitted and scattered from samples is widely used to determine their cellular or biological content. It is desirable to have a multi-colour detection system that allows analysis of a wide optical detection range of biological samples, with high resolution, instantaneously for a variety of assays in immunology, biochemistry, and haematology for example. Pre-defined algorithms combining multi-sensor outputs in a multi-colour detection platform would provide the end-user with the capability of running these assays without the need for detailed knowledge of the technology and with minimal interaction and analysing it with high resolution over an extended dynamic range.

Photodetectors are required to detect and quantify this light from a sample under test, when stimulated by some form of luminescence. Photodiodes, arrays of photodiodes, CCDs and other solid state sensors can be used to quantify this light; however Photomultiplier Tubes (PMT's) are the incumbent photodetector of choice to detect such light components. This is due to their low-end sensitivity and ability to resolve over the PMT's dynamic range due to their internal gain structure (>10e6).

However, a number of problems exist in that PMT's are expensive, require very high voltage operation, are not suited to point of care diagnostic instruments and settings due to their size, require complex and expensive optical arrangements, with PMT based instruments requiring specialised and expensive maintenance. A PMT's practical optical dynamic range is typically 3 decades, so to adjust its optical range of sensitivity to quantify the light emitted or scattered from a sample requires the bias voltage to be adjusted by a skilled operator trained in the use of each specific instrument.

Due to recent advances in high gain semiconductor optical sensors such as avalanche photodiodes (APDs), Geiger-mode avalanche photodiodes and arrays of these Geiger-mode detectors (referred to as Silicon Photomultipliers or SiPMs for short) they are beginning to replace PMT's in such analysers. For example EP2293032A1, assigned to the applicant of the present invention and incorporated herein by reference, describes an integrated cytometric sensor system using SiPMs and other advances to solve such aforementioned issues. The main advantages relate to lower cost and size, lower voltage operation, faster start-up times, scope for increased semiconductor integration and lower maintenance requirements. These advances open the possibility of bringing advanced central laboratory techniques, such as the gold standard method of clinical flow cytometry, into decentralised point-of-care environments for screening of patients' blood for various infectious diseases, chronic and acute conditions, viruses and blood ailments.

Similar to PMTs, solid state sensors that contain an internal gain structure, like APDs and SiPMs, allow their region of sensitivity to be selected for the sample under test, by altering their operating voltage. This is controlled somewhat by setting their voltage bias with respect to their breakdown voltage.

By setting the bias voltage higher than the breakdown voltage (placing the SiPM in Geiger mode) these high gain semiconductor optical sensors can detect dimmer light, but saturate easily in the presence of bright light. By lowering the bias voltage towards the breakdown voltage or below it (placing the SiPM in its linear mode of operation), brighter light can be detected before saturation occurs, but the solid state sensor will be less sensitive to dimmer light as a consequence.

Lowering the bias of a high gain semiconductor optical sensor extends its dynamic range, enabling the detection of brighter light at the expense of reducing resolution. This is due to the reduction in the sensor's internal gain with reducing bias voltage and hence reduction of the optical sensor's output photocurrent and responsivity. This compromise between resolution and dynamic range limits the ability of an optical system using two or more sensors biased at different bias levels to resolve between biological samples or

cells with similar but distinct high light level intensities, thereby limiting the range and/or resolution of the system. EP 2293032A1 uses such a method of adjusting the bias voltage of the sensor for wide dynamic range operation in an integrated multi-colour cytometric sensor based on high gain semiconductor optical sensors such as SiPMs. A problem with this method is that the reduction in bias will minimize the resolution of the system.

Additional systems where a multi-sensor approach is used to extend the dynamic range of an optical detection system is disclosed in US 2005/0151964 which outlines the expansion of its dynamic range by splitting the fluorescent light over multiple paths with different intensities onto multiple sensors/channels. It is then determined which channel is operating in its linear range and the output signal adjusted according to the intensity of light, using post processing techniques.

US 5491548 similarly produces a wide dynamic range output from two optical sensors where a percentage of light is split onto the first sensor and the remainder is transferred onto the second sensor and the outputs are combined digitally to produce a composite signal. However this invention uses two different types of sensor to detect the light. Additionally, the use of a switch ensures that only the data from one of these sensors can be used at a time.

US 6355921B1 describes a method where the output signals from multiple PMTs are combined to increase the dynamic as in the other prior art. Also the dynamic range of each PMT individually can also be increased by using a control circuit to combine the output from a low light level detection circuit and a similar bright light level detection circuit where needed. EP 1928167A1 involves using multiple detectors again and adjusting their parameters using a signal processing unit in a targeted manner. One detector is adjusted to a dynamic range for a maximum level of electromagnetic radiation expected, while the other detector is adjusted to a reduced dynamic range for small and middle signal levels in order to obtain a higher signal to noise ratio (SNR) for these. These systems rely on a number of physically different detectors with different surface area and active areas and cannot work using a single type of detector. Additionally the sensors are mounted as a single array and does not use a beam splitter.

It is an object of the invention to provide a system and method to overcome at least one of the above mentioned problems.

Summary of the Invention

According to the invention there is provided, as set out in the appended claims, a microfluidic platform comprising a multi-colour detection system which uses a combination of multiple high gain semiconductor optical sensors such as SiPMs using pre-determined algorithms to maintain high resolution while extending its dynamic range and methods are provided. These methods expand the system's dynamic range beyond what can be achieved with any single photodetector, while ensuring a low cost, miniaturised instrument for instantaneous high resolution detection of a wide range of optical intensities from cellular or biological samples.

In summary, the present invention provides a high resolution, wide dynamic range, multi-colour and sensor detection system designed specifically for high gain semiconductor optical sensors such as SiPMs. This is due to the non-linear internal gain profile of SiPMs compared to the linear profile of competing high gain photodetectors such as PMTs, such that a pre-defined algorithm per colour is required to select the high gain region of these sensors. The high gain region is used to maintain the resolution in this semiconductor based system and present a wide dynamic range by combining and weighting the outputs from the multiple sensors per colour.

This multi-colour detection system can be predominantly used for luminescence detection in microfluidics systems such as flow cytometers and centrifugal microfluidic platforms but not limited to these.

The invention provides a high resolution multi-colour detection system while providing wide dynamic range for each colour by combining the outputs of multiple high gain semiconductor sensors using pre-defined algorithms in a signal processing unit. This negates the need for sensor bias adjustment by the end user as done in the prior art, simplifying instrument usage particularly for POCT and clinical research.

In one embodiment there is provided multi-colour luminescence detection system in a microfluidic system, said detection system comprising:

means for splitting light into at least two light paths for each detected colour of luminescence from a cellular or biological sample in the microfluidic system;

a first high gain semiconductor optical sensor, for each luminescence colour component, adapted to be biased at an optimal operating voltage or gain for detecting low light levels of luminescence emitted from the cellular or biological sample with high resolution;

a second high gain semiconductor optical sensor for each colour component adapted to detect higher light levels of luminescence, such that the light level falling on the second sensor is attenuated while maintaining resolution with a high gain setting; and

means for processing per luminescence colour by combining and weighting the outputs from the first and second high gain semiconductor optical sensors using a signal processing unit, and providing an instantaneous multi-colour high resolution detection system over an extended dynamic range.

The optical dynamic range of the detection system for any specific wavelength of interest can be increased by using multiple high gain semiconductor optical sensors, collecting the emitted or scattered light from the luminescence sample at one or multiple points and directing the light to these sensors. Simultaneous measurements from two or more of these sensors per colour are collected, from the same sample of interest, and coupled together by a pre-defined algorithm in a signal processing unit to construct a wide dynamic range output for each colour. The algorithm for each colour is defined at an initial calibration stage where the algorithm coefficients that combine and weight the sensor combinations are determined by a monochromatic linear light source. The algorithms are then applied to the signal processing unit for each colour.

To pass the data from the multiple sensors to the signal processing unit, the photocurrent passed from the high gain semiconductor optical sensors due to luminescence stimulus are first amplified and converted into a voltage using a trans-impedance amplifier or similar arrangement. The outputs are digitized using an analogue to digital converter and then passed to the signal processing unit to process these individual outputs to

create a composite output per colour.While extending the dynamic range is possible by splitting the light to multiple high gain semiconductor optical sensors, the gain setting for each of these will be optimised to maintain high resolution over the complete dynamic range as part of the pre-calibration sequence in defining the algorithms for each colour.

The primary sensor(s) of the system are optimised to detect low optical intensities to maximise the sensitivity of the system by selecting an appropriate gain setting that also maintains high resolution. Further sensor(s) are then used, with similar high gain settings, to detect brighter luminescence levels and the light falling on these sensor(s) is attenuated to maintain the resolution of the sensor's optimal gain setting without saturating the sensor's output.

This is specifically important for high gain semiconductor optical sensors like SiPMs as they have an optimal gain region, which also provides high resolution, as determined by their bias voltage. The outputs from these sensors which is digitized and then combined by a pre-defined algorithm, provides instantaneous, high resolution, multi-colour luminescence detection over a wide optical range of interest. This high resolution, wide dynamic range detection system would be pre-configured in a microfluidic based instrument and alleviates the need for end-user intervention in setting the optimal gain of the sensor(s) for each sample under test. This will allow the quantification of biological samples over many decades of dynamic range without any user intervention as required in current instruments/analysers

In one embodiment the collected luminescence is split into specific wavelengths of interest by using coated beamsplitters or dichroic mirrors and additional optical filters where required. These mirrors and filters are grouped with the high gain semiconductor optical sensors and additional optical components, where required, to create a filter cell.

In one embodiment the light collected from the sample is directed to a single light path using light collection optics and focused onto 2 or more high gain semiconductor optical sensors. The photocurrent output is converted to the digital domain from these sensors

and merged together using the pre-determined algorithm in the signal processing unit to produce a composite output.

Other embodiments show the light collected from the sample from multiple locations or collection points and merged to produce a high resolution, wide dynamic range detection system also.

A key criterion in each embodiment is to maximize the amount of light collected by sensor(s) of the detection system, particularly for low-light level detection systems where the light budget is limited.

In one embodiment the first and second optical sensors generate signals and adapted to be fed into a signal processing means to generate a wide dynamic range high resolution signal for each colour.

In one embodiment the sensors in the detection system are SiPMs.

In one embodiment more than one collection point is used to gather the light emitted from the luminescence sample to generate a high resolution, wide dynamic range output per colour.

In one embodiment, light emitted from the sample is coupled to a collector and passed through at least one filter adapted to attenuate the light level and delivered to said second high gain semiconductor optical sensor for each colour.

In one embodiment a collector is adapted to collect light from the sample and directed to a beam splitter.

In one embodiment the collector comprises a lens system.

In one embodiment the beam splitter is adapted with two or more output ports for delivering light to said first and second high gain semiconductor optical sensors for each colour.

In one embodiment the beam splitter comprises an uncoated mirror, said uncoated mirror adapted to deliver maximum amount of light via a first port to the first high gain semiconductor optical sensor detecting low-end light levels while bright light components of the sample are detected simultaneously at the second port by said second high gain semiconductor optical sensor.

In one embodiment the amount of light delivered from said first and second ports is dependent on the transmission or reflectance of the uncoated mirror.

In another embodiment of the present invention there is provided a system for detecting specific biological targets in a fluid sample or a non-fluidic sample adapted for luminescence detection at different wavelengths, said system comprising the detection system having:

a first optical sensor biased at an optimal operating voltage or gain adapted for detecting low light levels of luminescence emitted from a cellular or biological sample; and

a second optical sensor adapted to detect higher light levels of luminescence, such that the light level falling on the second sensor is attenuated; wherein processing of cellular or biological samples over many decades of dynamic range is achieved at high resolution.

In one embodiment, the light collected from the sample is coupled in free-space to two or more high gain semiconductor optical sensors for each colour on the same light path. 1 or more of these sensors contains an optical component such as a neutral density filter or similar in the light path to reduce the light level reaching the secondary sensor(s) here. By biasing the sensors in the attenuated section of this light path at a similar voltage bias to the primary sensor detecting low light levels, this ensures high resolution over the entire dynamic range of interest for each colour. The attenuation component(s) ensure the secondary sensor(s) do not saturate with this high resolution/gain setting.

In one embodiment, the light can be collected from multiple points and coupled to one or more sensors per colour with or without attenuating components attached.

Interleaving multiple sensor outputs for each colour from multiple collection points is carried out here to further enhance the dynamic range, while maintaining high resolution.

In one embodiment, the light collected from a single point is coupled to an array of high gain semiconductor optical sensors. The central sensor receives the maximum proportion of the collected light, particularly if the light beam has a Gaussian profile. This configuration serves to attenuate the light levels collected to the adjacent secondary sensors compared to the centre one which is used to collect the majority of the light from the sample. As the collected light to the secondary sensors is effectively attenuated using this method, a high bias/gain setting can still be applied to maintain resolution in the detection system, without saturating the outputs. The outputs from the arrays of sensors per colour are then coupled together in the processing unit to generate the high resolution, wide dynamic range output from the system. The arrangement of the sensors can be optimised to suit the light measurement and is not restricted to the embodiment shown here.

In one embodiment the secondary high gain semiconductor optical sensors may be different from the main sensor. They may be different in one or more aspects including, but not limited to pixel density, active area, sensor size, photon detection efficiency (PDE) or spectral responsivity.

In one embodiment the lens which couples the collected light to the high gain semiconductor optical sensor array may be adjustable, either manually or automatically. In one embodiment, some of the adjacent high gain semiconductor optical sensors of the sensor array per colour may contain attenuation filters also.

In one embodiment, the light can be collected from multiple points but just one high gain semiconductor optical sensor at each colour location, including attenuation filters if required.

In one embodiment, the light from the sample can be coupled from multiple points to multiple high gain semiconductor sensor arrays, with or without attenuation filters, to generate the high resolution, wide dynamic range output per colour.

In one embodiment, a beam splitter can be used to split the light collected from the sample simultaneously and couple it to the high gain semiconductor sensors for each colour. The split ratio of the beam splitter is used to attenuate the light to the secondary sensor(s) used to detect the higher optical power components from a sample. The processing unit again delivers a high dynamic range, high resolution output from these high gain semiconductor sensors for multiple wavelengths of interest.

In one embodiment the light is collected from multiple points and transferred to multiple beam splitters.

In one embodiment multiple sensors can detect the light from each port of the beam splitter and can also include a light attenuating component.

In a further embodiment there is provided a system comprising:

a first optical sensor biased at an optimal operating voltage or gain adapted for detecting low light levels of luminescence emitted from a cellular or biological sample; and

a second optical sensor adapted to detect higher light levels of luminescence, such that the light level falling on the second sensor is attenuated; wherein processing of cellular or biological samples over many decades of dynamic range is achieved at high resolution.

Brief Description of the Drawings

The invention will be more clearly understood from the following description of an embodiment thereof, given by way of example only, with reference to the accompanying drawings, in which:-

Figure 1 illustrates a block diagram of the arrangement showing the multi-colour luminescence collected from a cellular or biological sample and distributed to wavelength specific filter cells that contain 1 or more high gain semiconductor

optical sensors. The collected light is split between multiple light paths to extend the dynamic range for each luminescence colour of interest while keeping a high gain setting in all the sensors to maintain resolution;

Figure 2 illustrates how the wide dynamic range signal for the first luminescence colour, described in Figure 1, is created. The outputs from the high gain semiconductor optical sensors for this luminescence colour are converted to analogue voltages and then digitized. These digital signals are transferred to a signal processing unit that contains a pre-determined algorithm which combines and weights these to produce an instantaneous wide dynamic range signal with high resolution, according to one embodiment of the invention;

Figure 3 illustrates the difference between the gain versus bias voltage profile of an industry standard high gain sensor photomultiplier tube (PMT) compared to the high gain semiconductor optical sensors used in the multi-colour detection described in this invention;

Figure 4 illustrates the resolution limitations of high gain semiconductor optical sensors, according to the invention, when reducing their bias voltage or gain to detect bright optical signals;

Figure 5 illustrates the reconstruction of a wide dynamic range signal from multiple high gain semiconductor optical sensors using the method of changing the bias voltage to detect bright optical signals, the resolution limitations with this, or using beam splitting or light attenuation while maintaining the high internal gain in the sensors, to construct an ideal high resolution wide dynamic range signal;

Figure 6 illustrates another embodiment of the present invention using multiple collection points to transfer this luminescence to multiple high gain semiconductor optical sensors per colour. Again, splitting the light using a beamsplitter or similar over 1 or more sensors as described in the embodiment of Figure 1 applies here. In this embodiment, the dynamic range can be extended further using multiple collection points as the method for generating a multi-colour high resolution, wide dynamic range detection system;

Figure 7 illustrates an embodiment of the present invention using multiple light collection points instead of a beam splitting method for generating a high resolution, wide dynamic range detection system. Attenuating components are

used on some of the sensors to extend the dynamic range of the system, while maintaining high resolution throughout; and

Figure 8 illustrates another embodiment of the present invention using an array of high gain semiconductor optical sensors for each spectral range of interest at any collection point as a method for generating a multi-colour high resolution, wide dynamic range detection system. The area of the sensor array illuminated by the collected light beam can be varied and will determine the dynamic range of a given sensor array when the outputs of these are combined in a pre-determined algorithm per colour.

Detailed Description of the Drawings

Figure 1 illustrates the fundamental block diagram of the multi-colour luminescence detection system presented in this invention. In this, the luminescence 2 is collected from a biological or cellular sample 1 in a microfluidic platform, that this detection system is housed in (not shown). The luminescence from the sample 1 is transferred to the detection system using a lens system 3. The collected luminescence 2 is then transferred to a beamsplitter or uncoated dichroic mirror 4 which diverts a percentage of this light along two light paths. The percentage of light sent along each light path depends on the transmission or the reflectance of the beamsplitter 4. The split ratio of the beamsplitter 4 can be such that the majority of light can be sent along either light path. It is assumed that the majority of the light is transmitted to the spectrally sensitive filter cells 5, 10 and 15. The remaining reflected light is sent to the corresponding filter cells 24, 29 and 34, via a reflective mirror 23. Each filter cell shown uses a combination of dichroic mirrors and bandpass filters, where required, to transfer wavelength specific light to 1 or more high gain semiconductor optical sensors. In any high gain semiconductor optical sensor system described in this invention, there can be between 1 and C colours of luminescence to discriminate between using the spectrally sensitive filter cells. For the first colour detected, the majority of light is detected using filter cell 5 which consists of a dichroic mirror 6 which reflects the wavelengths of interest and transmits the remaining spectrum to the adjacent filter cell 10. The reflected light from the dichroic mirror 6 in this filter cell 5, is transferred to 1 or more high gain semiconductor optical sensors 9 via a bandpass filter 7 and an attenuating component 8, in embodiments where required. An attenuating component is included in filter cells for

detecting particular dynamic ranges of interest, while maintaining high resolution in the sensors. The remaining light from the beamsplitter 4 for the first colour of interest is detected by filter cell 24. Again, the light is reflected from the dichroic mirror 25 and transferred to 1 or more high gain semiconductor optical sensors 28 via a bandpass filter 26 and an attenuating component 27 in embodiments where required. The secondary sensor 28 for the first colour maintains a high gain setting but can detect bright luminescence samples without saturating, due to the reduction in light coming from the beamsplitter 4. The outputs 20 and 39 from the high gain semiconductor optical sensors 9 and 28 are then digitized and interleaved using a pre-determined algorithm, to create a high resolution, wide dynamic range signal dedicated to this spectral region of interest (see Figure 2 description). The second of the C colours in the detection system is determined using the filter cells 10 and 29. The transmitted light from the dichroic mirrors 6 and 25 from the first colour strike the dichroic mirrors 11 and 30 respectively. The reflected light from the dichroic mirrors 11 and 30 for the spectral range of interest are transferred to the high gain semiconductor optical sensors 14 and 33 respectively. The outputs 21 and 40 from these are digitized and interleaved using a pre-determined algorithm, to create a high resolution, wide dynamic range signal dedicated to a second spectral region of interest. Bandpass filters 12 and 31 and attenuating components 13 and 32 are included in embodiments where required. The C^{th} luminescence colour in the detection system is determined using the filter cells 15 and 34. The transmitted light from the dichroic mirrors from the previous filter cells strike the mirrors 16 and 35 respectively. The reflected light from the dichroic mirrors 16 and 35 for the spectral range of interest are transferred to the high gain semiconductor optical sensors 19 and 38 respectively. The outputs 22 and 41 from these are digitized and interleaved using a pre-determined algorithm, to create a high resolution, wide dynamic range signal dedicated to the C^{th} spectral region of interest. Bandpass filters 17 and 37 and attenuating components 18 and 37 are included in embodiments where required. Additional beamsplitters with associated filter cells, 42, can be added to extend the dynamic range for each of the C colours of interest. In this case the reflective mirror 23 can be replaced with a beamsplitter.

Figure 2 illustrates in more detail how the wide dynamic range signal for the first luminescence colour, described in Figure 1, is created. The collected luminescence 2 is

again transferred to a beamsplitter 4 that splits the light into 2 paths. Again for this first colour, the majority of light is detected using filter cell 5 which consists of a dichroic mirror 6 which reflects the wavelengths of interest and transmits the remaining spectrum to adjacent filter cells (not shown). The reflected light from the dichroic mirror 6 in this filter cell 5 is transferred to 1 or more high gain semiconductor optical sensors 9 via a bandpass filter 7 and an attenuating component 8. The remaining light from the beamsplitter 4 for the first colour of interest is detected by filter cell 24. Again, the light is reflected from the dichroic mirror 25 and transferred to 1 or more high gain semiconductor optical sensors 28 via a bandpass filter 26 and an attenuating component 27. Each of the outputs 20 and 39 from the sensor components 9 and 28 are connected to analogue front-end (AFE) components, 43 and 47, to convert the respective photocurrents 20 and 39 to analogue voltages 44 and 48 using transimpedance amplifiers or similar. These analogue signals are digitized using analogue to digital convertors (ADC) 45 and 49 and these digital signals 46 and 50 are transferred to a signal processing unit 51 that contains a pre-determined algorithm for this first colour. This algorithm combines and weights the digital outputs 46 and 49 for the spectral range of interest. This produces a pre-determined, instantaneous, wide dynamic range signal 52 for this colour of interest. The high gain settings of the sensors 9 and 28 maintain high resolution in the detection system over this dynamic range.

Figure 3 illustrates the difference between the gain versus bias voltage profile of an industry standard high gain sensor photomultiplier tube (PMT) compared to a high gain semiconductor optical sensor. The PMT 57 produces a linearly increasing internal gain 53 profile with increasing PMT bias 54. The internal gain profile for high gain semiconductor optical sensors such as SiPMs 56 is non-linear and varies greatly with SiPM bias 55 as shown in Figure 5. There is a limited region 58 for SiPMs where the gain profile is linear and of similar magnitude to PMTs. Therefore, to preserve high resolution in detection system using high gain semiconductor optical sensors, it is important to pre-tune the sensors' biases to be in this high gain region 58. As demonstrated in Figure 4 and 5, moving away from this high gain region 58 in this type of detection system can increase dynamic range but at the expense of resolution.

Figure 4 illustrates that a compromise between resolution and dynamic range is required for high gain semiconductor optical sensors, when varying their bias voltage or gain. This is of particular importance for solid-state sensors with internal gain such as SiPMs, where the gain is increased in the sensor by increasing its bias which enhances its ability to resolve between cellular samples of similar but different optical intensities.

It will be appreciated that high resolution in the detection system can be achieved by increasing the bias voltage to raise the detected luminescence from dimly stained samples above the noise floor of the system while also keeping a brighter sample of interest within the linear range of an individual sensor. To maximise the resolution in the sensor, requires increasing this bias to the point where the detected luminescence intensity between these two samples no longer increases, which is termed high resolution in this specification.

However, this increase in resolution 59 comes at the expense of dynamic range 60 for an individual high gain semiconductor optical sensor9, as the sensor output would reach its saturation limit 61 at lower optical intensities. Resolution 59 here corresponds to the photocurrent output 20 of an optical sensor, its analogue voltage output 44 or its digitised equivalent 46. The saturation limit 61 corresponds to the optical sensor's photocurrent limit. As the gain of the sensor 9 or its bias voltage is reduced to detect brighter optical components and ensure it does not reach its saturation limit 61, the resolution 59 of the sensor decreases even though its dynamic range 60 increases. Therefore, reducing the bias voltage of the secondary sensor(s) to detect brighter optical powers from cellular or biological samples decreases the resolution of the sensor(s) despite expanding the dynamic range of the system. To counteract this resolution versus dynamic range compromise, the present invention uses optical power splitting to multiple sensors using beam splitters or uncoated mirrors to maintain the resolution of the sensors in high gain mode.

Figure 5 also illustrates the resolution versus dynamic range compromise of the high gain semiconductor optical sensors. This figure shows the output response or resolution 59 from a single sensor 63 over the first optical range of interest 66. The output response from a secondary sensor 64 over the second optical range of interest 67 is also

shown. The output response 64 generated in this case is done so by reducing the bias voltage or the gain of the sensor to look at this range 67 without saturating the sensor's output. The combination of these 63, 64 would produce a wide dynamic range detection system over these two optical ranges, 66, 67 and up to an N^{th} optical range of interest 68 if N optical sensors were used. However, this system would be limited with regard to resolving between samples of interest with bright optical components. To overcome these resolution limitations, an ideal wide dynamic range detection system with additional high resolution is depicted by the system's output 65, where the light falling on the sensors in the optical ranges 67up to 68 is attenuated using beamsplitter components or similar instead of reducing the sensors' gain. The system's output 65 per colour of interest is a combination of the sensor output 63 over the first optical range 66 and this is interleaved with the output from a secondary sensor which has the light levels falling on it attenuated, as described.

Defining wide dynamic range is achieved by using two or more sensors. The output 65 can produce a dynamic range dependent on the number of the sensors used in the detection system, while keeping the optimal resolving capability of the first sensor over the complete range. This compares to just three decades of dynamic range for any single sensor based system Any roll-off at the saturation limit of any of the single sensors 17 are pre-determined and eliminated by the pre-determined algorithm per colour in the processing unit.

Figure 6 illustrates another embodiment of the present invention using multiple collection points M to collect luminescence from N high gain semiconductor optical sensors per colour C. Again, splitting the light using a beamsplitter or similar over 1 or more sensors as described in Figure 1 applies here as the method for generating a multi-colour high resolution, wide dynamic range detection system for use in microfluidic applications. Luminescence 69 and 70 emanating from a biological or cellular sample 1 is collected at 1 or M collection points using lens systems 3 to 71. For the luminescence 69 collected at 3, the pre-determined interleaved output for each colour is as described in the description of Figure 1 and 2. In this embodiment the dynamic range is extended further by collecting from M collection points with the dynamic range now determined by N sensors from these M collection points. The detection system described in Figure 1

and 2 can also be represent the detection system 72 connected to the lens system 71 for the M^{th} collection point. In Figure 5 the dynamic range 60 was determined by 1 or more high gain semiconductor optical sensors from 1 collection point, where in relation to Figure 6 the dynamic range 60 represents N sensors from M collection points for each luminescence colour C where required. For each colour C, the outputs of these N sensors are similarly interleaved as described for Figure 2 with a resulting extended dynamic range.

Figure 7 illustrates an embodiment of the present invention using a plurality of light collection points instead of a beam splitting method for generating a high resolution, wide dynamic range detection system. Luminescence 69 to 70 emanating from a biological or cellular sample 1, is collected at 1 or M collection points. The light collected from the first collection point is done by a lens system 3 while the lens system 71 collects the light from the M^{th} collection point. At each of these collection points, the collected luminescence for each colour is transferred to 1 or more sensors. The primary collection lens system 3 transfers the luminescence to filter cells 5, 10 and 15 for the C colours of interest. For the first spectral region of interest, the light is detected using filter cell 5 which consists of a dichroic mirror 6 which reflects the wavelengths of interest and transmits the remaining spectrum to the adjacent filter cell 10. The reflected light from the dichroic mirror 6 is transferred to 1 or more high gain semiconductor optical sensors 9 via a bandpass filter 7 and an attenuating component 8, in embodiments where required. For the second spectral region of interest, the light is detected using filter cell 10 which consists of a dichroic mirror 11 which reflects the wavelengths of interest and transmits the remaining spectrum to the adjacent filter cell 15. The reflected light from the dichroic mirror 11 is transferred to 1 or more high gain semiconductor optical sensors 14 via a bandpass filter 12 and an attenuating component 13, in embodiments where required. For the C^{th} spectral region of interest, the light is detected using filter cell 15 which consists of a reflective mirror 16 which reflects the remaining wavelengths of interest. The reflected light from the dichroic mirror 16 is transferred to 1 or more high gain semiconductor optical sensors 19 via a bandpass filter 17 and an attenuating component 18, in embodiments where required. Typically the sensors 19 in this first collection point do not use an attenuating component as these sensors will be used to detect any dim light components from the luminescence sample

1. For some embodiments this will not be the case. The attenuating components will therefore be typically used in the sensors connected to the remaining M collection points to provide a wide dynamic range while maintaining the sensors in their high internal gain region to sustain resolution for the C colours of interest. Therefore, for additional collection points such as the M^{th} one lens system 71 transfers the luminescence from the sample 1 to filter cells 24, 29 and 34 for the same C colours of interest. For the first spectral region of interest of the M^{th} collection point, the light is detected using filter cell 24 which consists of a dichroic mirror 25 which reflects the wavelengths of interest and transmits the remaining spectrum to the adjacent filter cell 29. The reflected light from the dichroic mirror 25 is transferred to 1 or more high gain semiconductor optical sensors 28 via a bandpass filter 26 and an attenuating component 27 where required. For the second spectral region of interest in the M^{th} collection point, the light is detected using filter cell 29 which consists of a dichroic mirror 30 which reflects the wavelengths of interest and transmits the remaining spectrum to the adjacent filter cell 34. The reflected light from the dichroic mirror 30 is transferred to 1 or more high gain semiconductor optical sensors 33 via a bandpass filter 31 and an attenuating component 32, in embodiments where required. For the C^{th} spectral region of interest for the lens system 71, the light is detected using filter cell 34 which consists of a reflective mirror 35 which reflects the remaining wavelengths of interest. The reflected light from the dichroic mirror 35 is transferred to 1 or more high gain semiconductor optical sensors 38 via a bandpass filter 36 and an attenuating component 37, in embodiments where required.

For each colour component, the photocurrent outputs 20 to 39, 21 to 40 and 22 to 41 from these N sensors are digitized as shown in the Figure 2 description and interleaved using pre-determined algorithms, to create high resolution, wide dynamic range signals.

Figure 8 illustrates another embodiment of the present invention using an array of high gain semiconductor optical sensors for each spectral range of interest at any collection point as a method for generating a multi-colour high resolution, wide dynamic range detection system. Luminescence 69 to 70 emanating from a biological or cellular sample 1 is collected at 1 or M collection points, and focused on an array of main 73, and secondary 74, high gain semiconductor optical sensors. The main sensor, 73, is

positioned and configured so that it receives the maximum amount of collected luminescence. The secondary sensors, 74, are positioned such that they receive light not collected by the main sensor, 73. The main sensor, 73, should have the highest photon collection performance and will be used to measure dim levels of luminescence. The secondary sensors shall be designed to be the main measurement system for the brighter levels of luminescence. The luminescence collected shall be distributed over the sensor array. The area illuminated by the collected luminescence is indicated by the dotted regions 75 and 76. The array may be configured such that the main sensor receives all or most of the collected luminescence as indicated by region 76. It may also be configured such that a greater portion of the luminescence is distributed over the secondary sensors, 74 using the illumination region 75. The beam size will ultimately determine the dynamic range associated with each sensor array per colour when the outputs combined and weighted using pre-determined algorithms as discussed in other embodiments. The illumination region should ideally match the shape of the sensor array to optimize the amount of light collected; however in this embodiment a Gaussian beam is used for illustration purposes. An attenuating component, such as a lens system in this case, as used in the filter cells of Figure 1-2, 6-7 adjusts the illumination spot sizes 75 and 76. To achieve such spot size adjustment, the lens system can be moved relative to sensor array or otherwise the array is moved relative to the lens system.

It will be appreciated that the invention can be incorporated into multiple microfluidic analytical instruments including portable point of care analysers. This invention enables the various embodiments of such instruments presented herein. Such instruments include, for example, and without limitation, immunoassay analysers, clinical haematology analysers, flow and scanning cytometers, fluorimeters, and chemistry analysers. The specific biological targets can be in a microfluidic platform, where luminescence detection at different wavelengths over a wide optical range while maintaining high resolution can be achieved with the present invention.

The invention is not limited to the embodiments hereinbefore described but may be varied in both construction and detail.

Claims

1. A multi-colour luminescence detection system in a microfluidic system, said detection system comprising:

means for splitting light into at least two light paths for each detected colour of luminescence from a cellular or biological sample in the microfluidic system;

a first high gain semiconductor optical sensor, for each luminescence colour component, adapted to be biased at an optimal operating voltage or gain for detecting low light levels of luminescence emitted from the cellular or biological sample with high resolution;

a second high gain semiconductor optical sensor for each colour component adapted to detect higher light levels of luminescence, such that the light level falling on the second sensor is attenuated while maintaining resolution with a high gain setting; and

means for processing per luminescence colour by combining and weighting the outputs from the first and second high gain semiconductor optical sensors using a signal processing unit, and providing an instantaneous multi-colour high resolution detection system over an extended dynamic range.

2. A detection system as claimed in claim 1 wherein the high gain semiconductor optical sensor comprises of a SiPM sensor.

3. A detection system as in claim 1 or 2 comprising means for splitting the collected luminescence into specific wavelengths of interest using a coated beamsplitters/dichroic mirror and at least one optical filter.

4. A detection system as claimed in claim 1 comprising a beam splitter adapted to split light collected from the sample and couple it to the high gain semiconductor sensors for each colour, wherein the split ratio of the beam splitter is used to attenuate the light to the second sensor and used to detect the higher optical power components from the sample.

5. A detection system as claimed in any preceding claim wherein more than one optical detection point is used to collect the light from the emitted sample to generate an instantaneous high resolution signal over a wide dynamic range per colour.

6. A detection system as claimed in any preceding claim wherein light emitted from the sample is coupled to a collector and passed through at least one optical component to attenuate the light level and delivered to said second optical sensor.

7. A detection system as claimed in any preceding claim comprising a collector adapted to collect light from the sample and directed to a beam splitter before being passed to each filter cell.

8. A detection system as claimed in claim 6 wherein the collector comprises a lens system.

9. A detection system as claimed in claims 6 or 7 wherein the beam splitter is adapted with two or more output ports for delivering light to each filter cell per colour

10. A detection system as claimed in claim 7 wherein the beam splitter comprises an uncoated mirror, said mirror adapted to deliver maximum amount of light via a first port to the first high gain semiconductor optical sensor detecting low-end light levels while bright light components of the sample are detected simultaneously using the remaining light at the second port by said second sensor.

11. A detection system as claimed in claim 9 wherein the amount of light delivered from said first and second ports is dependent on the transmission or reflectance of the uncoated beam splitter.

12. A method of multi-colour luminescence detection in a microfluidic system, said method comprising the steps of:

splitting light into at least two light paths for each detected colour of luminescence from a cellular or biological sample in the microfluidic system;

biasing a first high gain semiconductor optical sensor, for each luminescence colour component, at an optimal operating voltage or gain for detecting low light levels of luminescence emitted from the cellular or biological sample with high resolution;

biasing a second high gain semiconductor optical sensor for each colour component adapted to detect higher light levels of luminescence, such that the light level falling on the second sensor is attenuated while maintaining resolution with a high gain setting; and

processing per luminescence colour by combining and weighting the outputs from the first and second high gain semiconductor optical sensors using a signal processing unit, and providing an instantaneous multi-colour high resolution detection system over an extended dynamic range.

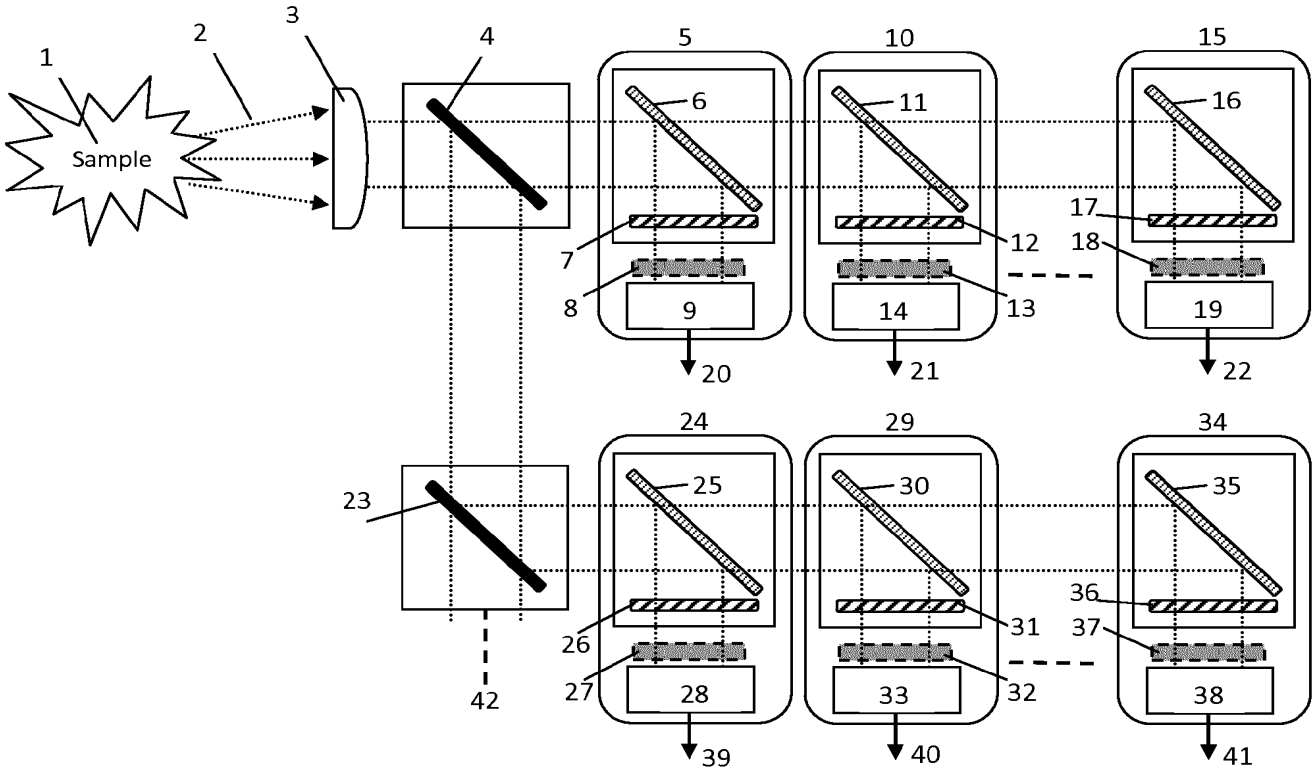

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Figure 1

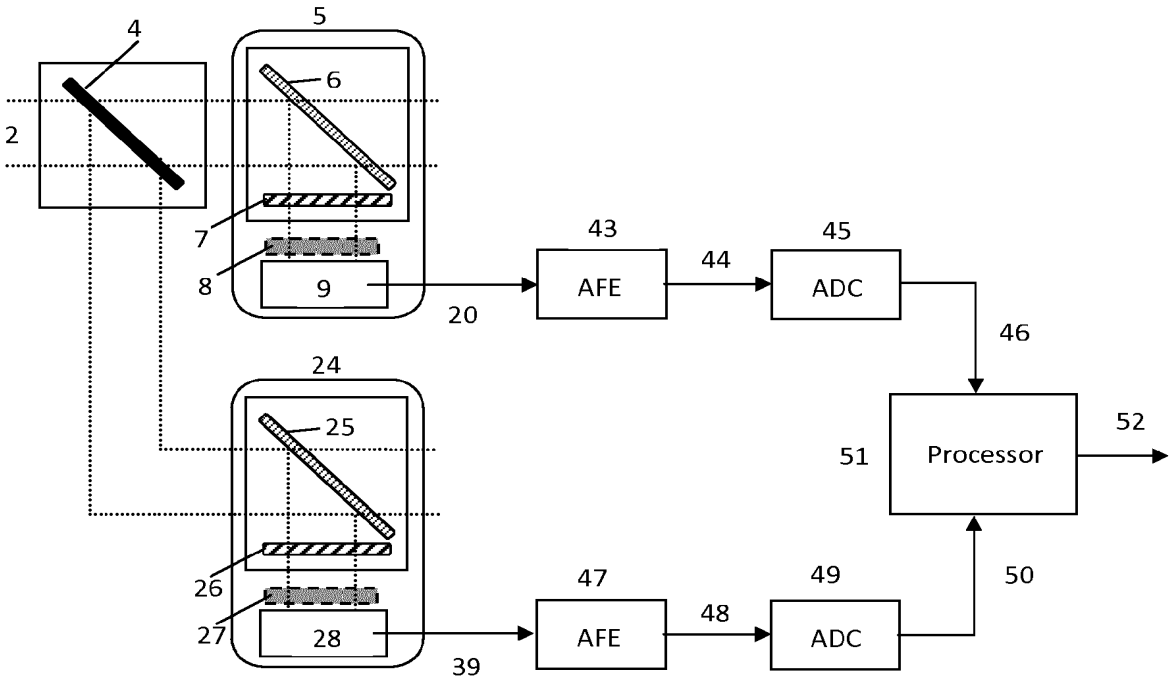

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Figure 2

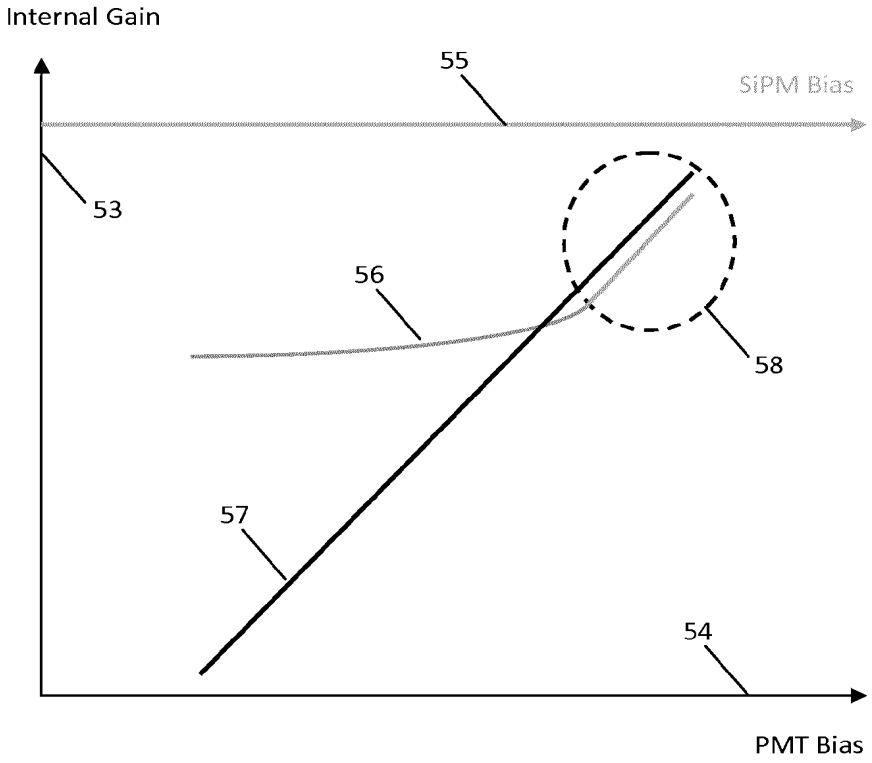


Figure 3

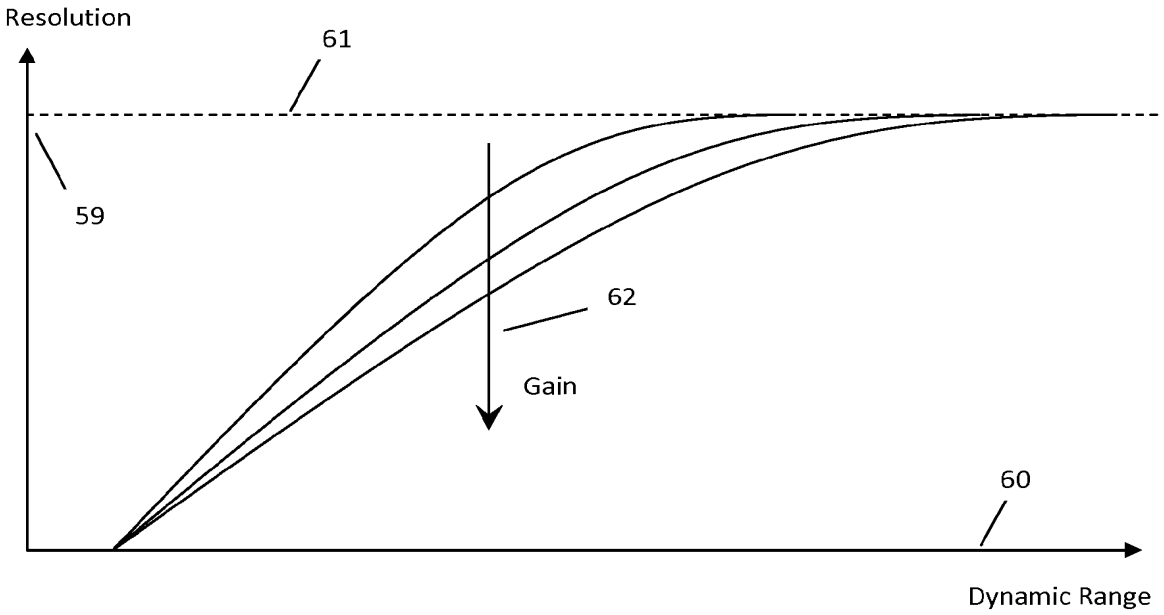


Figure 4

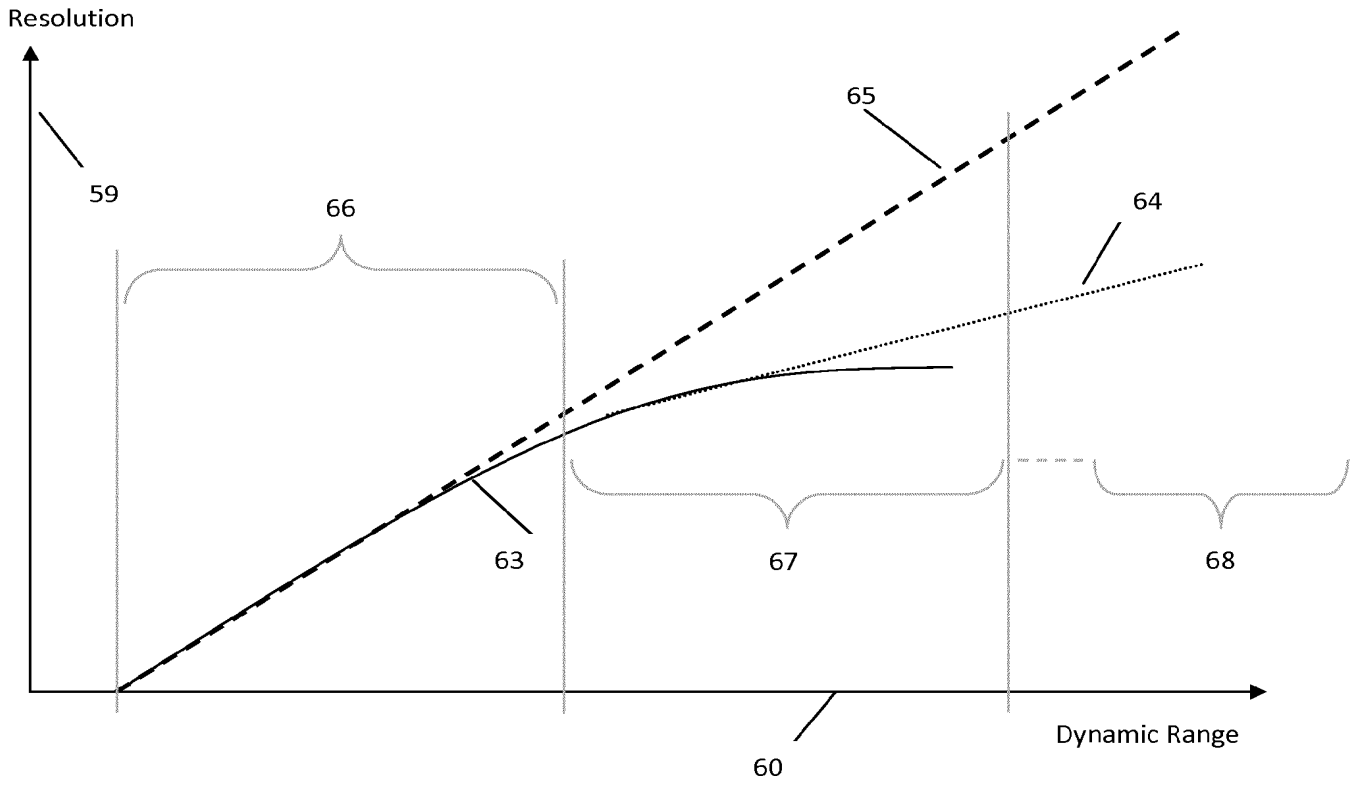


Figure 5

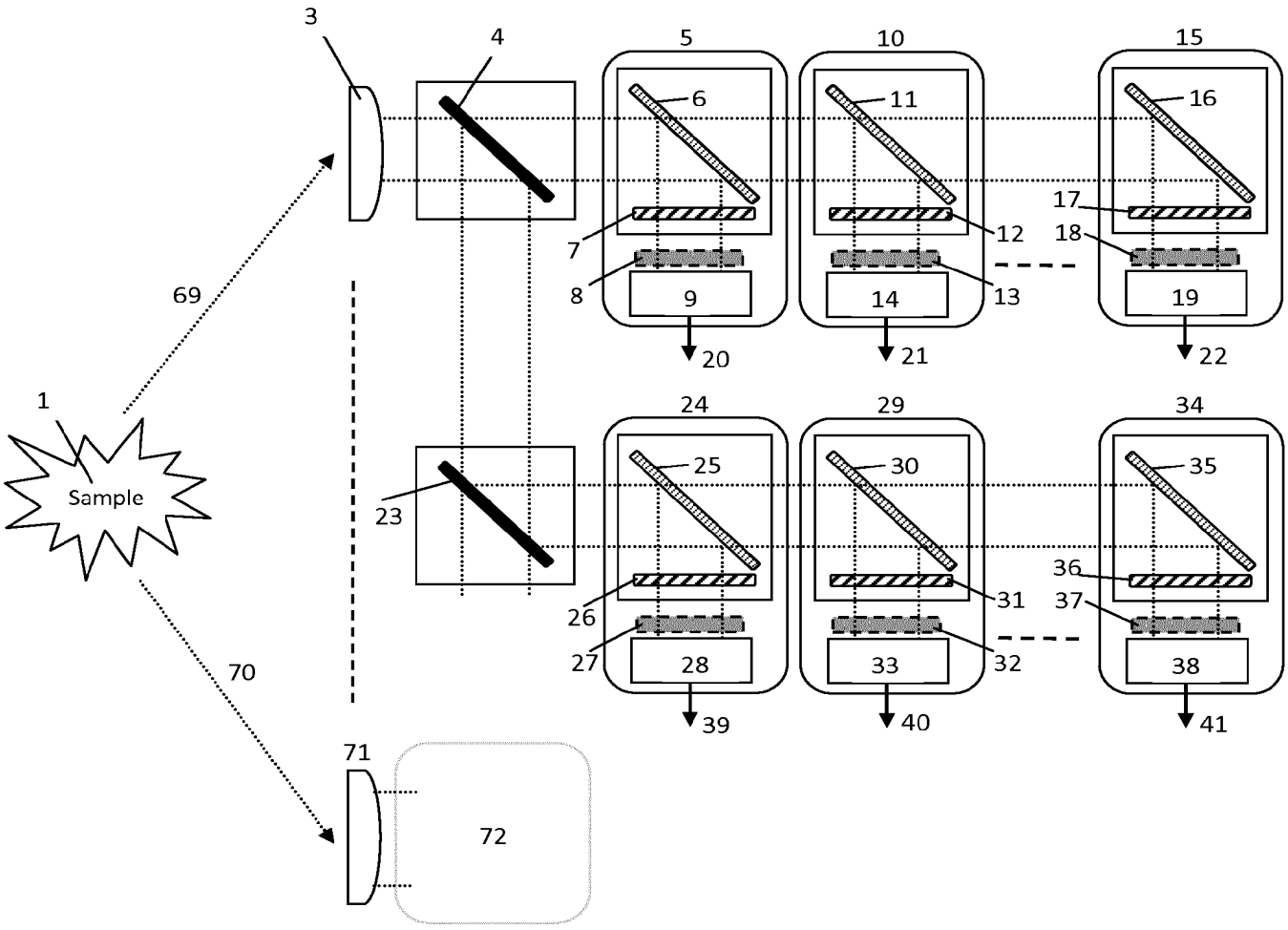

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Figure 6

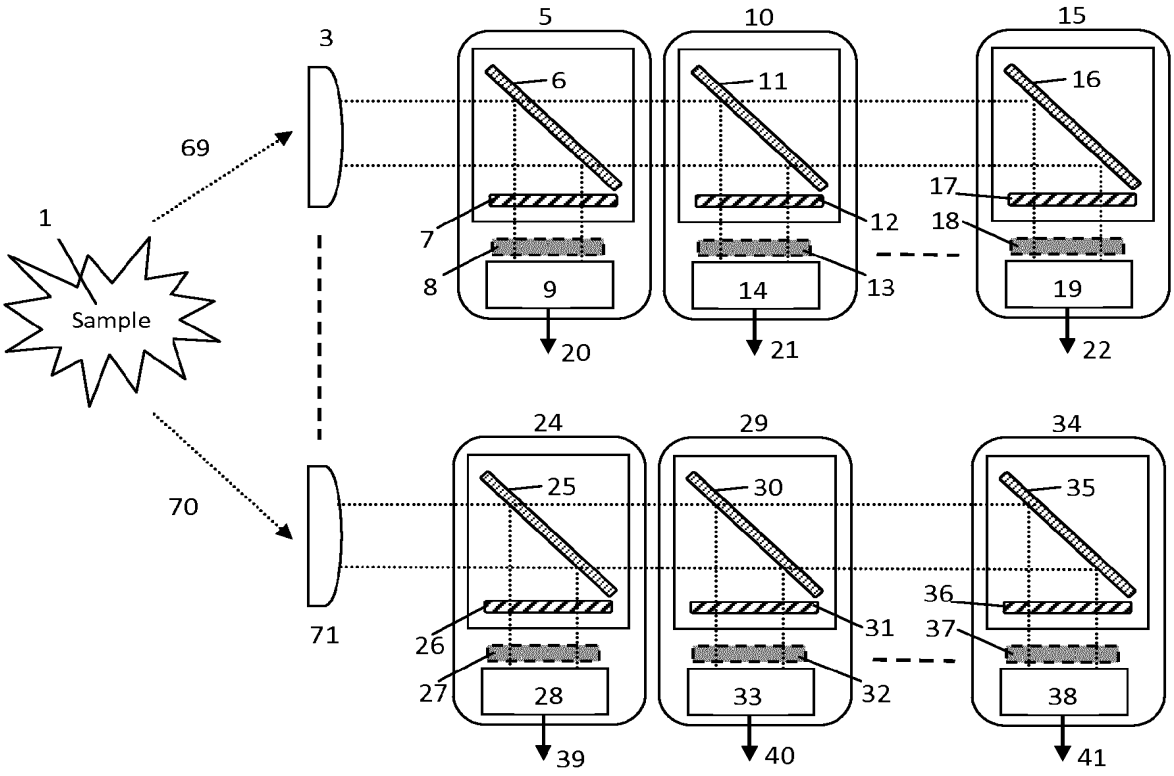

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Figure 7

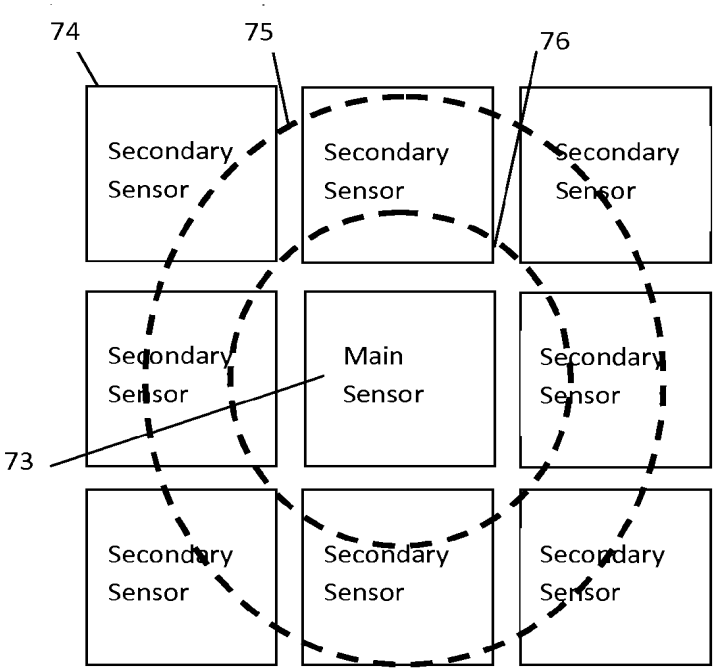

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Secondary Sensor
Secondary Sensor
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Secondary Sensor

Figure 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/076719

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01J1/18 G01J1/42 G01J1/04 G01J3/36 G01N15/14
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01J G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/026942 A2 (RADISENS DIAGNOSTICS LTD [IE]; BARRY LEE [IE]; BRIEN JEREMIAH O [IE]) 10 March 2011 (2011-03-10) cited in the application page 4, line 18 - line 27 page 13, line 13 - page 15, line 11 page 20, line 20 - page 21, line 27; figures 1,11	1,2,5,6, 8

X	US 2005/151964 A1 (ROTH WAYNE D [US]) 14 July 2005 (2005-07-14)	1-12
Y	paragraph [0037] - paragraph [0048] paragraph [0053] - paragraph [0058]; figure 1	1,2,12

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 April 2013	24/04/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Besser, Veronika

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2003/058433 A1 (ALMOGY GILAD [IL] ET AL) 27 March 2003 (2003-03-27) paragraph [0026] paragraph [0052] - paragraph [0054] paragraph [5863]; figure 1 -----	1,2,12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/076719

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011026942 A2	10-03-2011	EP 2293032 A1	09-03-2011
		EP 2473825 A2	11-07-2012
		JP 2013504051 A	04-02-2013
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		WO 03027651 A1	03-04-2003

Electronic Acknowledgement Receipt

EFS ID:	22546358
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	129381
Filer:	Gina N. Shishima/Ben Horton
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US.CIP(12US0040
Receipt Date:	05-JUN-2015
Filing Date:	26-NOV-2014
Time Stamp:	10:57:34
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		IDS.pdf	961901 0f4668146293 6ed398246d5f4fabeb439b76480f	yes	12

	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
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	Information Disclosure Statement (IDS) Form (SB08)		3	12	
Warnings:					
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2	Non Patent Literature	Brogioli_2002.PDF	129664 ba76ece2c16fc3aa788890fff2f5a9497b8b770f	no	3
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3	Foreign Reference	CN102087198A.PDF	417874 7d53ffde60c76c1f8215d39dfd7c7537664a3be7	no	7
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8	Foreign Reference	CN201936066U.PDF	417948 8ee75967e2b818331eecaa1f6c6c09bb296cf141	no	9
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13	Foreign Reference	EP0757346B1.PDF	764687 febdcf654a91dda6753d150df4013c78047feab1	no	12
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14	Foreign Reference	EP0758755A2.PDF	389920 c69647083b2d05aa81517614cedddfcc30c3e134	no	8
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16	Foreign Reference	EP1004907B1.PDF	96477 47f861bfe44872361738c6b4be42f1b50409db8a	no	11
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17	Foreign Reference	EP1953526A2.PDF	408797 2467eb599eaa2eefb37eb9d47165492e4482ce60	no	31
Warnings:					
Information:					

18	Foreign Reference	EP2381475B1.PDF	753878 bb91e379393df7932484c88701a62deb6558742c	no	14
Warnings:					
Information:					
19	Foreign Reference	JPH05333245A.PDF	196161 00718e5242f8c2d146f5a065503d1641e455ac63	no	7
Warnings:					
Information:					
20	Other Reference-Patent/App/Search documents	PCT_US2013_043453_ISR.PDF	560541 ecc6846c41536da6e96534adbdec34b16a7f0985	no	11
Warnings:					
Information:					
21	Other Reference-Patent/App/Search documents	PCT_US2014_067682_ISR.PDF	1321827 98d2bf6e45683e0a58d3d31b1b9bdff546848591	no	17
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22	Non Patent Literature	Schmidt_1932.PDF	4747622 91324b04c93c7dd13648580458O5c5b36ca01cb6	no	5
Warnings:					
Information:					
23	Non Patent Literature	Smiley_1936.PDF	190030 ba929cf4203038d3e6fea517ae9a6c9e70d6fe37	no	7
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24	Foreign Reference	JP08029726A.pdf	1841765 10e34fe0d97b399943a643d5f477392e5da0e69b	no	16
Warnings:					
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25	Foreign Reference	WO0057173A1.PDF	521767 5d5b1a5429a8022b2ed84dadec5052385544d073	no	13
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26	Foreign Reference	WO0127590.PDF	705227 527d9a460d11b3521969089a3496c2e85604314f	no	20
Warnings:					
Information:					

27	Foreign Reference	WO0140764.PDF	317274 430a7ca526260150d9f3569e092d8384d5ffa34b	no	29
Warnings:					
Information:					
28	Foreign Reference	WO03012403A1.PDF	1380407 9acef3eb0a706470bae41a05308bd531a66e2788	no	30
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29	Foreign Reference	WO9307471A1.PDF	430459 980bef2b24fe7375fbfe689b683c5d22b48a9e4d	no	35
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30	Foreign Reference	WO9316368A1.PDF	996048 94e0bdd961ce99a3f5b508fab9e51f67155f3dee	no	34
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31	Foreign Reference	WO9429695A1.PDF	195196 72f652ec9edcb498fd51541542e8f4b633ec0ddf	no	17
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32	Foreign Reference	WO9714066A2.PDF	722443 3a22d7ffdcc32cecc557f9e231d233f9e7bd8659	no	17
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36	Foreign Reference	WO2009098867A1.PDF	798954 80dc378093fc97f3186cda7c691ae03c7866e905	no	23
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37	Foreign Reference	WO2011026942A2.PDF	230184 4e83faf433a10c8f0a3b78aa6c1ff185b651d9e5	no	34
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38	Foreign Reference	WO2012056217A1.PDF	1326693 fcb931645766a9421c0da6fbbe79b958a0b9ef06	no	37
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39	Foreign Reference	WO2012177367A2.PDF	1934559 d0c190d95e9d0723f5bd814c859933b382b1a31e	no	39
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40	Foreign Reference	WO2013013229A1.PDF	5110407 dde479dc31414e0067fe56588538ef28f55dc402	no	102
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41	Foreign Reference	WO2013093035A1.PDF	209038 965ee6504fbbc231d800e47c6bf7b560490db5c0	no	34
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Information:					
		Total Files Size (in bytes):	37247614		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Yong Qin Chen	Group Art Unit: 2884
Serial No.: 14/555,102	Examiner: Mindy D. Vu
Filed: November 26, 2014	Atty. Dkt. No.: BECC.P0002US.CIP
For: Flow Cytometer	Confirmation No.: 4589

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

6/5/15
Date

Gina N. Shishima

INFORMATION DISCLOSURE STATEMENT

Commissioner:

In compliance with the duty of disclosure under 37 C.F.R. § 1.56, it is respectfully requested that this Information Disclosure Statement be entered and the documents listed on attached Form PTO-1449 be considered by the Examiner and made of record. Copies of the listed documents required by 37 C.F.R. § 1.98(a)(2) are enclosed for the convenience of the Examiner.

In accordance with 37 C.F.R §§ 1.97(g), (h), this Information Disclosure Statement is not to be construed as a representation that a search has been made, and is not to be construed to be an admission that the information cited is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

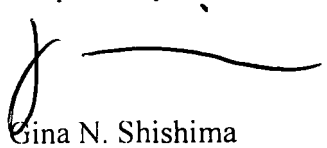
The present Information Disclosure Statement is being filed prior to the receipt of a first Official Action reflecting an examination on the merits, and hence is believed to be timely filed

in accordance with 37 C.F.R. § 1.97(b). No fees are believed to be due in connection with the filing of this Information Disclosure Statement, however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be deemed necessary for any reason relating to these materials, the Commissioner is authorized to deduct the appropriate fees from Norton Rose Fulbright US LLP Deposit Account No.: 50-1212/BECC.P0002US.CIP.

Please note that an English translation of reference C4 (Schmidt, 1932) is not included per 37 C.F.R. § 1.98(a)(3)(ii) because it is not readily available. The explanation of relevance of this reference can be found in the Specification of the subject application. Similar contents to the Schmidt reference can be found in reference C5 (Smiley, 1936) submitted herewith.

Applicants respectfully request that the listed documents be made of record in the present case.

Respectfully submitted,



Gina N. Shishima
Reg. No. 45,104
Attorney for Applicants

NORTON ROSE FULBRIGHT US LLP
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Austin, TX 78701
(512) 474-5201

Date: 6/5/15

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040	4589

129381 7590 04/20/2016

NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

EXAMINER
VU, MINDY D

ART UNIT	PAPER NUMBER
2884	

NOTIFICATION DATE	DELIVERY MODE
04/20/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

aoipdocket@nortonrosefulbright.com
PatentDocket@Beckman.com

Office Action Summary	Application No. 14/555,102	Applicant(s) CHEN, YONG QIN	
	Examiner MINDY VU	Art Unit 2884	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *364-386* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *364-386* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 364- 382, drawn to a flow cytometer having a wavelength division multiplexer, classified in G01N 15/14.

II. Claim 383, drawn to a flow cytometer having a composite microscope objective, classified in G01N 2015/1452.

III. Claim 384, drawn to a flow cytometer having a fluidic system, classified in G01N 15/1404.

IV. Claim 385, drawn to a flow cytometer having a peristaltic pump, classified in G01N 15/1404.

V. Claim 386, drawn to a flow cytometer having a laser diode system, classified in G01N 15/1434.

The inventions are distinct, each from the other because of the following reasons:

Inventions I and II, III, IV, V are related as combination and subcombination. Inventions in this relationship are distinct if it can be shown that (1) the combination as claimed does not require the particulars of the subcombination as claimed for patentability, and (2) that the subcombination has utility by itself or in other combinations (MPEP § 806.05(c)). In the instant case, the combination as claimed does not require the particulars of the subcombination as claimed because a composite

microscope objective, a fluidic system, a peristaltic pump, or a laser diode based optical system are not required by the claimed combination. The subcombination has separate utility such as can be used in systems other than flow cytometer.

The examiner has required restriction between combination and subcombination inventions. Where applicant elects a subcombination, and claims thereto are subsequently found allowable, any claim(s) depending from or otherwise requiring all the limitations of the allowable subcombination will be examined for patentability in accordance with 37 CFR 1.104. See MPEP § 821.04(a). Applicant is advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

Inventions II, III, IV and V are related as subcombinations disclosed as usable together in a single combination. The subcombinations are distinct if they do not overlap in scope and are not obvious variants, and if it is shown that at least one subcombination is separately usable. In the instant case, subcombination II has separate utility such as a composite microscope objective. In the instant case, subcombination III has separate utility such as a fluidic system. In the instant case, subcombination IV has separate utility such as a peristaltic pump. In the instant case, subcombination V has separate utility such as a laser diode based optical system. See MPEP § 806.05(d).

The examiner has required restriction between subcombinations usable together. Where applicant elects a subcombination and claims thereto are subsequently found allowable, any claim(s) depending from or otherwise requiring all the limitations of the allowable subcombination will be examined for patentability in accordance with 37 CFR 1.104. See MPEP § 821.04(a). Applicant is advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and/or examination burden if restriction were not required because one or more of the following reasons apply:

As set forth above.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention.**

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time

of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other invention.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINDY VU whose telephone number is (571)272-8539. The examiner can normally be reached on M-F 9am - 5:30pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Porta can be reached on 571-272-2444. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/DAVID PORTA/
Supervisory Patent Examiner, Art Unit 2884

/M. V./
Examiner, Art Unit 2884

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14555102	CHEN, YONG QIN
	Examiner	Art Unit
	MINDY VU	2884

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	04/13/2016								
	364	÷								
	365	÷								
	366	÷								
	367	÷								
	368	÷								
	369	÷								
	370	÷								
	371	÷								
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	381	÷								
	382	÷								
	383	÷								
	384	÷								
	385	÷								
	386	÷								

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Yong Qin Chen

Serial No.: 14/555,102

Filed: November 26, 2014

For: FLOW CYTOMETER

Group Art Unit: 2884

Examiner: Vu, Mindy D.

Atty. Dkt. No.: BECC.P0002US.CIP

Confirmation No.: 4589

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

June 3, 2016 — Date

Elizabeth Beasterfield

RESPONSE TO RESTRICTION REQUIREMENT DATED APRIL 20, 2016

Commissioner:

Applicants submit this paper in response to the Restriction Requirement dated April 20, 2016, for which the date for response is June 20, 2016.

Amendments to the Claims begin on page 2.

Remarks begin on page 6.

AMENDMENT TO THE CLAIMS

The listing of claims will replace all prior versions, and listing of claims in the application.

1-363. (Canceled)

364. (Previously presented) A flow cytometer having a wavelength division multiplexer (WDM), the WDM comprising:

an extended light source providing light that forms an object;

a collimating optical element that captures light from the extended light source and projects a magnified image of the object as a first light beam; and

a first focusing optical element configured to focus the first light beam to a size smaller than the object of the extended light source to a first semiconductor detector.

365. (Previously presented) The flow cytometer of claim 364, wherein the first focusing optical element is configured to focus the first light beam to a size smaller than 1 mm in diameter.

366. (Previously presented) The flow cytometer of claim 364, further comprising an image relay optical element arranged to receive a color band of interest of the first light beam, the image relay optical element configured to project a second image as a second light beam, wherein the second light beam is substantially a unit magnification of the first light beam.

367. (Previously presented) The flow cytometer of claim 366, wherein the image relay optical element is one of a refractive optical device and a concave mirror.

368. (Previously presented) The flow cytometer of claim 366, wherein the image relay optical element is a concave mirror having a radius of curvature approximately equal to the distance between the collimating optical element and the magnified image projected thereby.

369. (Previously presented) The flow cytometer of claim 366, further comprising a second focusing optical element configured to focus the second light beam to a size smaller than the object of the extended light source to a second semiconductor detector.

370. (Previously presented) The flow cytometer of claim 369, wherein the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the image relay optical element and the second focusing optical element.

371. (Previously presented) The flow cytometer of claim 366, wherein the image relay optical element is a concave shaped dichroic filter.

372. (Previously presented) The flow cytometer of claim 366, further comprising a dichroic filter disposed between the collimating optical element and the first focusing optical element.

373. (Previously presented) The flow cytometer of claim 372, wherein the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the collimating optical element, the dichroic filter, and the image relay optical element.

374. (Previously presented) The flow cytometer of claim 372, wherein the dichroic filter is bonded to a filter holder such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder.

375. (Previously presented) The flow cytometer of claim 374, wherein the reference surface of the filter holder rests against an optically flat surface of a reference block included in the WDM, thereby providing consistent optical alignment when installing the dichroic filter into the WDM.

376. (Previously presented) The flow cytometer of claim 364, wherein the object is formed by one of light passing through a pinhole and light emitted from a facet of a multimode optical fiber.

377. (Previously presented) The flow cytometer of claim 364, wherein the first light beam focused by the first focusing optical element is approximately the same size as the effective size of the collimating optical element.

378. (Previously presented) The flow cytometer of claim 364, wherein the size of the magnified image of the object is less than approximately ten times the size of the object of the extended light source.

379. (Previously presented) The flow cytometer of claim 364, further comprising a composite microscope objective to direct light emitted by a particle in a flow channel in a viewing zone of the composite microscope to the extended light source, the particle illuminated by an illumination light, the composite microscope objective including,

a spherical mirror located on a first side of the viewing zone, the spherical mirror configured to reflect the emitted light, and,

an aberration corrector plate located on a second side of the viewing zone opposite the first side, the aberration corrector plate configured to reduce optical aberrations in the reflected light caused by the spherical mirror.

380. (Previously presented) The flow cytometer of claim 379, further comprising a fluidic system configured to supply liquid sheath to the flow channel, the fluidic system having a T-coupling arrangement configured to receive the liquid sheath from a reservoir and to direct a first fraction of the liquid sheath back to the reservoir via a bypass conduit and a second fraction of the liquid sheath to the flow channel.

381. (Previously presented) The flow cytometer of claim 379, further comprising a peristaltic pump configured to supply liquid sample to the flow channel, the peristaltic pump including a pump housing having a arcuate curved track formed therein, a plurality of rollers,

and a compressible tube sandwiched between the rollers and the arcuate curved track, the arcuate curved track further including at least one recess section located between at least two pumping sections along the arcuate curved track.

382. (Previously presented) The flow cytometer of claim 379, further comprising a beam compressing optical element configured to compress the illumination light along an axis perpendicular to the direction of the flow channel.

383-386. (Canceled)

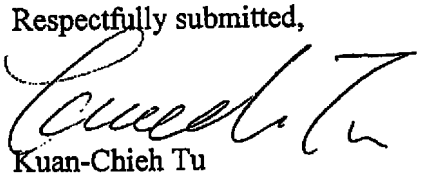
REMARKS

In response to the restriction requirement, Applicants hereby elect the claims identified with respect to Group I, Claims 364-382 for continued examination, without traverse Accordingly, claims 383-386 are canceled, all of which are directed to non-elected inventions. No new matter is added. Applicants reserve the right to reintroduce these claims upon an allowance of an appropriate generic claim.

It is believed that no fees are due; however, should any fees be required under 37 C.F.R. §§ 1.16 to 1.21 in connection with the filing of this paper, the Commissioner is authorized to deduct said fees from Norton Rose Fulbright US LLP Account No.: 50-1212/BECC.P0002US.CIP/11412161.

The Examiner is invited to contact the undersigned attorney with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



Kuan-Chieh Tu
Reg. No.: 70,414
Attorney for Applicants

NORTON ROSE FULBRIGHT US LLP
98 San Jacinto Blvd., Suite 1100
Austin, Texas 78701
(512) 474-5201

Date: 6.3.2016

Electronic Acknowledgement Receipt

EFS ID:	25968671
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	129381
Filer:	R Ross Viguet/Elizabeth Beasterfield
Filer Authorized By:	R Ross Viguet
Attorney Docket Number:	BECC.P0002US.CIP(12US0040
Receipt Date:	03-JUN-2016
Filing Date:	26-NOV-2014
Time Stamp:	17:30:49
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		BECCP0002USCIP_RespToRestriction_6-3-16.pdf	385379 faddd4bce4474faabc01643ebab612d5c951529e	yes	6

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response to Election / Restriction Filed	1	1
	Claims	2	5
	Applicant Arguments/Remarks Made in an Amendment	6	6
Warnings:			
Information:			
	Total Files Size (in bytes):	385379	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP	Serial No.: 14/555,102
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: Iris International, Inc.	
	Filing Date: November 26, 2014	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*none*	*See Page 1*

U.S. Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A167	2004165828	8/26/04	Capewell et al.	385	47	3/1/04
	A168	6159686	12/12/00	Kardos et al.	435	6	1/30/98

Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C6	Extended European Search Report for EP 13796734.5, mailed March 18, 2016.
	C7	Goda et al., "Serial time-encoded amplified imaging for observation of fast dynamic phenomena", *Nature* 458:1145-1149, 2009.

42271887.1

EXAMINER:	DATE CONSIDERED:

EXAMINER: INITIAL IF REFERENCE CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED. INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

Electronic Acknowledgement Receipt

EFS ID:	25968632
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	129381
Filer:	Gina N. Shishima/Ben Horton
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US.CIP(12US0040
Receipt Date:	03-JUN-2016
Filing Date:	26-NOV-2014
Time Stamp:	17:29:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		IDS.pdf	261098 874063112cd1a97031bc0cc0716b5bc3360892e5	yes	3

	Multipart Description/PDF files in .zip description			
	Document Description	**Start**	**End**	
	Transmittal Letter	1	2	
	Information Disclosure Statement (IDS) Form (SB08)	3	3	
Warnings:				
Information:				
2	Other Reference-Patent/App/Search documents	EESR.pdf	431053 175e0b1b131b29120802dae7156b0d51601ed6d8	no	9
Warnings:				
Information:				
3	Non Patent Literature	Goda_2009.pdf	745218 3fde7d1a1629243529011060l959a43dace6a85d	no	6
Warnings:				
Information:				
		Total Files Size (in bytes):	1437369	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Iris International, Inc.	Group Art Unit: 2884
Serial No.: 14/555,102	Examiner: Mindy D. Vu
Filed: November 26, 2014	Atty. Dkt. No.: BECC.P0002US.CIP
For: FLOW CYTOMETER	Confirmation No.: 4589

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

June 3, 2016	/Kuan-Chieh Tu/
Date	Kuan-Chieh Tu

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Commissioner:

In compliance with the duty of disclosure under 37 C.F.R. § 1.56, it is respectfully requested that this Supplemental Information Disclosure Statement be entered and the documents listed on attached Form PTO-1449 be considered by the Examiner and made of record. Copies of the listed documents required by 37 C.F.R. § 1.98(a)(2) are enclosed for the convenience of the Examiner.

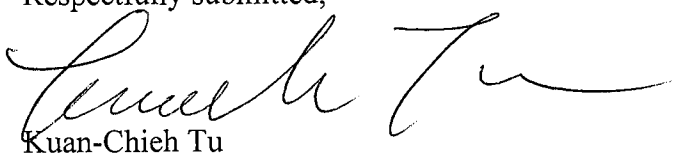
In accordance with 37 C.F.R §§ 1.97(g), (h), this Supplemental Information Disclosure Statement is not to be construed as a representation that a search has been made, and is not to be construed to be an admission that the information cited is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

The present Supplemental Information Disclosure Statement is being filed prior to the receipt of a first Official Action reflecting an examination on the merits, and hence is believed to

be timely filed in accordance with 37 C.F.R. § 1.97(b). No fees are believed to be due in connection with the filing of this Supplemental Information Disclosure Statement, however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be deemed necessary for any reason relating to these materials, the Commissioner is authorized to deduct the appropriate fees from Norton Rose Fulbright US LLP Deposit Account No.: 50-1212/BECC.P0002US.CIP/11412161.

Applicants respectfully request that the listed documents be made of record in the present case.

Respectfully submitted,



Kuan-Chieh Tu
Reg. No.: 70,414
Attorney for Applicants

NORTON ROSE FULBRIGHT US LLP
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701
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Date: 6-3-2016

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/555,102	Filing Date 11/26/2014	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

		(Column 1)		(Column 2)	(Column 3)		
	06/03/2016	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	* 19	Minus	** 23	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***5	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

		(Column 1)		(Column 2)	(Column 3)		
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/FLORENCE PATTERSON/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040	4589

129381 7590 06/27/2016

NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

EXAMINER	
VU, MINDY D	

ART UNIT	PAPER NUMBER
2884	

NOTIFICATION DATE	DELIVERY MODE
06/27/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

aoipdocket@nortonrosefulbright.com
PatentDocket@Beckman.com

Office Action Summary	Application No. 14/555,102	Applicant(s) CHEN, YONG QIN	
	Examiner MINDY VU	Art Unit 2884	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06/03/16*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *364-382* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *364-382* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *11/26/14* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *06/05/15,06/03/16*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

Claims 383-386 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected inventions, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on June 03, 2016.

Claims 383-386 are canceled in the reply filed on June 03, 2016.

Information Disclosure Statement

Where the IDS citations are submitted but not described, the examiner is only responsible for cursorily reviewing the references. The initials of the examiner on the PTO-1449 indicate only that degree of review unless the reference is either applied against the claims, or discussed by the examiner as pertinent art of interest, in a subsequent office action. See Guidelines for Reexamination of Cases in View of In re Portola Packaging, Inc., 110 F.3d 786, 42 USPQ2d 1295 (Fed. Cir. 1997), 64 FR at 15347, 1223 Off. Gaz. Pat. Office at 125 (response to comment 6). Consideration by the examiner of the information submitted in an IDS means that the examiner will consider the documents in the same manner as other documents in Office search files are considered by the examiner while conducting a search of the prior art in a proper field of search. The initials of the examiner placed adjacent to the citations on the PTO-1449 or PTO/SB/08A and 08B or its equivalent mean that the information has been considered by the examiner to the extent noted above. MPEP § 609 (Eighth Edition, August 2001).

The examiner notes that due to the unusually large number of references cited, and the absence of any description of the relevance of the references, it should be assumed that only the most cursory review of the cited documents consistent with these guidelines has been performed. If applicant is aware of any information that might be of particular relevance, it should be pointed out in order to insure a higher degree consideration.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 364 and 366-379 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Oostman, Jr. et al. (US 6,683,314, hereafter Oostman) in view of Capewell et al. (US 2004/0165828, hereafter Capewell).

With respect to independent Claim 364, Oostman discloses a flow cytometer (column 9 lines 4-5, fig. 15) having a wavelength division multiplexer (WDM) (col. 6 lines 40-65, fig. 5), the WDM comprising:

an extended light source providing light that forms an object (fig. 5, optical fiber 123);

a collimating optical element that captures light from the extended light source and projects a magnified image of the object as a first light beam (fig. 5, collimating lens 145); and

a first focusing optical element configured to focus the first light beam to a first semiconductor detector (fig. 5, focusing element 158).

Oostman does not explicitly disclose the focusing optical element configured to focus the first light beam to a size smaller than the object of the extended light source to a first semiconductor detector. In a similar field of endeavor, Capewell discloses an optical system comprising a WDM having a paraxial optical assembly and an aspheric lens to focus the first beam on the detector [0027,0039] (figs. 5a, 5b, 6). Moreover, Capewell discloses that with the paraxial optical assembly, the size of the focused light beam can be minimized in order to maximize the tolerance to detector misalignment [0027, 0038]. In particular, with the WDM of Capewell, it is possible to de-magnify the image of the object of the extended light source on the photodetector. In view of this teaching, it would have been obvious to one of ordinary skill in the art at the time of the invention was made to modify the focusing element of Oostman to be able to focus to a size smaller than the object of the extended light source in view of increasing the tolerance to detector misalignment.

With respect to Claim 366, Oostman discloses an image relay optical element (beam splitting element 151) arranged to receive a color band of interest of the first light beam, the image relay optical element (1511) configured to project a second image as a

second light beam, wherein the second light beam is substantially a unit magnification of the first light beam (fig. 5, col. 6 lines 40-65).

With respect to Claim 367, Oostman discloses the image relay optical element is one of a refractive optical device and a concave mirror (col. 11 lines 1-3).

With respect to Claim 368, Oostman discloses the image relay optical element is a concave mirror but does not explicitly disclose a radius of curvature approximately equal to the distance between the collimating optical element and the magnified image projected thereby. However, it is within the ordinary skill of one in the art to choose any suitable size for the intended application. Therefore, it would have been obvious to one of ordinary skill in the art before the effective filing date of the claimed invention to adapt the recited ranges in view of the feature is merely a variation in design and the results would have been predictable.

With respect to Claim 369, Oostman discloses a second focusing optical element configured to focus the second light beam to a second semiconductor detector (fig. 5). Oostman does not explicitly disclose a second focusing optical element configured to focus the second light beam to a size smaller than the object of the extended light source to a second semiconductor detector. Capewell discloses an optical system comprising a WDM having a paraxial optical assembly and an aspheric lens to focus the first beam on the detector [0027, 0039] (figs. 5a, 5b, 6). Moreover, Capewell discloses that with the paraxial optical assembly, the size of the focused light beam can be minimized in order to maximize the tolerance to detector misalignment [0027, 0038]. In particular, with the WDM of Capewell, it is possible to de-magnify the image of the

object of the extended light source on the photodetector. In view of this teaching, it would have been obvious to one of ordinary skill in the art at the time of the invention was made to modify the focusing element of Oostman to be able to focus to a size smaller than the object of the extended light source in view of increasing the tolerance to detector misalignment.

With respect to Claim 370, Oostman discloses the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the image relay optical element and the second focusing optical element (fig. 5).

With respect to Claim 371, Oostman discloses the image relay optical element is a concave shaped dichroic filter (col. 6 lines 45-46).

With respect to Claim 372, Oostman discloses a dichroic filter (151) disposed between the collimating optical element and the first focusing optical element (fig. 5, col. 6 lines 40-50).

With respect to Claim 373, Oostman discloses the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the collimating optical element, the dichroic filter, and the image relay optical element (fig. 5).

With respect to Claim 374, Oostman discloses the dichroic filter is bonded to a filter holder such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder (fig. 7, col. 8 lines 33-37).

With respect to Claim 375, Oostman discloses the reference surface of the filter holder rests against an optically flat surface of a reference block included in the WDM, thereby providing consistent optical alignment when installing the dichroic filter into the WDM (fig. 7).

With respect to Claim 376, Oostman discloses the object is formed by one of light passing through a pinhole and light emitted from a facet of a multimode optical fiber (fig. 5).

With respect to Claim 377, Oostman discloses the first light beam focused by the first focusing optical element is approximately the same size as the effective size of the collimating optical element (col. 6 lines 40-50)

With respect to Claim 378, Oostman discloses the size of the magnified image of the object is less than approximately ten times the size of the object of the extended light source (co. 10 lines 48-50).

With respect to Claim 379, Oostman discloses a composite microscope objective to direct light emitted by a particle in a flow channel in a viewing zone of the composite microscope to the extended light source (col. 5 lines 33-38), the particle illuminated by an illumination light, the composite microscope objective including, a spherical mirror located on a first side of the viewing zone, the spherical mirror configured to reflect the emitted light, and. an aberration corrector plate located on a second side of the viewing zone opposite the first side, the aberration corrector plate configured to reduce optical aberrations in the reflected light caused by the spherical mirror (col. 10 line 61- col. 11 line 20).

Claim 365 is rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Oostman and Capewell as applied to claim 364 above, and further in view of Flanders (US 2002/0067895).

With respect to Claim 365, Oostman as modified by Capewell does not explicitly disclose wherein the first focusing optical element is configured to focus the first light beam to a size smaller than 1 mm in diameter. However, focusing the light to a spot of less than 1mm is considered to be easily made by a skilled person by simply choosing the appropriate lens. In addition, Flanders discloses focusing to spot in the recited range is known [0037]. In view of this teaching, it would have been obvious to one of ordinary skill in the art at the time of the invention was made to focus the beam to a size smaller than 1 mm and the claim would have been obvious because a particular known technique was recognized as part of the ordinary capabilities of one skilled in the art.

Claim 380 is rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Oostman and Capewell as applied to claim 379 above, and further in view of Heuer, Jr. et al. (US 3,199,341, hereafter Heuer).

With respect to Claim 380, Oostman as modified by Capewell discloses a fluidic system but does not disclose a T-coupling arrangement configured to receive the liquid sheath from a reservoir and to direct a first fraction of the liquid sheath back to the reservoir via a bypass conduit and a second fraction of the liquid sheath to the flow channel. In a similar field of endeavor, Heuer discloses such arrangement is known (fig.

1). Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention was made to include the recited arrangement and the claim would have been obvious because a particular known technique was recognized as part of the ordinary capabilities of one skilled in the art.

Claim 381 is rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Oostman and Capewell as applied to claim 379 above, and further in view of Minarik et al. (US 5,257,917).

With respect to Claim 381, Oostman as modified by Capewell discloses the flow cytometer but does not disclose a peristaltic pump configured to supply liquid sample to the flow channel, the peristaltic pump including a pump housing having a arcuate curved track formed therein, a plurality of rollers, and a compressible tube sandwiched between the rollers and the arcuate curved track, the arcuate curved track further including at least one recess section located between at least two pumping sections along the arcuate curved track. In a similar field of endeavor, Minarik discloses such peristaltic pump is known (figs. 1-4, col. 3 line 42-col. 5 line 30). In view of this teaching, it would have been obvious to one of ordinary skill in the art at the time of the invention was made to include the recited peristaltic pump as taught by Minarik in view of pumping fluids through tubing without any contact between pump components and the fluid being pumped.

Claim 382 is rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Oostman and Capewell as applied to claim 379 above, and further in view of Chu (US 7,876,436).

With respect to Claim 382, Oostman as modified by Capewell discloses the illumination light but omits a beam compressing optical element configured to compress the illumination light along an axis perpendicular to the direction of the flow channel. In a similar field of endeavor, Chu discloses the benefit of using two cylindrical lenses capable of compressing and magnifying the light beam in X direction enable the size of the irradiated spot (figs. 5a-5b, col. 4 lines 1-3, col. 6 lines 43-50). In view of this teaching, it would have been obvious to one of ordinary skill in the art at the time of the invention was made to include a beam compressing optical element in view of enable the size of the irradiated spot.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINDY VU whose telephone number is (571)272-8539. The examiner can normally be reached on M-F 9am - 5:30pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Porta can be reached on 571-272-2444. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/DAVID PORTA/
Supervisory Patent Examiner, Art Unit 2884

/M. V./
Examiner, Art Unit 2884

Notice of References Cited	Application/Control No. 14/555,102	Applicant(s)/Patent Under Reexamination CHEN, YONG QIN	
	Examiner MINDY VU	Art Unit 2884	Page 1 of 1

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*	B	US-2002/0067895 A1	06-2002	Flanders, Dale C.	G01J3/08	385/88
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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
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*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP	Serial No.: 14/555,102
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: Iris International, Inc.	
	Filing Date: November 26, 2014	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*none*	*See Page 1*

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Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A167	2004165828	8/26/04	Capewell et al.	385	47	3/1/04
	A168	6159686	12/12/00	Kardos et al.	435	6	1/30/98

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	C6	Extended European Search Report for EP 13796734.5, mailed March 18, 2016.
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42271887.1

EXAMINER: /MINDY D VU/	DATE CONSIDERED: 06/17/2016

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14/555,102	11/26/2014	250	2884	BECC.P0002US.CIP(12US0040

APPLICANTS
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INVENTORS
Yong Qin Chen, San Jose, CA;

**** CONTINUING DATA ***************************
This appln claims benefit of 61/911,859 12/04/2013
and is a CIP of PCT/US2013/043453 05/30/2013
which claims benefit of 61/653,245 05/30/2012
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and claims benefit of 61/715,819 10/18/2012
and claims benefit of 61/715,836 10/19/2012
and claims benefit of 61/816,819 04/29/2013

**** FOREIGN APPLICATIONS ***************************

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Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /MINDY D VU/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		CA	35	23	5

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UNITED STATES

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EAST Search History

EAST Search History (Prior Art)

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L7	1	t$1coupl$3 same reservoir same liquid same sheath	US-PGPUB; USPAT; USOCR	OR	ON	2016/06/21 12:57
L8	33	coupl$3 same reservoir same liquid same sheath same conduit	US-PGPUB; USPAT; USOCR	OR	ON	2016/06/21 12:58
L9	325501	flow near2 (cell cytomet$3 channel)	US-PGPUB; USPAT; USOCR	OR	ON	2016/06/21 12:59
L10	4	L9 and 8	US-PGPUB; USPAT; USOCR	OR	ON	2016/06/21 12:59
L11	4	coupl$3 same reservoir same liquid same sheath same bypass	US-PGPUB; USPAT; USOCR	OR	ON	2016/06/21 13:03
L12	227	peristaltic and pump and liquid and arcuate and curv$3 and roller and compress$4 and recess$3	US-PGPUB; USPAT; USOCR	OR	ON	2016/06/21 13:20
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		"20120274925" | "20130050782" | "20130070243" | "3953727" | "4188543" | "4515274" | "4920275" | "5251060" | "5470211" | "5748372" | "5781351" | "5850292" | "6608682" | "6748133" | "6954722" | "7212343" | "8149525" | "8229707" | "8253938" | "8405048" | "20030142720" | "20070124947" | "20090091746" | "20120281204" | "20120282126" | "20120287419" | "20130204538" | "3411293" | "3542491" | "4834630" | "5177641" | "5299066" | "5373395" | "5396487" | "5467225" | "5548395" | "5746585" | "5777674" | "6252719" | "6572255" | "6618143" | "6839367" | "6876079" | "6897954" | "7305018" | "7453915" | "7496463" | "7623243" | "7645127" | "7835000" | "20130010181" | "0315667" | "4482994" | "4778253" | "4997275" | "5050963" | "5245318" | "5786915" | "5971713" | "6315952" | "6647175" | "6768593" | "6813017" | "7505131" | "7580120" | "7668422" | "7768120" | "8432541" | "8436993" | "8507279" | "20060256335" | "20090190128" | "20110032522" | "20120156074" | "3726613" | "5142462" | "5369476" | "5475210" | "5805363" | "6008920" | "6510007" | "6713019" | "7072540" | "7110192" | "7456960" | "7507588" | "7758324" | "7981661" | "8213472" | "8284402" | "20040218184" | "20080024758" | "20110014075" | "20130094102" | "4564342" | "5915925" | "6135734" | "6794671" | "7038778" | "7113266" | "7129505" | "7262838" | "7876436" | "8187888" | "8233146" | "8270098" | "8345237" | "2669709" | "20060221325" | "20080241911" | "3661460" | "3826593" | "3873204" | "4623225" | "4871249" | "4976590" | "5317162" | "6017194" | "6102678" | "6542306" | "6638481" | "6767188" | "6788409" | "7127356" | "7561267" | "7781227" | "7787197" | "7894047" | "8049185").PN.				
S29	26	S27 and S28	US-PGPUB; USPAT; USOCR	OR	ON	2016/06/21 00:01

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List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: Yong Qin Chen	
	Filing Date: November 26, 2014	Group: 2884

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Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A1	20020141902	10/3/02	Ozasa et al.	422	82.09	3/27/02
	A2	20030142720	7/31/03	Bradburn et al.	372	108	5/24/02
	A3	20040218184	11/4/04	Joregenson et al.	356	419	2/27/04
	A4	20050084402	4/21/05	Vanek	417	477.3	10/27/04
	A5	20060221325	10/5/06	Wells	356	73	3/30/05
	A6	20060245964	11/2/06	Koslov	417	477.1	6/29/06
	A7	20060256335	11/16/06	Chen	356	369	5/3/06
	A8	20060292021	12/28/06	Tu	417	477.1	11/7/02
	A9	20070124947	6/7/07	Munroe et al.	33	286	10/25/06
	A10	20080024758	1/31/08	Tabata	356	39	7/26/07
	A11	20080241911	10/2/08	Ueno et al.	435	287.1	3/28/08
	A12	20090091746	4/9/09	Fukuda et al.	356	73	10/1/08
	A13	20090190128	7/30/09	Cerni et al.	356	336	11/24/08
	A14	20110014075	1/20/11	Reif et al.	417	477.12	7/1/10
	A15	20110032522	2/10/11	Graves et al.	356	338	10/12/10
	A16	20120156074	6/21/12	Kusch et al.	417	477.3	2/4/10
	A17	20120274925	11/1/12	Chen et al.	356	73	11/1/12
	A18	20120281204	11/8/12	Hoshishima et al.	356	72	1/13/11
	A19	20120282126	11/8/12	Brandt et al.	417	477.9	11/4/10
	A20	20120287419	11/15/12	Sharpe et al.	356	51	5/11/12
	A21	20130010181	1/10/13	Baba	348	360	3/14/11
	A22	20130020498	1/24/13	Ebi et al.	250	435	7/18/12
	A23	20130050782	2/28/13	Heng et al.	358	494	2/17/12

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	A24	20130070243	3/21/13	Goehde	356	338	11/9/12
	A25	20130094102	4/18/13	Baba	359	784	7/13/10
	A26	20130204538	8/8/13	Rich	702	21	10/25/11
	A27	2385495	9/25/45	Brian	250	487.1	8/21/43
	A28	2669709	2/16/54	Glancy et al.	348	784	10/19/60
	A29	2764147	9/25/56	Brunner	126	247	2/23/51
	A30	315667	4/14/85	Serdinko	417	475	10/20/84
	A31	3411293	11/19/68	Akins	60	454	10/20/66
	A32	3542491	11/24/70	Newman	417	295	5/27/69
	A33	3661460	5/9/72	Elking et al.	356	36	8/28/70
	A34	3726613	4/10/73	von Casimir	417	477.1	10/12/70
	A35	3826593	7/30/74	von Casimir	417	53	5/11/73
	A36	3873204	3/25/75	Friedman et al.	356	39	11/29/72
	A37	3946239	3/23/76	Salzman et al.	250	461.2	1/24/75
	A38	3953727	4/27/76	d'Auria et al.	398	88	1/17/75
	A39	3989381	11/2/76	Fulwyler	356	39	5/5/75
	A40	4073574	2/14/78	Clarke et al.	359	728	10/1/76
	A41	4188543	2/12/80	Brunsting et al.	250	458.1	4/24/78
	A42	4189236	2/19/80	Hogg et al.	356	317	3/20/78
	A43	4244045	1/6/81	Nosu et al.	398	86	1/31/79
	A44	4482994	11/13/84	Ishikawa	398	86	6/7/82
	A45	4515274	5/7/85	Hollinger et al.	209	3.1	12/2/81
	A46	4564342	1/14/86	Weber et al.	417	477.6	7/24/84

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	A47	4576556	3/18/86	Thompson	417	477.12	4/2/80
	A48	4623225	11/18/86	Forkner	359	669	6/29/84
	A49	4727020	2/23/88	Recktenwald	435	6.11	2/25/85
	A50	4745285	5/17/88	Recktenwald et al.	250	458.1	8/21/86
	A51	4778253	10/18/88	Siga et al.	359	819	3/26/86
	A52	4834630	5/30/89	Godwin	417	475	2/16/88
	A53	4871249	10/3/89	Watson	356	73	7/1/88
	A54	4920275	4/24/90	Itoh	250	574	12/30/88
	A55	4950136	8/21/90	Haas et al.	417	477.7	8/14/89
	A56	4976590	12/11/90	Baldwin	417	53	6/8/88
	A57	4997275	3/5/91	Gaucher et al.	356	72	9/23/88
	A58	5050963	9/24/91	Murakami	359	808	10/9/90
	A59	5142462	8/25/92	Kashima	362	268	4/27/90
	A60	5177641	1/5/93	Kobayashi et al.	359	820	10/30/90
	A61	5230614	7/27/93	Zanger et al.	417	477.9	6/3/92
	A62	5245318	9/14/93	Tohge et al.	340	611	12/15/92
	A63	5251060	10/5/93	Uenishi et al.	359	328	9/30/92
	A64	5299066	3/29/94	Rombult	359	819	8/12/92
	A65	5317162	5/31/94	Pinsky et al.	250	461.2	9/9/92
	A66	5369476	11/29/94	Bowers et al.	399	49	1/28/92
	A67	5373395	12/13/94	Adachi	359	652	5/10/93
	A68	5396487	3/7/95	Abe et al.	359	819	10/31/91
	A69	5467225	11/14/95	Manabe	359	661	11/1/94

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Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A70	5470211	11/28/95	Knott et al.	417	477.9	8/11/94
	A71	5475210	12/12/95	Taguchi et al.	250	205	6/8/93
	A72	5548395	8/20/96	Kosaka	356	73	8/31/92
	A73	5583683	12/10/96	Scobey	398	79	6/15/95
	A74	5615200	3/25/97	Hoshino et al.	369	112.04	4/14/95
	A75	5746585	5/5/98	McDunn et al.	417	477.11	12/31/96
	A76	5748372	5/5/98	Kitagawa	359	565	4/17/96
	A77	5777674	7/7/98	Ohmuro	348	338	4/10/95
	A78	5781351	7/14/98	Murakami et al.	359	808	5/31/96
	A79	5786915	7/28/98	Scobey	398	82	10/22/96
	A80	5788927	8/4/98	Farrell et al.	422	63	6/30/96
	A81	5805363	9/8/98	Okuda et al.	359	819	7/17/97
	A82	5850292	12/15/98	Braun	356	419	11/13/97
	A83	5915925	6/29/99	North Jr.	417	36	1/7/97
	A84	5971713	10/26/99	North Jr.	417	36	12/29/98
	A85	6008920	12/28/99	Hendrix	398	79	3/11/98
	A86	6017194	1/25/00	North Jr.	417	36	12/31/98
	A87	6102678	8/15/00	Peclat	417	477.7	4/3/98
	A88	6135734	10/24/00	Isozumi et al.	417	542	9/23/98
	A89	6200101	3/13/01	North Jr.	417	36	7/6/99
	A90	6252719	6/26/01	Eichenbaum	359	634	3/19/99
	A91	6315952	11/13/01	Sklar et al.	422	63	6/10/99
	A92	6510007	1/21/03	Blasenheim	359	659	8/21/01

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	A93	6542306	4/1/03	Goodman	359	634	3/16/01
	A94	6572255	6/3/03	Husher	366	132	4/24/01
	A95	6608682	8/19/03	Ortyn et al.	356	419	10/12/01
	A96	6618143	9/9/03	Roche et al.	356	339	10/2/01
	A97	6638481	10/28/03	Sklar et al.	422	63	9/19/01
	A98	6647175	11/11/03	LoRegio et al.	385	24	10/8/01
	A99	6683314	1/27/04	Oostman Jr. et al.	250	461.2	8/28/01
	A100	6713019	3/30/04	Ozasa et al.	422	82.09	3/27/02
	A101	6748133	6/8/04	Liu et al.	385	24	11/26/01
	A102	6767188	7/27/04	Vrane et al.	417	40	8/15/02
	A103	6768593	7/27/04	Jutamulia	359	641	7/24/03
	A104	6788409	9/7/04	Goodwin	356	339	9/3/02
	A105	6794671	9/21/04	Nicoli et al.	250	574	7/17/02
	A106	6813017	11/2/04	Hoffman et al.	356	317	10/19/00
	A107	6839367	1/4/05	Nagamatsu et al.	372	34	3/16/01
	A108	6870679	3/22/05	Randall et al.	359	580	6/10/02
	A109	6870976	3/22/05	Chen et al.	385	14	3/13/01
	A110	6897954	5/24/05	Bishop et al.	356	317	12/20/02
	A111	6941047	9/6/05	Capewell et al.	385	47	3/1/04
	A112	6954722	10/11/05	Parks et al.	702	194	10/17/03
	A113	6975400	12/13/05	Ortyn et al.	356	419	7/28/03
	A114	7038778	5/2/06	Yamauchi	356	419	8/13/02
	A115	7072540	7/4/06	Szapiel et al.	385	24	4/22/03

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	A116	7110192	9/19/06	Sauter et al.	359	754	1/12/05
	A117	7113266	9/26/06	Wells	356	73	3/30/05
	A118	7127356	10/24/06	Nicoli et al.	702	26	5/18/04
	A119	7129505	10/31/06	Oostman Jr. et al.	250	458.1	10/17/03
	A120	7212343	5/1/07	He et al.	359	618	7/11/03
	A121	7260328	8/21/07	Kropp	398	82	2/24/03
	A122	7262838	8/28/07	Fritz	356	73	1/16/04
	A123	7268953	9/11/07	Matthae et al.	359	656	6/7/06
	A124	7305018	12/4/07	Otoma	372	50.1	12/29/05
	A125	7381565	6/3/08	Kurabayashi et al.	436	63	3/20/06
	A126	7385682	6/10/08	Chu et al.	356	73	9/7/06
	A127	7430048	9/30/08	Reel et al.	356	432	2/16/06
	A128	7450229	11/11/08	Ortyn et al.	356	326	5/4/05
	A129	7453915	11/18/08	Imai	372	50.1	12/5/06
	A130	7456960	11/25/08	Cerni et al.	356	336	11/15/05
	A131	7496463	2/24/09	Nicoli et al.	702	104	9/12/06
	A132	7505131	3/17/09	Roth	356	317	3/6/08
	A133	7507588	3/24/09	Mehrpouyan et al.	436	518	4/14/06
	A134	7561267	7/14/09	Luo et al.	356	336	12/22/06
	A135	7580120	8/25/09	Hamada et al.	356	73	4/6/06
	A136	7623243	11/24/09	Kato	356	419	9/13/04
	A137	7645127	1/12/10	Hagen et al.	417	477.12	6/29/06
	A138	7668422	2/23/10	Kropp	385	47	3/12/04

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	A139	7758324	6/20/10	Baumann et al.	417	477.2	5/12/05
	A140	7768120	8/3/10	Takashima	257	706	12/27/07
	A141	7781227	8/24/10	Mehrpouyan et al.	436	523	3/4/09
	A142	7787197	8/31/10	Chen	359	793	9/4/08
	A143	7835000	11/16/10	Graves et al.	356	338	11/3/06
	A144	7876436	1/25/11	Chu	356	338	10/31/07
	A145	7894047	2/22/11	Hamada et al.	356	39	7/24/09
	A146	7952981	3/31/11	Suzuki	369	121	10/23/08
	A147	7981661	7/19/11	Rich	435	286.5	4/17/07
	A148	8009189	8/30/11	Ortyn et al.	348	80	6/3/08
	A149	8049185	11/1/11	Hayashi et al.	250	459.1	2/4/09
	A150	8149525	4/3/12	Do	359	797	3/28/07
	A151	8157547	4/17/12	Vrielink	417	477.3	4/23/07
	A152	8187888	5/29/12	Rich	436	63	7/8/11
	A153	8213472	7/3/12	Hirai et al.	372	36	1/23/08
	A154	8229707	7/24/12	Olson et al.	702	189	7/4/11
	A155	8233146	7/31/12	Chen	356	246	1/5/10
	A156	8253938	8/28/12	Vacca et al.	356	337	5/26/11
	A157	8270098	9/18/12	Fukuta et al.	359	784	2/19/09
	A158	8284402	10/9/12	Frazier et al.	356	419	2/26/10
	A159	8337096	12/25/12	Shen et al.	385	89	11/30/09
	A160	8345237	1/1/13	Tsukii et al.	356	338	12/23/11
	A161	8405048	3/26/13	Hayashi	250	458.1	2/4/09

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.V/

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP	Serial No.: 14/555,102
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: Yong Qin Chen	
	Filing Date: November 26, 2014	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 9*	*See Page 10*

U.S. Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A162	8432541	4/30/13	Rich	356	246	11/4/10
	A163	8436371	5/7/13	Medendorp Jr., et al.	257	88	5/24/07
	A164	8436993	5/7/13	Kaduchak et al.	356	337	2/3/12
	A165	8488244	7/16/13	Li et al.	359	618	2/17/11
	A166	8507279	8/13/13	Ball et al.	436	10	6/2/10

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U.S. Patent Documents	Foreign Patent Documents	Other Art
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Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	B1	CN 102087198	6/8/11	China	Chinese (English abstract)
	B2	CN 102818794	12/12/12	China	Chinese (English abstract)
	B3	CN 102818795	12/12/12	China	Chinese (English abstract)
	B4	CN 102818796	12/12/12	China	Chinese (English abstract)
	B5	CN 201917509U	8/3/11	China	Chinese (English abstract)
	B6	CN 201936066U	8/17/11	China	Chinese (English abstract)
	B7	CN 201984209U	9/21/11	China	Chinese (English abstract)
	B8	EP 0889319	1/7/99	EPO	German (English abstract)
	B9	EP 1004907	4/22/09	EPO	English
	B10	EP 1953526	8/6/08	EPO	English
	B11	EP 2381475	3/4/15	EPO	English
	B12	EP 459764A2	12/4/91	EPO	English
	B13	EP 459764B1	12/11/96	EPO	English
	B14	EP 757346A1	2/5/97	EPO	English
	B15	EP 757346B1	11/14/01	EPO	English
	B16	EP 758755A2	2/19/97	EPO	English
	B17	JP 05333245	12/17/93	Japan	Japanese (English abstract)
	B18	JP 829726	2/2/96	Japan	Japanese (English translation)
	B19	WO 0057173	9/28/00	WIPO	English
	B20	WO 0127590	4/19/01	WIPO	English
	B21	WO 0140764	6/7/01	WIPO	English
	B22	WO 03012403	2/13/03	WIPO	French (English abstract)
	B23	WO 2005033654	4/14/05	WIPO	English
	B24	WO 2006013316	2/9/06	WIPO	English
	B25	WO 2009095358	8/6/09	WIPO	German (English abstract)
	B26	WO 2009098867	8/13/09	WIPO	Japanese (English abstract)

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Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP	Serial No.: 14/555,102
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U.S. Patent Documents	Foreign Patent Documents	Other Art
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Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	B27	WO 2011026942	3/10/11	WIPO	English
	B28	WO 2012056217	5/3/12	WIPO	English
	B29	WO 2012177367	12/27/12	WIPO	English
	B30	WO 2013013229	1/24/13	WIPO	English
	B31	WO 2013093035	6/27/13	WIPO	English
	B32	WO 9307471	4/15/93	WIPO	English
	B33	WO 9316368	8/19/93	WIPO	English
	B34	WO 9429695	12/22/94	WIPO	English
	B35	WO 9714066A2	4/17/97	WIPO	English

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C1	International Search Report and Written Opinion for PCT/US2013/043453 mailed December 20, 2013.
	C2	International Search Report and Written Opinion for PCT/US2014/067682 mailed April 9, 2015.
	C3	Brogioli et al., "Heterodyne near-field scattering," *Applied Physics Letters* 81(22):4109-4111, 2002.
	C4	Schmidt, B., "Ein lichtstarkes komafreies Spiegelsystem," *Mitteilungen der Haburger Sternwarte in Bergedorf* 7(36):15-17, 1932.
	C5	Smiley, C. H., "The Schmidt Camera." *Popular Astronomy* 44:415-421, 1936.

/MINDY D VU/ 06/20/2016

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.V/

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14555102	CHEN, YONG QIN
	Examiner	Art Unit
	MINDY VU	2884

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	04/13/2016	06/20/2016							
	364	÷	✓							
	365	÷	✓							
	366	÷	✓							
	367	÷	✓							
	368	÷	✓							
	369	÷	✓							
	370	÷	✓							
	371	÷	✓							
	372	÷	✓							
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	378	÷	✓							
	379	÷	✓							
	380	÷	✓							
	381	÷	✓							
	382	÷	✓							
	383	÷	-							
	384	÷	-							
	385	÷	-							
	386	÷	-							

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14555102	CHEN, YONG QIN
	Examiner	Art Unit
	MINDY VU	2884

CPC- SEARCHED		
Symbol	Date	Examiner
G01N 21/64, 15/1434, 15/1436, 15/1459, 2015/1452	06/20/16	MV
G02B 6/26, 6/29365	06/20/16	MV

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Invenvor name search	06/19/16	mv
EAST BRS - See search history printout	06/21/16	mv

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

Amendment in Response to Non-Final Office Action
Under 37 CFR 1.111
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: October 27, 2016 Signature: (Greg McCormack)

Docket No.: BECC.P0002US.CIP/11412161
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Yong Qin Chen

Application No.: 14/555,102 Confirmation No.: 4589

Filed: November 26, 2014 Art Unit: 2884

For: FLOW CYTOMETER Examiner: Vu, Mindy D.

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 CFR 1.111

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Commissioner:

INTRODUCTORY COMMENTS

In response to the Office Action dated June 27, 2016, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENT TO THE CLAIMS

The listing of claims will replace all prior versions, and listing of claims in the application.

1-363. (Canceled)

364. (Previously presented) A flow cytometer having a wavelength division multiplexer (WDM), the WDM comprising:

an extended light source providing light that forms an object;

a collimating optical element that captures light from the extended light source and projects a magnified image of the object as a first light beam; and

a first focusing optical element configured to focus the first light beam to a size smaller than the object of the extended light source to a first semiconductor detector.

365. (Previously presented) The flow cytometer of claim 364, wherein the first focusing optical element is configured to focus the first light beam to a size smaller than 1 mm in diameter.

366. (Previously presented) The flow cytometer of claim 364, further comprising an image relay optical element arranged to receive a color band of interest of the first light beam, the image relay optical element configured to project a second image as a second light beam, wherein the second light beam is substantially a unit magnification of the first light beam.

367. (Previously presented) The flow cytometer of claim 366, wherein the image relay optical element is one of a refractive optical device and a concave mirror.

368. (Previously presented) The flow cytometer of claim 366, wherein the image relay optical element is a concave mirror having a radius of curvature approximately equal to the distance between the collimating optical element and the magnified image projected thereby.

369. (Previously presented) The flow cytometer of claim 366, further comprising a second focusing optical element configured to focus the second light beam to a size smaller than the object of the extended light source to a second semiconductor detector.

370. (Previously presented) The flow cytometer of claim 369, wherein the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the image relay optical element and the second focusing optical element.

371. (Previously presented) The flow cytometer of claim 366, wherein the image relay optical element is a concave shaped dichroic filter.

372. (Previously presented) The flow cytometer of claim 366, further comprising a dichroic filter disposed between the collimating optical element and the first focusing optical element.

373. (Previously presented) The flow cytometer of claim 372, wherein the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the collimating optical element, the dichroic filter, and the image relay optical element.

374. (Previously presented) The flow cytometer of claim 372, wherein the dichroic filter is bonded to a filter holder such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder.

375. (Previously presented) The flow cytometer of claim 374, wherein the reference surface of the filter holder rests against an optically flat surface of a reference block included in the WDM, thereby providing consistent optical alignment when installing the dichroic filter into the WDM.

376. (Previously presented) The flow cytometer of claim 364, wherein the object is formed by one of light passing through a pinhole and light emitted from a facet of a multimode optical fiber.

377. (Previously presented) The flow cytometer of claim 364, wherein the first light beam focused by the first focusing optical element is approximately the same size as the effective size of the collimating optical element.

378. (Previously presented) The flow cytometer of claim 364, wherein the size of the magnified image of the object is less than approximately ten times the size of the object of the extended light source.

379. (Previously presented) The flow cytometer of claim 364, further comprising a composite microscope objective to direct light emitted by a particle in a flow channel in a viewing zone of the composite microscope to the extended light source, the particle illuminated by an illumination light, the composite microscope objective including,

a spherical mirror located on a first side of the viewing zone, the spherical mirror configured to reflect the emitted light, and,

an aberration corrector plate located on a second side of the viewing zone opposite the first side, the aberration corrector plate configured to reduce optical aberrations in the reflected light caused by the spherical mirror.

380. (Previously presented) The flow cytometer of claim 379, further comprising a fluidic system configured to supply liquid sheath to the flow channel, the fluidic system having a T-coupling arrangement configured to receive the liquid sheath from a reservoir and to direct a first fraction of the liquid sheath back to the reservoir via a bypass conduit and a second fraction of the liquid sheath to the flow channel.

381. (Previously presented) The flow cytometer of claim 379, further comprising a peristaltic pump configured to supply liquid sample to the flow channel, the peristaltic pump

including a pump housing having a arcuate curved track formed therein, a plurality of rollers, and a compressible tube sandwiched between the rollers and the arcuate curved track, the arcuate curved track further including at least one recess section located between at least two pumping sections along the arcuate curved track.

382. (Previously presented) The flow cytometer of claim 379, further comprising a beam compressing optical element configured to compress the illumination light along an axis perpendicular to the direction of the flow channel.

383.-386. (Canceled)

387. (New) The flow cytometer of claim 364, wherein the extended light source has an etendue at least a hundred times greater than an etendue of a laser beam out of a single mode optical fiber.

REMARKS

I. GENERAL

Claims 364-382 are presently pending in the application. Claim 387 is newly added herein. Claims 1-363 and 383-386 were previously canceled. The issues in the current Office Action are as follows:

- Claims 364 and 366-379 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Oostman, Jr. et al., U.S. Patent No. 6,683,314 (hereinafter "*Oostman*") in view of Capewell et al., U.S. Publication No. 2004/0165828 (hereinafter "*Capewell*").

- Claim 365 is rejected under 35 U.S.C. § 103(a) as being unpatentable over *Oostman* and *Capewell* as applied to claim 364 above, and further in view of Flanders, U.S. Publication No. 2002/0067895 (hereinafter "*Flanders*").

- Claim 380 is rejected under 35 U.S.C. § 103(a) as being unpatentable over *Oostman* and *Capewell* as applied to claim 379 above, and further in view of Heuer, Jr. et al., U.S. Patent No. 3,199,341 (hereinafter "*Heuer*").

- Claim 381 is rejected under 35 U.S.C. § 103(a) as being unpatentable over *Oostman* and *Capewell* as applied to claim 379 above, and further in view of Minarik et al., U.S. Patent No. 5,257,917 (hereinafter "*Minarik*").

- Claim 382 is rejected under 35 U.S.C. § 103(a) as being unpatentable over *Oostman* and *Capewell* as applied to claim 379 above, and further in view of Chu, U.S. Patent No. 7,876,436 (hereinafter "*Chu*").

Applicant hereby traverses the rejections and requests reconsideration and withdrawal in light of the amendments and remarks contained herein.

II. CLAIM AMENDMENTS

Claim 387 has been newly added to recite that "the extended light source has an etendue at least a hundred times greater than an etendue of a laser beam out of a single mode optical fiber." Support for these amendments is found at least on page 69, lines 1-5 of the originally filed specification ("specification"). No new matter is added.

III. CLAIM REJECTIONS

A. 35 U.S.C. § 103(a) Rejections (Oostman in view of Capewell)

Claims 364 and 366-379 are rejected under 35 U.S.C. § 103(a) as being unpatentable over *Oostman* in view of *Capewell*. To render a claim unpatentable under 35 U.S.C. § 103, all the claim limitations must be taught by the prior art, M.P.E.P. § 2143.03. Moreover, the Examiner must provide analysis supporting any rationale why a person skilled in the art would combine the prior art to arrive at the claimed invention, and "[such] analysis should be made explicit," *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 82 U.S.P.Q.2d 1385 (2007). The Examiner bears the initial burden of factually supporting any *prima facie* conclusion of obviousness, M.P.E.P. § 2142; *In re Peehs*, 204 U.S.P.Q. 835, 837 (C.C.P.A. 1980).

1. Independent claim 364

Claim 364 recites that "a first focusing optical element configured to focus the first light beam to ***a size smaller than the object of the extended light source to a first semiconductor detector***." (Emphasis added.) The Office Action admits that *Oostman* fails to teach these features, but offers *Capewell* to cure the deficiencies. *See* Office Action, on p. 4. Applicant respectfully asserts that a *prima facie* conclusion of obviousness has not been established because the Office Action has not established any explicit analysis supporting any rationale why the combination of *Oostman* with *Capewell* would be obvious to one of ordinary skill in the art.

Applicant respectfully asserts that the fluorescence detection instrument, as taught by *Oostman*, and the optical communication system, as taught by *Capewell*, are actually different kinds of optical systems with different sizes of light sources. *Oostman* teaches fluorescence detection instrument that includes collimating lens, beam splitting elements, dichroic mirrors,

and focusing elements to form a beam emitted from a light source, and a photomultiplier tube to detect the beam. *See Oostman*, at col. 6, lns. 40-49. In *Oostman*, the photomultiplier tube is used to detect light emitted from a pinhole or a multimode optical fiber (common light source of the detector) in fluorescence detection instrument, e.g., flow cytometer. The size of an object of such light source is usually measured in millimeters. *See* specification, on p. 69, lns. 19-22; *see also Oostman*, at col. 10, lns. 25-47. However, such light source has an etendue hundreds of times greater than that of a laser beam out of a single mode optical fiber, and, therefore, it is more difficult to focus a beam from such light source down to a small size. *See* specification, on p. 69, lns. 1-8. As such, a photomultiplier tube, as illustrated by *Oostman* in detail, instead of a semiconductor detector with a small active area, is normally used to detect light with greater etendue in fluorescence detection instrument in the prior art. *Id.*

On the contrary, *Capewell* teaches an optical communication system that includes multiple optical assemblies to collimate and redirect a beam emitted from a light source, and a detector to detect the beam. *See Capewell*, at ¶s [0002] and [0039]. In *Capewell*, the detector is used to detect light emitted from a point light source, such as a single mode optical fiber, in a WDM to be used with a communication system. *Id.*, at ¶ [0030]. The size of an object of such point light source is usually measured in micrometers. *See* specification, on p. 69, lns. 22-23; *see also Capewell*, at ¶ [0049]. As such, such point light source has much less etendue than a bigger light source. *See* specification, on p. 69, lns. 1-8.

From the foregoing, it is clear that *Oostman* and *Capewell* discuss different kinds of optical systems (fluorescence detection instrument v. optical communication system) with different sizes of light sources. The Office Action fails to provide any explicit analysis to support the combination of optical elements from different kinds of optical systems, as taught by *Oostman* and *Capewell*. For instance, the Office Action asserts that since *Capewell* has disclosed that the size of the focused light beam can be minimized in order to maximize the tolerance to detector misalignment, it would have been obvious to one of ordinary skill in the art to modify the focusing element of *Oostman* to be able to focus to a size smaller than the object of the extended light source. *See* Office Action, on p. 4. However, the Office Action fails to address ***how*** to modify a focusing element in ***fluorescence detection instrument*** with a focusing

element in an ***optical communication system*** to focus a beam to a size smaller than the object of the extended light source to a first semiconductor detector, as claimed. *See In re Kumar,* 418 F.3d 1361, 1369 (Fed. Cir. 2005) ("To render a later invention unpatentable for obviousness, the prior art must enable a person of ordinary skill in the field to make and use the later invention."). As discussed above, it is more difficult to focus a beam with greater etendue in fluorescence detection instrument than to focus a beam with less etendue in an optical communication system.

According to the above, a *prima facie* conclusion of obviousness has not been established. *Oostman* in view of *Capewell* fails to render claim 364 obvious. Hence, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claim 364.

2. Dependent claims 366-379

a. Claim 366

Claim 366 recites that "an image relay optical element arranged to receive a color band of interest of the first light beam, the image relay optical element configured to project a second image as a second light beam, wherein the second light beam is substantially a unit magnification of the first light beam." The Office Action asserts that *Oostman* has disclosed the above features. *See* Office Action, on ps. 4-5. In particular, the Office Action asserts that beam splitting element 151 in *Oostman* meets the image relay optical element as claimed. *Id.* Applicant respectfully disagrees with such assertion.

For example, *Oostman*'s beam splitting element 151 does ***not receive a color band of interest*** of the first light beam as the claimed image relay optical element does. Instead, *Oostman*'s beam splitting element 151 receives beam 147 output from a fiber ***in its entirety*** and splits beam 147 into beam 155 and beam 153. *See Oostman*, at col. 6, lns. 45-47 and 55-57. Further, the cited selections of *Oostman* do not teach or suggest the size of beam 147 received by beam splitting element 151, or beams 155 and 153 output from beam splitting element 151. In fact, controlling the beam size is important for a WDM. As specified in the specification, utilizing an image relay optical element to extend a collimated beam path without expanding the

beam's diameter can make such collimated beam to be easily focused down to a small spot. *See* specification, on p. 47, lns. 24-27. *Oostman* is wholly silent as to beam size control for an extended light path in a WDM. Therefore, *Oostman* fails to teach that "the second light beam is substantially a unit magnification of the first light beam," as recited by claim 364. *Capewell* is not offered to teach these features, nor does it. Therefore, *Oostman* in view of *Capewell* fails to teach or suggest that "an image relay optical element arranged to receive a color band of interest of the first light beam, the image relay optical element configured to project a second image as a second light beam, wherein the second light beam is substantially a unit magnification of the first light beam," as recited by claim 366.

Further, claim 366 depends from independent claim 364. Therefore, claim 366 includes all the features of its respective base claim. It is respectfully submitted that claim 366 is also allowable at least because of its dependence from claim 364 for the reasons discussed above. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claim 366.

b. Claim 367

Claim 367 recites that "the image relay optical element is one of a refractive optical device and a concave mirror." The Office Action asserts that *Oostman*, at col. 10, lns. 1-3, teaches these features. *See* Office Action, on p. 5. However, the cited selections of *Oostman* do not discuss the beam splitting element 151, which has been asserted by the Office Action to meet the image relay optical element as claimed. Instead, the lens combination as cited is used to magnify and view cellular material within a cytometry flow cell or cuvette. *Id*, at col. 9, lns. 44-46. *Capewell* is not offered to teach these features of claim 367, nor does it. Therefore, *Oostman* in view of *Capewell* fails to teach or suggest that "wherein the image relay optical element is one of a refractive optical device and a concave mirror," as recited by claim 367.

Further, claim 367 depends from independent claim 364. Therefore, claim 367 includes all the features of its respective base claim. It is respectfully submitted that claim 367 is also allowable at least because of its dependence from claim 364 for the reasons discussed above.

Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claim 367.

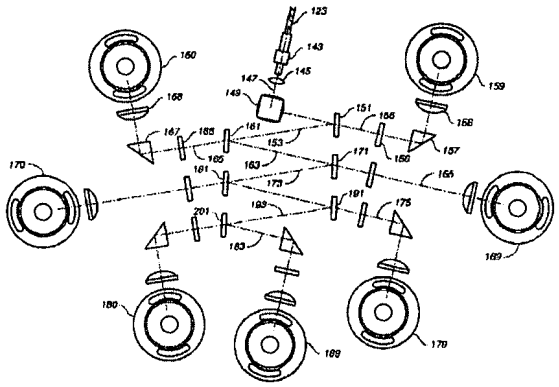
c. Claims 370 and 373

Claim 370 recites that "the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the image relay optical element and the second focusing optical element." Claim 373 recites that "the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the collimating optical element, the dichroic filter, and the image relay optical element." The Office Action asserts that *Oostman*, at FIG. 5, teaches these features of claims 370 and 373. Applicant respectfully disagrees.

In the Office Action, collimating lens 145, focusing element 158, and beam splitting element 151 in *Oostman* have been asserted to meet the claimed collimating optical element, first focusing optical element, and image relay optical element, respectively. *See* Office Action, on ps. 4-5. Accordingly, FIG. 5 of *Oostman* (as reproduced below) has clearly shown that the optical path length between collimating lens 145 and focusing element 158 is ***shorter*** than the optical path length between beam splitting element 151 and focusing element 168 or other focusing elements. *See Oostman*, at FIG. 5.

In the Office Action, collimating lens 145 and focusing element 158 in *Oostman* have been asserted to meet the claimed collimating optical element and first focusing optical element, respectively. *See* Office Action, on p. 4. Also, the Office Action asserts that beam splitting element (dichroic mirror) 151 meets both the claimed image replay optical element and dichroic filter. *Id*, on ps. 4-5. Accordingly, FIG. 5 of *Oostman* has also clearly shown that the optical path length between collimating lens 145 and focusing element 158 is ***longer*** than the optical path length between collimating lens 145, dichroic mirror 151, and beam splitting element 151. *See Oostman*, at FIG. 5. In particular, beam splitting element (dichroic mirror) 151 is located in the middle of the optical path between collimating lens 145 and focusing element 158. *Id*, at col.

6, lns. 42-49.



Capewell is not offered to teach these features of claim 370 or 373, nor does it. Therefore, *Oostman* in view of *Capewell* fails to teach or suggest that "the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the image relay optical element and the second focusing optical element," as recited by claim 370. Also, *Oostman* in view of *Capewell* fails to teach or suggest that "the optical path length between the collimating optical element and the first focusing optical element is substantially the same as the optical path length between the collimating optical element, the dichroic filter, and the image relay optical element," as recited by claim 373.

Further, claims 370 and 373 depend from independent claim 364. Therefore, claims 370 and 373 include all the features of its respective base claim. It is respectfully submitted that claims 370 and 373 are also allowable at least because of their dependence from claim 364 for the reasons discussed above. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claims 370 and 373.

d. Claims 374 and 375

Claim 374 recites that "the dichroic filter is bonded to a filter holder such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder." Claim 375 recites that "the reference surface of the filter holder rests against an optically flat surface of a reference block included in the WDM, thereby providing consistent

optical alignment when installing the dichroic filter into the WDM." The Office Action asserts that *Oostman*, at FIG. 7 and col. 8, lns. 33-37, teaches these features of claims 374-375. *See* Office Action, on ps. 6-7. However, the cited selections of *Oostman* are wholly silent as to the structure/shape of the dichroic filter and alignment of the dichroic filter by an optically flat surface of a reference block as claimed. The cited selections of *Oostman*, at most, discuss that dichroic mirror holders are mounted in vertically removable housing so that dichroic mirrors may be interchanged or replaced. *See Oostman*, at FIG. 7 and col. 8, lns. 33-37.

Capewell is not offered to teach these features of claim 374 or 375, nor does it. Therefore, *Oostman* in view of *Capewell* fails to teach or suggest that "the dichroic filter is bonded to a filter holder such that a coated filter surface of the dichroic filter is indented and optically parallel to a reference surface of the filter holder," as recited by claim 374, and "the reference surface of the filter holder rests against an optically flat surface of a reference block included in the WDM, thereby providing consistent optical alignment when installing the dichroic filter into the WDM," as recited by claim 375.

Further, claims 374-375 depend from independent claim 364. Therefore, claims 374-375 include all the features of its respective base claim. It is respectfully submitted that claims 374-375 are also allowable at least because of their dependence from claim 364 for the reasons discussed above. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claims 374-375.

e. Claim 377

Claim 377 recites that "the first light beam focused by the first focusing optical element is approximately the same size as the effective size of the collimating optical element." The Office Action asserts that *Oostman*, col. 6, lns. 40-50, teaches these features of claim 377. *See* Office Action, on p. 7. However, the cited selections of *Oostman* are wholly silent as to the size of the first light beam focused by focusing element 158 and the size of collimating element 151. On the contrary, as discussed in the specification, the above features can advantageously keep the beam of light propagating between the collimating optical element and the first focusing

optical element collimated. *See* specification, on p. 70, lns. 1-4. *Capewell* is not offered to teach these features of claim 377, nor does it. Therefore, *Oostman* in view of *Capewell* fails to teach or suggest that "the first light beam focused by the first focusing optical element is approximately the same size as the effective size of the collimating optical element," as recited by claim 377.

Further, claim 377 depends from independent claim 364. Therefore, claim 377 includes all the features of its respective base claim. It is respectfully submitted that claim 377 is also allowable at least because of its dependence from claim 364 for the reasons discussed above. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claim 377.

f. Claim 379

Claim 379 recites:

> comprising a composite microscope objective to direct light emitted by a particle in a flow channel in a viewing zone of the composite microscope ***to the extended light source*** . . . the composite microscope objective including,
>
> a spherical mirror located ***on a first side of the viewing zone*** . . . and an aberration corrector plate located ***on a second side of the viewing zone opposite the first side.***

(Emphasis added.) The Office Action asserts that *Oostman*, at col. 5, lns. 33-38 and col. 10, ln. 61 to col. 11, ln. 20, teaches these features of claim 379. *See* Office Action, on p. 7. However, *Oostman*, at col. 5, lns. 33-38, actually discusses that multiple beams from multiple lasers can be ***directed toward*** flow stream for illuminating the stream, instead of light ***emitted from a flow channel*** to be directed to the extended light source as claimed. *See Oostman*, at col. 5, lns. 33-38. Further, *Oostman*, at col. 10, ln. 61 to col. 11, ln. 20, discusses lens combination located on ***one side*** of flow cell/cuvette 103. *Id*, at col. 10, ln. 61 to col. 11, ln. 20 and FIG. 15. *Capewell* is not offered to teach these features of claim 379, nor does it. Therefore, *Oostman* in view of *Capewell* fails to teach or suggest the above features of claim 379.

Further, claim 379 depends from independent claim 364. Therefore, claim 379 includes all the features of its respective base claim. It is respectfully submitted that claim 379 is also allowable at least because of their dependence from claim 364 for the reasons discussed above. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claim 379.

g. Claims 368-369, 371-372, 376, and 378

Claims 368-369, 371-372, 376, and 378 depend from independent claim 364. Each dependent claim includes all the features of its respective base claim. It is respectfully submitted that dependent claims 368-369, 371-372, 376, and 378 are allowable at least because of its dependence from claim 364 for the reasons discussed above. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claims 368-369, 371-372, 376, and 378.

B. 35 U.S.C. § 103(a) Rejections (Oostman and Capewell in view of Flanders)

Claim 365 is rejected under 35 U.S.C. § 103(a) as being unpatentable over *Oostman* and *Capewell* as applied to claim 364 above, and further in view of *Flanders*.

Claim 365 recites that "the first focusing optical element is configured to focus the first light beam to a size smaller than 1 mm in diameter." The Office Action admits that *Oostman* in view of *Capewell* fails to teach these features of claim 365, but offers *Flanders*, at paragraph [0037], to cure the deficiencies. *See* Office Action, on p. 8. However, the cited selections of *Flanders* merely discuss that focusing lens 60 can focus to a spot smaller than 50μm. *See Flanders*, at ¶ [0037]. But such focusing lens 60 in *Flanders* is not part of a WDM device. Focusing lens 60 is merely used with a WDM device. *Id. Flanders*, at most, suggests that the size of ***input beam*** of WDM is smaller than 50μm. *Id.* None of *Oostman, Capewell*, and *Flanders* teach or suggest a focusing element ***in a WDM*** focusing to a size smaller than 1mm in diameter as claimed. Therefore, claim 365 is allowable over *Oostman, Capewell, Flanders*, or any combination thereof.

Also, claim 365 depends from independent claim 364, and thus, includes every feature of its base claim. Claim 364 has been shown above to be patentable over *Oostman* in view of *Capewell*. The tertiary reference *Flanders* introduced in the 35 U.S.C. § 103 rejection of claim 365 is not relied upon to cure the above-identified deficiencies in the foregoing reference, nor does it. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claim 365.

C. 35 U.S.C. § 103(a) Rejections (Oostman and Capewell in view of Heuer)

Claim 380 is rejected under 35 U.S.C. § 103(a) as being unpatentable over *Oostman* and *Capewell* as applied to claim 379 above, and further in view of *Heuer*.

Claim 380 depends from independent claim 364, and thus, includes every feature of its base claims. Claim 364 has been shown above to be patentable over *Oostman* in view of *Capewell*. The tertiary reference *Heuer* introduced in the 35 U.S.C. § 103 rejection of claim 380 is not relied upon to cure the above-identified deficiencies in the foregoing reference, nor does it. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claim 380.

D. 35 U.S.C. § 103(a) Rejections (Oostman and Capewell in view of Minarik)

Claim 381 is rejected under 35 U.S.C. § 103(a) as being unpatentable over *Oostman* and *Capewell* as applied to claim 379 above, and further in view of *Minarik*.

Claim 381 depends from independent claim 364, and thus, includes every feature of its base claims. Claim 364 has been shown above to be patentable over *Oostman* in view of *Capewell*. The tertiary reference *Minarik* introduced in the 35 U.S.C. § 103 rejection of claim 381 is not relied upon to cure the above-identified deficiencies in the foregoing reference, nor does it. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claim 381.

E. 35 U.S.C. § 103(a) Rejections (Oostman and Capewell in view of Chu)

Claim 382 is rejected under 35 U.S.C. § 103(a) as being unpatentable over *Oostman* and *Capewell* as applied to claim 379 above, and further in view of *Chu*.

Claim 382 depends from independent claim 364, and thus, includes every feature of its base claims. Claim 364 has been shown above to be patentable over *Oostman* in view of *Capewell*. The tertiary reference *Chu* introduced in the 35 U.S.C. § 103 rejection of claim 382 is not relied upon to cure the above-identified deficiencies in the foregoing reference, nor does it. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) of claim 382.

IV. NEW CLAIM

Claim 387 has been newly added to recite that "the extended light source has an etendue at least a hundred times greater than an etendue of a laser beam out of a single mode optical fiber." Applicant respectfully submits that the cited art fails to teach or suggest these features of claim 387. For example, neither *Oostman* nor *Capewell* discusses an etendue of its light source. Further, claim 387 depends from independent claim 364, and thus, includes every feature of its base claim. As such, claim 387 is also allowable at least because of its dependence from claim 364 for the reasons discussed above. Accordingly, Applicant respectfully requests that the Examiner allow claim 387.

V. CONCLUSION

In view of the above, Applicant believes the pending application is in condition for allowance. Applicant believes $200.00 for a one month extension of time is due with this response as stated in the Extension of Time filed concurrently herewith. The fee will be paid online by Credit Card. Please charge any additional fees required or credit any overpayment during the pendency of this Application pursuant to 37 CFR 1.16 through 1.21 inclusive, and any other sections in Title 37 of the Code of Federal Regulations that may regulate fees to Deposit Account No. 06-2380, under Order No. BECC.P0002US.CIP/11412161 from which the undersigned is authorized to draw.

Dated: October 27, 2016

Respectfully submitted,

By _____
Kuan-Chieh Tu
Registration No.: 70,414
NORTON ROSE FULBRIGHT US LLP
2200 Ross Avenue, Suite 3600
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Attorney for Applicant

Electronic Patent Application Fee Transmittal

Application Number:	14555102
Filing Date:	26-Nov-2014
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Filer:	Gina N. Shishima/Greg McCormack
Attorney Docket Number:	BECC.P0002US.CIP(12US0040

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 1 month with $0 paid	1251	1	200	200
Miscellaneous:				
			Total in USD ($)	**200**

Electronic Acknowledgement Receipt

EFS ID:	27345231
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	129381
Filer:	Gina N. Shishima/Greg McCormack
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US.CIP(12US0040
Receipt Date:	27-OCT-2016
Filing Date:	26-NOV-2014
Time Stamp:	16:35:45
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$200
RAM confirmation Number	102816INTEFSW16363400
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		BECCP0002USCIP.pdf	2504858 b41b3c2b55d88b3a35b8bdd62a6b6172ecc81cae	yes	19

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Extension of Time	1	1
Amendment/Req. Reconsideration-After Non-Final Reject	2	2
Claims	3	6
Applicant Arguments/Remarks Made in an Amendment	7	19

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30632 43de18c0dee7ec5a4ea433b471f1aaad98a59b84	no	2

Warnings:

Information:

Total Files Size (in bytes):	2535490

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) BECC.P0002US.CIP/11412161
Application Number 14/555,102-Conf. #4589	Filed November 26, 2014
For FLOW CYTOMETER	
Art Unit 2884	Examiner M. D. Vu

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
[x] One month (37 CFR 1.17(a)(1))	$200	$100	$50	$ 200.00
[ ] Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
[ ] Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$
[ ] Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
[ ] Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

[ ] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

[ ] A check in the amount of the fee is enclosed.

[x] Payment by credit card.

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account.

[x] The Director is hereby authorized to charge any underpayment or credit any overpayment, to Deposit Account Number 06-2380 .

[ ] Payment made via EFS-Web.

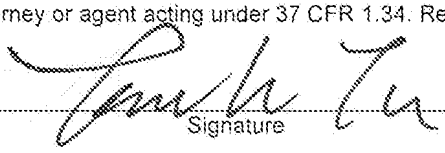
WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

[ ] applicant.

[ ] attorney or agent of record. Registration number _____ .

[x] attorney or agent acting under 37 CFR 1.34. Registration number 70,414 .



Signature — October 27, 2016 (Date)

Kuan-Chieh Tu (Typed or printed name) — (214) 855-8032 (Telephone Number)

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

[ ] * Total of 1 forms are submitted.

One Month Request for Extension of Time under 37 CFR 1.136(a)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: October 27, 2016 Signature: _____ (Greg McCormack)

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/555,102	Filing Date 11/26/2014	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	10/27/2016	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 20	Minus	** 23	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***5	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
NINA RATANAVONG

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
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NOTICE OF ALLOWANCE AND FEE(S) DUE

129381 7590 04/05/2017

NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

EXAMINER
VU, MINDY D

ART UNIT	PAPER NUMBER
2884	

DATE MAILED: 04/05/2017

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040	4589

TITLE OF INVENTION: FLOW CYTOMETER

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	07/05/2017

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

129381 7590 04/05/2017
NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040	4589

TITLE OF INVENTION: FLOW CYTOMETER

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	07/05/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
VU, MINDY D	2884	250-458100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29 — NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27 — NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status. — NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040	4589

129381 7590 04/05/2017

NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

EXAMINER	
VU, MINDY D	

ART UNIT	PAPER NUMBER
2884	

DATE MAILED: 04/05/2017

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Notice Requiring Inventor's Oath or Declaration	Application No. 14/555,102	Applicant(s) Yong Qin Chen	
	Examiner VU, MINDY D	Art Unit 2884	

This notice is an attachment to the Notice of Allowability (PTOL-37), or the Notice of Allowability For A Design Application (PTOL-37D).

An inventor's oath or declaration in compliance with 37 CFR 1.63 or 1.64 executed by or with respect to each inventor has not yet been submitted.

An oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each inventor (for any inventor for which a compliant oath, declaration, or substitute statement has not yet been submitted) MUST be filed no later than the date on which the issue fee is paid. See 35 U.S.C. 115(f). Failure to timely comply will result in ABANDONMENT of this application.

A properly executed inventor's oath to declaration has not been received for the following inventor(s):

If applicant previously filed one or more oaths, declarations, or substitute statements, applicant may have received an informational notice regarding deficiencies therein.

The following deficiencies are noted:

INFORMAL ACTION PROBLEMS

- A properly executed inventor's oath or declaration has not been received for the following inventor(s): **Yong Qin Chen**.
  Applicant may submit the inventor's oath or declaration at any time before the Notice of Allowance and Fee(s) Due, PTOL-85, is mailed.

Questions relating to this Notice should be directed to the Application Assistance Unit at 571-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 14/555,102	Applicant(s) CHEN, YONG QIN	
	Examiner MINDY VU	Art Unit 2884	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *submissions of 27 October 2016*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *364-382 and 387*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Response to Arguments

Applicant's arguments, see page 8, filed October 27, 2016, with respect to independent claim 364 have been fully considered and are persuasive. Upon reconsideration, Capewell suggests minimizing the size of the image to maximize the optical energy and tolerance to detector misalignment. However, there's no teaching to focus the light beam to **a size smaller than the object**. Therefore, the rejection of claim 364 and those depend from 364 has been withdrawn.

Thus, claims 364-382 and 387 are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINDY VU whose telephone number is (571)272-8539. The examiner can normally be reached on M-F 9am - 5:30pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Porta can be reached on 571-272-2444. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/DAVID PORTA/
Supervisory Patent Examiner, Art Unit 2884

/M. V./
Examiner, Art Unit 2884

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S40	1074	chen-yong$.in.	US-PGPUB; USPAT; USOCR	OR	ON	2017/01/27 15:33
S41	7	S40 and wdm	US-PGPUB; USPAT; USOCR	OR	ON	2017/01/27 15:34
S42	8797	(g01n15/1434 g01n15/1436 g01n15/1459).cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 15:47
S46	310	S43 and S44	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 15:48
S45	91	S42 and S44	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 15:48
S44	425417	multiplexer wdm	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 15:48
S43	1966	g02b6/26.cpc. g02b6/29365.cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 15:48
S47	17503	focus$3 with siz$3 with small$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 16:16
S48	560	S44 and S47	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 16:17
S50	335	S49 and S44	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 16:19
S49	112156	flow near3 cytomet$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 16:19
S53	1518	S47 and S51	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 16:49
S52	837	(S42 or S43) and S51	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 16:49
S51	1035405	multiplex$3 wdm	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 16:49
S55	127	S53 and S54	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 16:50
S54	122053	cytomet$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/01/27 16:50
S60	82	wdm and S59	US-PGPUB; USPAT;	OR	OFF	2017/03/29

			USOCR; FPRS; EPO; JPO; DERWENT			10:10
S59	4030	collimat$3 with magnif$7	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 10:10
S62	119	S61 and wdm	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 11:18
S61	27493	smaller near4 size near4 (spot diameter)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 11:18
S66	11	wdm and S65	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 13:46
S65	122	S63 and S64	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 13:46
S64	27493	smaller near4 size near4 (spot diameter)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 13:46
S63	4030	collimat$3 with magnif$7	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 13:46
S68	8	S63 and S67 and wdm	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 13:49
S67	4567	focus$3 with small$2 with size with (spot diameter)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 13:49
S69	66	S63 and S67	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/29 14:57
L2	38	1 and wdm	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/30 10:23
L1	2216	focus$3 with smaller with size with (spot diameter)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2017/03/30 10:23

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S56	7	(flow and cytomet$3 and source and collimat$3 and focus$3 and siz$3).clm.	US-PGPUB; USPAT	OR	ON	2017/01/27 16:41
S58	4	(wavelength and multiplex$3 and collimat$3 and focus$3 and detect$3 and magnif$6).clm.	US-PGPUB; USPAT	OR	ON	2017/01/27 16:46
S57	159	(wavelength and multiplex$3 and collimat$3 and focus$3 and detect$3).clm.	US-PGPUB; USPAT	OR	ON	2017/01/27 16:46

3/30/2017 10:59:02 AM
C:\Users\mvu\Documents\EAST\Workspaces\14555102.wsp

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14555102	CHEN, YONG QIN
	Examiner	**Art Unit**
	MINDY VU	2884

CPC						
Symbol					**Type**	**Version**
G01N		15	/	1436	F	2013-01-01
G01N		15	/	1434	I	2013-01-01
G01N		2015	/	1452	A	2013-01-01
G01N		2015	/	144	A	2013-01-01
G02B		6	/	29365	I	2013-01-01
G02B		6	/	26	I	2013-01-01
G01N		2015	/	1006	A	2013-01-01
G01N		15	/	1459	I	2013-01-01
G02B		21	/	04	I	2013-01-01
G02B		21	/	361	I	2013-01-01
G02B		27	/	0025	I	2013-01-01
			/			
			/			
			/			
			/			

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

/MINDY VU/ Examiner.Art Unit 2884	01/27/17	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	19	
/DAVID PORTA/ Supervisory Patent Examiner.Art Unit 2884	03/30/2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	25

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14555102	CHEN, YONG QIN
	Examiner	**Art Unit**
	MINDY VU	2884

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				

INTERNATIONAL CLASSIFICATION					
CLAIMED					NON-CLAIMED
G	0	1	N	15 / 14 (2006.01.01)	
G	0	2	B	6 / 26 (2006.0)	
G	0	2	B	6 / 293 (2006.0)	

/MINDY VU/ Examiner.Art Unit 2884	01/27/17	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	19	
/DAVID PORTA/ Supervisory Patent Examiner.Art Unit 2884	03/30/2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	25

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14555102	CHEN, YONG QIN
	Examiner	**Art Unit**
	MINDY VU	2884

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

/MINDY VU/ Examiner.Art Unit 2884	01/27/17	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	19	
/DAVID PORTA/ Supervisory Patent Examiner.Art Unit 2884	03/30/2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	25

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14555102	CHEN, YONG QIN
	Examiner	Art Unit
	MINDY VU	2884

CPC- SEARCHED		
Symbol	Date	Examiner
G01N 21/64, 15/1434, 15/1436, 15/1459, 2015/1452	06/20/16	MV
G02B 6/26, 6/29365	06/20/16	MV
Above updated	01/27/17	mv

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Invenvor name search	06/19/16	mv
EAST BRS - See search history printout	06/21/16	mv
EAST BRS (Updated) - see search history printout	01/27/17	mv

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
Interference search history printout		1/27/17	mv

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040	4589

7590 04/14/2017

NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

EXAMINER	
VU, MINDY D	
ART UNIT	PAPER NUMBER
2884	
NOTIFICATION DATE	DELIVERY MODE
04/14/2017	ELECTRONIC

Letter Withdrawing a Notice Requiring Inventor's Oath or Declaration

The Notice Requiring Inventor's Oath or Declaration mailed on 4-5-17 was sent in error, and is hereby withdrawn. The time period set forth in the Notice of Allowance and Fee(s) Due to file a reply and pay the required fees continues to run from the mailing date of the Notice of Allowance and Fee(s) Due. Any time period set forth in the Notice of Allowability continues to run from the mailing date of the Notice of Allowability.

Questions relating to this Notice should be directed to the Application Assistance Unit at 571-272-4200.

/Shonne Martin/

(571)-272-4200 or 1(888)-786-0101

Patent Publication Branch

Office of Data Management

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

129381 7590 04/05/2017
NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

Gina N. Shishima	(Depositor's name)
/Gina N. Shishima/	(Signature)
June 29, 2017	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040	4589

TITLE OF INVENTION: FLOW CYTOMETER

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	07/05/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
VU, MINDY D	2884	250-458100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
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(A) NAME OF ASSIGNEE: Iris International, Inc.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Chatsworth, CA

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Authorized Signature /Gina N. Shishima/ Date June 29, 2017
Typed or printed name Gina N. Shishima Registration No. 45,104

Electronic Patent Application Fee Transmittal

Application Number:	14555102
Filing Date:	26-Nov-2014
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Filer:	Gina N. Shishima/Deborah Roszkowski
Attorney Docket Number:	BECC.P0002US.CIP(12US0040

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	1501	1	960	960

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
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Electronic Acknowledgement Receipt

EFS ID:	29656351
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
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1	Issue Fee Payment (PTO-85B)	BECCP0002USCIP_Issue_Fee_Transmittal.pdf	1591672 2e493faa3ea3b93509e9d4646f84996a721eb164	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30811 0565944b6a980c868dfde29b528e78605ea40810	no	2
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New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP/11412161	Serial No.: 14/555,102
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: IRIS INTERNATIONAL, INC.	
	Filing Date: November 26, 2014	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
None	*See Page 1*	*See Page 1*

U.S. Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.

Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	B36	JP H04-184241	07/01/92	Japan	Japanese (English Abstract)
	B37	JP 2004-341204	12/02/04	Japan	Japanese (English Abstract)
	B38	JP 2012-026837	02/09/12	Japan	Japanese (English Abstract)
	B39	JP H10-73528	03/17/98	Japan	Japanese (English Abstract)
	B40	JP H09-502794	03/18/97	Japan	Japanese (English Abstract)

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C6	Office Action issued in Japanese Patent Application No. 2015-515208, issued May 29, 2017 (English Translation Provided) (Attorney Docket No. BECC.P0002JP)

42533006.1

EXAMINER:	DATE CONSIDERED:

EXAMINER: INITIAL IF REFERENCE CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED. INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

Espacenet

Bibliographic data: JPH04184241 (A) — 1992-07-01

PARTICLE ANALYSER

Inventor(s): YAMAZAKI ISAO; OKI HIROSHI; MIYAKE AKIRA ± (YAMAZAKI ISAO, ; OKI HIROSHI, ; MIYAKE AKIRA)

Applicant(s): HITACHI LTD ± (HITACHI LTD)

Classification: **- international:** ***G01N15/14;*** (IPC1-7): G01N15/14
- cooperative:

Application number: JP19900315099 19901120 Global Dossier

Priority number (s): JP19900315099 19901120

Abstract of JPH04184241 (A)

PURPOSE:To obtain high analyzing accuracy by simply adjusting the width and uniformity of a laser beam irradiation region by providing a converting lens system on this side of the beam splitting element on laser beam and providing a converging lens system behind the element. CONSTITUTION:Laser beam 3 is split into two beams by a beam splitting element (Wollaston polarizing prism) 8 and these beams are respectively focused to an irradiation surface by converging lenses 2a, 2b but the spot positions thereof are separated at the interval equal to the diameter of each of the beams. When the focal distances of the lenses 2a, 2b are changed, the beam diameter and the spot interval are changed in the same ratio and, when the focal distance or magnifying power of a converting lens 4 is changed, only the beam diameter is largely changed. Therefore, by altering the converting lens 4, the overlap degree of the beams is changed and the uniformity of the intensity in an irradiation region can be altered.

⑲日本国特許庁（JP）　⑪特許出願公開

⑫公開特許公報（A）　平4－184241

⑤1Int. Cl.[5]	識別記号	庁内整理番号	⑬公開　平成4年(1992)7月1日
G 01 N 15/14		D 7005－2J	
		B 7005－2J	
		C 7005－2J	

審査請求　未請求　請求項の数 9　（全9頁）

⑤4発明の名称　粒子分析装置

⑳1特　願　平2－315099

⑳2出　願　平2(1990)11月20日

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明　細　書

1．発明の名称

粒子分析装置

2．特許請求の範囲

1．フローセルに被検粒子の懸濁液を供給する液供給手段と、該懸濁液中の被検粒子にレーザ光を照射する光学手段と、該被検粒子の像を結像させる集光手段と、該被検粒子から発散する光を検出する光検出手段と、該検出された光から前記被検粒子を分析する分析手段とを備えた粒子分析装置において、前記光学手段と前記フローセルの間のレーザ光の経路上に、該レーザ光をビーム径と同等あるいはそれ以下の間隔を隔てたビームに分岐するビーム分岐素子系を含み、該光学手段と該ビーム分岐素子系の間のレーザ光の経路上に焦点距離または拡大率可変の変換レンズ系を、該ビーム分岐素子系と該フローセルとの間のレーザ光の経路上に収束レンズ系を含むことを特徴とする粒子分析装置。

2．前記変換レンズ系は拡大率の異なる複数の変換レンズ・サブ系を並列して構成し、該変換レンズ・サブ系はその光軸を前記レーザ光の光軸に平行とし、該レーザ光の光軸を含む面内で平行移動可能に設置されたことを特徴とする請求項1記載の粒子分析装置。

3．前記ビーム分岐素子系は分岐角度の異なる複数のビーム分岐素子・サブ系を並列して構成し、該ビーム分岐素子・サブ系はその光軸を前記レーザー光の光軸に平行とし、該レーザ光の光軸を含む面内で平行移動可能に設置されたことを特徴とする請求項1または2記載の粒子分析装置。

4．前記収束レンズ系は収束率の異なる収束レンズ・サブ系を並列して構成し、該収束レンズ・サブ系はその光軸を前記レーザ光の光軸に平行とし、該レーザ光の光軸を含む面内で平行移動可能に設置されたことを特徴とする請求項1、2または3いずれか記載の粒子分析装置。

5．前記ビーム分岐素子・サブ系はそれぞれレーザ光進行方向に順次配列された1／2波長波と

ウォラストンプリズムとで構成されていることを特徴とする請求項4記載の粒子分析装置。

6．前記ビーム分岐素子系はレーザ光進行方向に順次配列された1／2波長板とウォラストンプリズムとで構成されていることを特徴とする請求項1記載の粒子分析装置。

7．前記ビーム分岐素子系は分割プリズムから構成されたことを特徴とする請求項1記載の粒子分析装置。

8．前記収束レンズ系は2枚のシリンドリカルレンズで構成し、該2枚のシリンドリカルレンズの一方は懸濁液の流れ方向と平行に、他方は該流れ方向に光を収束するように設置したことを特徴とする請求項1記載の粒子分析装置。

9．前記収束レンズ系は拡大率可変のレンズで構成したことを特徴とする請求項1記載の粒子分析装置。

3．発明の詳細な説明

〔産業上の利用分野〕

本発明は細管を流れる粒子にレーザ光を照射し、粒子から発散する散乱光や蛍光の強度を分析する粒子分析装置に係り、特にガウス分布を持つレーザビームのビーム断面における強度分布形状を平坦化するに好適なビーム強度平坦化装置を備えた粒子分析装置に関する。

〔従来の技術〕

従来、懸濁液サンプル中の粒子を速く効率的に識別できる方法の1つに流動細胞測定法がある。この方法では、粒子の懸濁液、典型的には血液サンプル中の細胞がフローセルの微小断面の流路を通して流され、そこで血液サンプル中の個々の粒子がレーザビームで照射される。そして個々の粒子とレーザビームの相互作用の結果が、一つもしくは複数の光検出装置により検出される。通常、光検出装置は特定の散乱角度における光の散乱または特定の波長の蛍光を測定するように設計されている。こうして、フローセルを流れる個々の粒子は、散乱光、蛍光もしくは他の光学的または電気的特性について関連した一つ以上の特性について特徴づけられる。それらの特性によって、個々の粒子は検出器で測定される光の強さまたは他の特性を軸とする特徴空間の中に写像される。理想的にはサンプル中の異なった種類の粒子が特徴空間の中の重複しない領域に写像され、特徴空間におけるその写像に基づいて個々の粒子の種類が推定できるとよい。粒子の分析精度を向上させるため、フローセル内の流れをつくる方法としてシースフロー方式が採用され、この方式によれば微小断面流路の中の中心部分のみを血液サンプルが流れる。

レーザビームのビーム断面における強度分布は、第9図に示すように中心部が高く周辺部が低いガウス分布になっている。粒子分析装置などの光源として使う場合には強度分布が一様である必要があるために、従来レーザビーム径を血液の流れに対して垂直な方向（幅方向）にのみ拡大した楕円ビームを用いてその中心部分のみを利用することが行われてきた。ガウス分布のレーザビームは一般に強度分布が中心強度の1／e^2となる径dをもってビームの大きさとしている。照射領域幅Sの範囲内での強度の均一度Uをi／Iで定義すると、d／Sを大きくするほど均一度Uは高くなる。しかし、d／Sを大きくした場合、レーザビームの持つエネルギのうち、照射領域幅Sの中で利用できる割合は小さくなってしまう。従って、ガウス分布のレーザビームを用いる限り均一度Uとエネルギ効率Eを同時に高くすることはできない。均一度Uとエネルギ効率Eを同時に高くするためにはレーザビームの強度分布をガウス分布より効率のいい形に変換する必要がある。

レーザビームを平坦化する装置としては、特開平1－210923号公報に記載の装置などがある。これは、プリズムの稜線を用いて、ガウス分布のビームを分割し、強度分布を反転して重ね合わせることにより強度分布を平坦化するものであった。この方法でエネルギ効率Eを向上することができ、アライメントレーザ等に用いることができるが、粒子分析装置の光源として用いるには適していない。それは、粒子分析装置では、必要な照射領域幅が20μm程度と小さいため、ガウス

分布のビームを分割した場合干渉縞が発生して強度分布が滑らかにならないこと、及び、レーザビームの光軸とプリズムの中心軸がわずかでもずれると得られるビームの強度分布が非対称になり、均一度Uが低下してしまうことによる。

そこで本出願人は特願平１－２７９４８７にて簡単な構成でビーム強度を平坦化した粒子分析装置を提案した。これはガウス分布のレーザビームをウォラストンプリズムで微小角度を持たせて分離し、集光レンズで粒子の通過領域に照射するものである。この方法では、レーザビームを偏光成分によって分離し、偏光成分が直行したまま重ね合わせるために、照射領域では互いに干渉しないレーザビームがある間隔をおいて重なった状態になっている。個々のレーザビームはガウス分布を保っているために、干渉縞が生じない。またプリズムの稜線の部分を使わないので、光軸とプリズムの中心軸を高精度に一致させる必要がないなどの利点があった。

〔発明が解決しようとする課題〕

粒子分析装置では、分析対象によってレーザビームの照射領域の大きさやレーザビームの強度を変更する必要がある。例えば、血液中の赤血球の分析を行う場合には、赤血球は単位容積当りに含まれる数が非常に多いために、フローセルに流すサンプル液の量を小さくするので、照射領域を狭くしてよい。さらに、赤血球の中の網状赤血球の比率を検出するには、網状赤血球から放射される蛍光の強度を高めるために、照射するレーザビームのエネルギ密度を高める必要がある。この場合は、照射領域の均一度を多少落としてもエネルギ密度を高くした方が効果的である。また、血液中の白血球の分析を行うためには、白血球が赤血球に比べて単位容積当りの数が１／７００と少ないために、フローセルに流すサンプル液の量を多くしなければならない。そのため、サンプル液の流れる領域の幅が大きくなるので、照射領域を広くしなければならない。この場合は、レーザビームのエネルギ密度が低くても白血球から放射される蛍光の強度は高いが、別の種類の白血球細胞と精度よく分類するには、レーザビームの照射均一度を高める必要がある。また、リンパ球のサブセットの分類を行う場合には、照射均一度を多少下げても、照射領域を広げ、かつエネルギ密度を高くする必要がある。このように、粒子分析装置では分析対象によってレーザビームの照射領域の大きさと領域内の照射均一度を独立に変更することが必要である。

従来のビーム強度平坦化装置を用いた粒子分析装置では、レーザビームが均一に照射している領域の大きさと領域内での照射の均一度を自由に変更することができなかった。２つのガウス分布のビームを重ね合わせると、照射面では第２図に示すように、強度分布形状は個々のビームの径ｄとビーム間隔Ｈで決まってしまう。ビーム径ｄは、収束レンズ２に入射するレーザビームの径Ｄと収束レンズ２の焦点距離ｆ、レーザ光の波長λを用いて、

$$d = (4/\pi) \cdot f \cdot \lambda / D$$

であらわされる。また、照射面での２つのビームの間隔Ｈは、ウォラストンプリズム８の分岐角度 θd を用いて、

$$H = f \cdot \theta d$$

で近似できる。分岐角度 θd はウォラストンプリズム８の幾何学的形状で決定されてしまうので自由に変更できない。収束レンズ２に焦点距離ｆが異なるレンズを用いても強度分布は相似な形状のまま拡大、縮小するだけである。粒子分析装置では、照射領域の大きさを変更するだけでなく照射均一度も変更する必要があるので強度分布形状も変える必要がある。強度分布の形状を変更するためには、ビーム径Ｄの異なるレーザ光源を用いるか、分岐角度 θd の異なるウォラストンプリズムを用いねばならず、高価になるという欠点があった。

また、粒子分析装置の照射領域を変更する場合以外にも、レーザ光源を交換したときなどにも強度分布形状を変更する必要がある。第２図は個々のビーム径ｄとビーム間隔Ｈの比Ｈ／ｄが０．５３０の場合の比較的平坦な強度分布であるが、Ｈ／ｄが０．６０７の場合の強度分布形状は第３図

のように二つの山形になる。レーザ光源によってビーム径は差があるが、ビーム径dが15%変化するだけで強度分布が第3図のように変化し照射均一度が下がってしまう。従来の粒子分析装置では、レーザ光源のビーム径の誤差によって照射均一度が低下して、分析精度が悪くなるという欠点があった。

本発明の目的は、レーザ光が均一に照射する領域の大きさと、その領域内の照射均一度を簡単に変更でき、分析精度の高い粒子分析装置を提供することにある。

〔課題を解決するための手段〕

上記目的を達成するために、本粒子分析装置は、フローセルに被検粒子の懸濁液を供給する液供給手段と該懸濁液中の被検粒子にレーザ光を照射する光学手段と、該被検粒子の像を結像させる集光手段と、該被検粒子から発散する光を検出する光検出手段と、該検出された光から前記被検粒子を分析する分析手段とを備えた装置において、前記光学手段と前記フローセルの間のレーザ光の経路上に、該レーザ光をビーム径と同等あるいはそれ以下の間隔を隔てたビームに分岐するビーム分岐素子系を含み、該光学手段と該ビーム分岐素子系の間のレーザ光の経路上焦点距離または拡大率可変の変換レンズ系を、該ビーム分岐素子系と該フローセルの間のレーザ光の経路上に収束レンズ系を含むことを特徴している。

そして、変換レンズ系は拡大率の異なる複数の変換レンズ・サブ系から構成しそれぞれの光軸がレーザ光の光軸に平行でかつ該レーザ光の光軸を含む面内で移動させる機構としてもよく、またビーム分岐素子系は分岐角度の異なる複数のビーム分岐素子・サブ系から構成しそれぞれの光軸がレーザ光の光軸に平行でかつ該レーザ光の光軸を含む面内で移動させる機構としてもよく、さらに収束レンズ系は収束率の異なる収束レンズ・サブ系から構成しかつそれぞれの光軸が前記レーザ光の光軸に平行でかつ該レーザ光の光軸を含む面内で移動させる機構としてもよい。

またビーム分岐素子系及びそのサブ系はそれぞれレーザ光進行方向に順次配列された1／2波長板とウォラストンプリズムで構成するのがよく、あるいはビーム分岐素子系は分割プリズムから構成することもできる。

また収束レンズ系は2枚のシリンドリカルレンズで構成し、該2枚のシリンドリカルレンズの一方は懸濁液の流れ方向と平行に他方は該流れ方向に光を収束するように設置するのがよい。また収束レンズ系は拡大率可変のレンズ組み合わせで構成することもできる。

〔作用〕

本発明の粒子分析装置において、レーザ光はビーム分岐素子により2つのビームに分離される。それぞれのビームは収束レンズで照射面に焦点を結ぶが、そのスポット位置はそれらのビームの径と同等またはそれ以下の間隔で隔たっている。収束レンズはレーザ光源から見てビーム分岐素子の背後にあるため収束レンズの焦点距離を変えるとビーム径及びスポット間隔が同じ割合で変化する。変換レンズはビーム分岐素子よりもレーザ光源側にあるために、変換レンズの焦点距離や拡大率を変化させるとスポットの間隔はあまり変化せずにビーム径のみが大きく変化する。したがって、変換レンズを変更することによりビームの重なり具合いを変化させることができ、照射領域内の強度の均一度を変更することができる。

〔実施例〕

本発明の実施例を図面を用いて説明する。

第1図は本発明の第1実施例の細胞分析装置の主要部分の構成を示す図である。レーザ光源15から出射するレーザビーム3の光軸の延長上に変換レンズ系4、1／2波長板7、ウォラストンプリズム8、収束レンズ2a、2b、フローセル1、ビームトラップ11が順次配置されている。フローセル1からレーザビーム3の進行方向には、集光レンズ6、ダイクロイックミラー21a、21bが順次設置され、そのダイクロイックミラー21a，21bの透過光路上及び2つの反射光路上に、それぞれフィルタ22（22a，22b，22c）、結像レンズ23（23a，23b，23

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ｃ）、スリット２４（２４ａ，２４ｂ，２４ｃ）、光検出器１０（１０ａ，１０ｂ，１０ｃ）からなる検出系が３組配置されている。フローセル１にはシース液供給器１９とサンプル液供給器２０からそれぞれシース液と被検粒子を懸濁したサンプル液が送られる。光検出器１０ａ，１０ｂ，１０ｃには分析器１７が接続され分析器１７には表示器１８が接続されている。また、制御器１６が分析器１７およびシース液供給器１９、サンプル液供給器２０に接続されている。

フローセル１の中を、細胞を特異的に染色する蛍光染料で染色した細胞の懸濁液をシースフローを形成して流し、フローセル１中の観測点５においてレーザビーム３で照射すると細胞が光を発散する。細胞から発散する光には、蛍光、散乱光などが含まれるが、それらの強度は細胞の種類、性質によって変化する。それらの光は集光レンズ６で平行光線に変換された後、ダイックロイックミラー２１ａ，２１ｂで各検出系に導かれる。ダイックロイックミラー２１ａは、波長の長い蛍光を反射し、それ以外の光を透過する。ダイックロイックミラー２１ｂは、波長の短い蛍光を反射し、散乱光を透過する。フィルタ２２ａは、レーザビーム３と同じ波長の散乱光のみを透過する狭帯域フィルタである。結像レンズ２３ａは観測点５から発散される散乱光の像をスリット２４ａの面上に結ぶ。スリット２４ａは、観測点５から発する散乱光の結像領域よりも大きな矩形スリットであり、観測点５から出た散乱光は制限せずに光検出器１０ａの検出面上に透過し、他の迷光成分は除外される。こうして光検出器１０ａでは細胞から発散される散乱光のみが検出される。同様に、光検出器１０ｂ及び光検出器１０ｃでは、それぞれ細胞から発散される緑色蛍光、及び、赤色蛍光のみが検出される。ここで、フィルタ２２ｂ及び２２ｃは、それぞれ緑色蛍光及び赤色蛍光のみを透過する狭帯域フィルタである。個々の細胞からの散乱光、緑色蛍光、赤色蛍光の分析をすることにより血液サンプル中に含まれる種々の細胞の個数や比率を得ることができる。

第１実施例において変換レンズ系４はズームレンズ系を構成しており、レンズ間隔を変えることにより焦点距離を変更可能である。１／２波長板はレーザビーム３の偏光面を４５度回転する方向に結晶軸を向けて取り付けてあり、さらに結晶軸方向が回転可能になっている。ウォラストンプリズム８はフローセル１の流路方向に直交する面内で光線を分岐する向きに取り付けてある。収束レンズ系２ａ及び２ｂは光軸方向に移動可能に取り付けられた焦点距離の異なる２枚のシリンドリカルレンズであり２ａは懸濁液の流れに直交する方向、２ｂはその流れに平行な方向にレーザビームを収束させる。レーザ光源１５を出射したレーザビーム３は小さな角度で広がっている。変換レンズ系４により広がり角度を一旦変更された後、収束レンズ２によってビーム径が縮小され、フローセル１中の観測点５でレーザビーム３のビーム径は最も小さくなる。１／２波長板７、及びウォラストンプリズム８は両面が平行なためにビーム径に与える影響は小さい。また、レーザビーム３の偏光面はレーザ光源１５から出射した時点で水平方向であるが、１／２波長板を通過する際に垂直から４５度方向に偏光面が回転する。ウォラストンプリズム８は、レーザビーム３を水平な偏光成分と垂直な偏光成分とに分岐する。その分岐角度θdはプリズムの形状で決まっている。２つに分岐したレーザビームは収束レンズ系２で観測点５に収束されるが、収束レンズ系を構成している２枚のシリンドリカルレンズの焦点距離が違うために収束したビームは、懸濁流の流れに平行な方向より垂直な方向の方が径が小さい楕円ビームとなっている。レーザビームは２つに分岐しているために２つの楕円ビームとして収束する。

観測点５における２つのレーザビームの流れに垂直な面内のビーム径をｄ、間隔をＨとすると強度分布は第２図のようにほぼ平坦になる。図で個々のレーザビームの強度分布を破線、合成された強度分布を実線で示してある。第２図は、Ｈ／ｄが０．５３０の場合であるが、計算によると、Ｈ／ｄが０．５以上になると強度分布の中心に凹み

をもち、その両側に最大値がある形状になる。このような場合に最大値をとる点の外側まで含めた領域内の照射の均一度は中心上の強度と最大の強度の比以下になる。第４図はビーム間隔と光強度均一度の関係を示す図で、Ｈ／ｄを変化させた場合の中心上の強度と最大強度の比を計算した結果である。ビーム間隔とビーム径の比Ｈ／ｄを変えると強度比が大きく変化するので、必要な照射均一度に合わせてビーム間隔とビーム径の比を精度よく設定する必要がある。また、このような場合に最も効率よくレーザビームのエネルギを利用するには、強度が最大となる点の外側で、中心の凹みと同じ強度となる点までの領域を利用することである。その領域は第２図のＳで示した。レーザビームの持つエネルギの内、領域Ｓに含まれる割合がエネルギ効率Ｅである。第５図はこのように選んだ領域Ｓに対するエネルギ効率Ｅと均一度Ｕの関係を計算した結果である。均一度Ｕを１に近付けるとエネルギ効率Ｅが急激に小さくなるので均一度Ｕを必要以上に高くするとレーザビームのエネルギ密度を確保するためにはレーザ光源１５の出力を大きくする必要がある。したがって用途に合わせて必要な均一度Ｅ及び、照射領域の幅Ｓを定めて、それに合わせて、ビーム間隔Ｈとビーム径ｄを設定する必要がある。

第１図の実施例の場合はビーム間隔Ｈは、収束レンズ系２ａの焦点距離ｆａとウォラストンプリズム８の分岐角度 θd を用いて

$$H = fa \cdot \theta d$$

で近似できる。またビーム径ｄは収束レンズ２ａに入射するレーザビーム径Ｄとレーザの波長λを用いて、

$$d = (4/\pi) \cdot fa \cdot \lambda / D$$

で近似できる。変換レンズ系４はズームレンズを構成しているので、レンズ間の間隔を変えることによりレーザビーム径Ｄを無段階に変更できる。収束レンズ２ａは容易に交換可能なので、焦点距離ｆａを自由に選べる。したがって変換レンズ系４と収束レンズ２ａの焦点距離を個別に変化させることにより、観測点５におけるレーザビームの照射均一度Ｅと照射領域の幅Ｓを個別に変更することができる。そのため照射領域の幅Ｓをフローセル１の中をサンプル液が流れる太さに合わせることができ、無駄な部分をレーザ光が照射することを防げるので、出力の小さなレーザ光源１を用いることができ、小形で低価格の粒子分析装置を構成することができる。またレーザ光源を別のものと交換して元々のレーザビームの径が変わっても調整することができる。また、照射領域内の均一度を調整できるので分析の精度を向上できる。また、照射領域以外の部分を照射する割合が小さいので、細胞粒子以外からの散乱光などの迷光が光検出器１０に入射して分析精度を下げることを避けられる。また、レーザビームの強度が小さくてすむ上に狭い領域しか照射しないので、レンズやミラーなどの光学部品に与える損傷が小さい。

また、本実施例の場合は、収束レンズ系が２枚のシリンドリカルレンズで構成されているため観測点５におけるビーム径を流れに平行な方向と垂直な方向に対して別々に設定できる。そのため、流れに垂直な方向のビーム径を小さくしておけば、観測点を粒子が通過する時間が短くなり、分析時間を短縮できる。また観測点内を同時に複数の粒子が通過することによる分析誤差を防ぐことができる。

また、変換レンズの間隔を変えた場合には収束レンズ２ａ、２ｂによるビームの収束位置が変化するが収束レンズ２ａ、２ｂは光軸方向に移動可能なので、収束位置を容易に観測点５に一致させることができる。

また、ウォラストンプリズム８で分岐されるレーザビームは偏光成分によって分けられているため、それぞれの成分の強度が異なると観測点５における照射強度分布が対称でなくなり、照射の均一度が悪化する。しかし、本実施例の場合は、１／２波長板７を回転させることによりそれぞれの成分の強度を一致させることができる。この場合に、ウォラストンプリズム８の後方に光検出器をおき、偏光子を用いて垂直偏光成分と水平偏光成分の強度測定し、それらが一致するように調整

するのみで照射強度分布が対称になるので、調整に特殊な測定器が要らないという利点がある。

なお、この実施例において、被検粒子を特定の一つに限定するような場合には、変換レンズ系の拡大率を一定にするため、各レンズを固定する手段を設けておくのがよい。

第６図は本発明の第２実施例のビーム整形光学系の構成を説明する図である。この場合は３つのスライダ２６、２７、２８が配置されている。スライダ２６は３つブロックで構成されそれぞれのブロックには変換レンズ系４が固定されており、スライダ２６を移動することで１組の変換レンズ系がレーザビーム３の光路上にくる。それぞれの変換レンズ系は拡大率の異なるビーム拡大光学系をサブ系として構成しており、出射するビームは平行ビームとなるように調整されている。スライダ２７はビーム分岐素子系のサブ系としての３つのブロックで構成され、そのうち２つには１／２波長板７と分岐角度 θd の異なるウォラストンプリズム８が固定されている。スライダ２７を移動することで、レーザビーム３の光路上に３つのうち１つのブロックが選ばれる。スライダ２８も収束レンズ系のサブ系として３つのブロックで構成されており、それぞれには収束レンズ系が固定されている。そのうち２組は、シリンドリカルレンズ２ａ，２ｂで構成されており、もう１組は球面レンズ２ｃで構成されている。スライダ２８を移動することで、どれか１組の収束レンズ系が選ばれる。スライダ２６、２７、２８はそれぞれ独立に動かすことができ、光軸に対して十分な位置の再現性がある。

この実施例の場合は、スライダを動かすことで簡単に観測点５における照射領域の幅と均一度を変更できる。また、変換レンズ系４を出射したレーザビームが平行ビームになっているため、光路上のウォラストンプルズムなどが変わってもレーザビームの収束位置が変化しないので、３つのスライダをどの様な組合せで用いてもビーム収束位置の補正が不要であるという利点がある。また粒子分析の対象によって最適な照射強度分布を得るように、スライダを自動的に動かす手段を設けることも可能である。またスライダ式の代りにレボルバの如く回転式にすることも可能である。

第７図は本発明の第３実施例のビーム整形光学系の構成を示す図である。第１実施例と異なるのは、１／２波長板とウォラストンプリズムを用いる代わりに、分割プリズム９を用いている点である。分割プリズム９は山形をしているので、稜線の上下でレーザビームが分岐角度 θd をもって分割され、収束レンズ２ａによって観測点５において間隔Ｈだけ隔てて収束する。この場合の観測点５における照射強度分布は第８図のようになる。個々のビーム第８図の破線で示したように、ガウス分布を中心で切断した形状をしている。それを重ね合わせると実線のように中心部が平坦な分布になる。照射領域の幅と均一度を調整する必要があることは第１実施例と同じであり、それは変換レンズ系４と収束レンズ系２ａ、２ｂを変えることで可能である。この実施例の場合は、分割したレーザビームの偏光面が一致しているため、粒子分析に偏光面の方向が影響を与えることを防ぐことができる。また、偏光面を回転させる１／２波長板が不要なため小型化、低価格化が可能である。また、レーザビームを半分に切断して用いるため、照射領域の外を照射する割合が小さく、エネルギ効率が高くなるという利点がある。

〔発明の効果〕

本発明によれば、粒子分析装置のレーザビーム上のビーム分岐素子の手前に変換レンズ系、ビーム分岐素子の背後に収束レンズ系を備えているため、必要な幅と均一度に合わせてレーザビームの照射領域の幅と均一度を調整でき、分析精度の高い粒子分析装置を提供することができる。

４．図面の簡単な説明

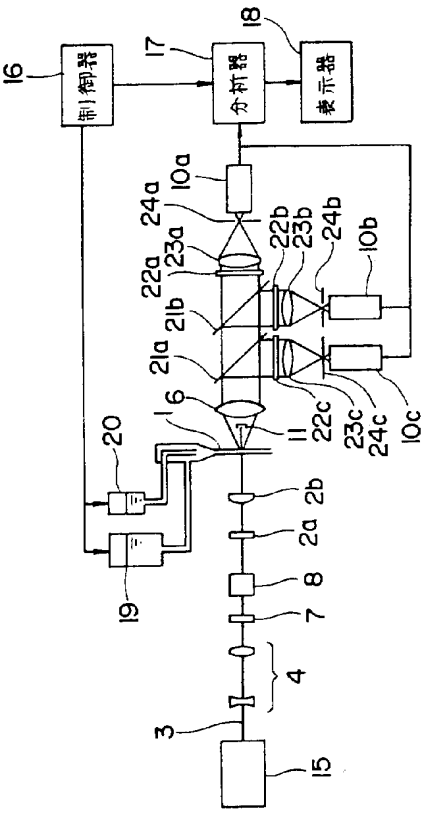
第１図は本発明の第１実施例を示す粒子分析装置の構成図、第２図～第５図は第１実施例の作用を説明する図、第６図は本発明の第２実施例の主要部分の構成図、第７図は第３実施例の主要部分の構成図、第８図は第３実施例の作用を説明する図、第９図は従来の光学系の作用を説明するため

の図。

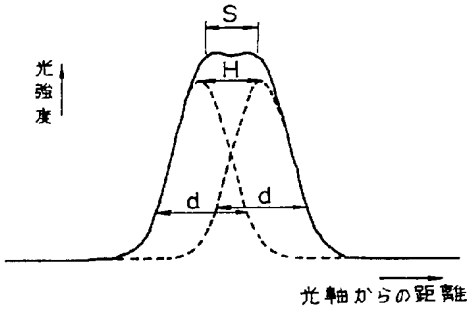
1…フローセル　　2…収束レンズ系
3…レーザビーム　　4…変換レンズ系
5…観測点　　6…集光レンズ
7…1／2波長板
8…ウォラストンプリズム
9…分割プリズム　　10…光検出器
15…レーザ光源　　19…シース液供給器
20…サンプル液供給器

代理人　鵜沼辰之

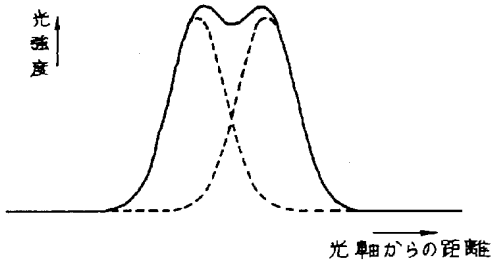
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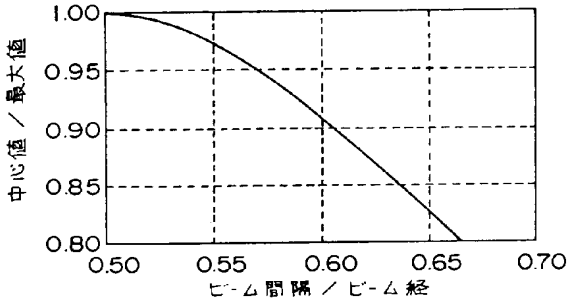
第2図



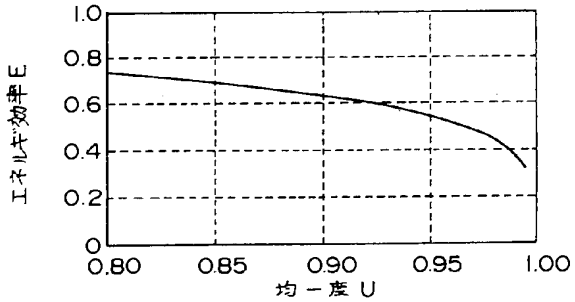
第3図



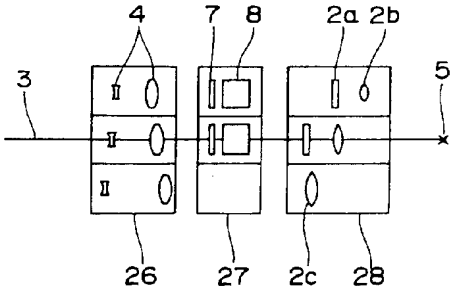
第4図



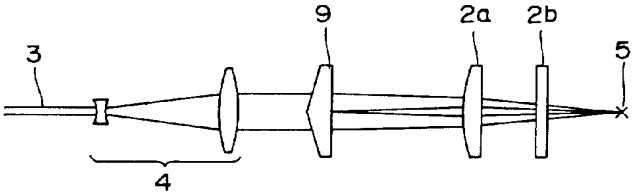
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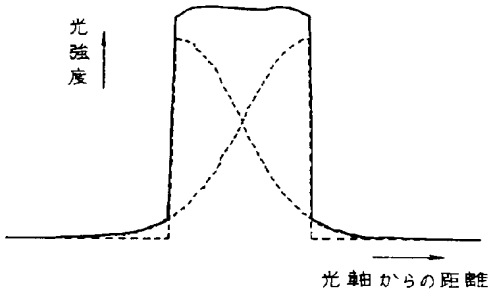
第 6 図



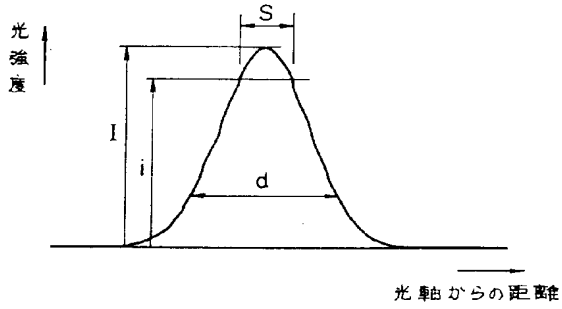
第 7 図



第 8 図



第 9 図



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Bibliographic data: JP2004341204 (A) — 2004-12-02

FLUORESCENT MICROSCOPE

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Applicant(s): OLYMPUS CORP ± (OLYMPUS CORP)

Classification: **- international:** ***G01N21/64; G02B21/06; G02B21/16;***
(IPC1-7): G01N21/64; G02B21/06; G02B21/16
- cooperative:

Application number: JP20030137483 20030515 Global Dossier

Priority number (s): JP20030137483 20030515

Abstract of JP2004341204 (A)

PROBLEM TO BE SOLVED: To provide a fluorescent microscope in which much fluorescent light reaches an optical detector which detects fluorescent light although the quantity of an undesired stimulating light reaching the optical detector is suppressed. ;SOLUTION: A scanning type fluorescent microscope has a light source part 1 which emits a light beam including stimulating light, a stimulating light irradiation optical system which irradiates a sample S with the light beam from the light source part 1, a separating optical element 5 which separates a fluorescent light generated by the sample S from the light beam traveling from the light source part 1 to the sample S, a light beam splitting part 15 which splits the light beam including the fluorescent light separated by the separating optical element 5 into two light beams, two fluorescent light selective transmission parts 18 and 23 which select fluorescent lights in specified wavelengths from the two split light beams respectively and transmit them, and two optical detectors 19 and 24 which detects the fluorescent lights transmitted through the fluorescent selective transmission parts 18 and 23 respectively. Further, the microscope has two stimulating light blocking optical elements 13 and 14 which are disposed between the separating optical element 5 and light beam splitting pat 15 and selectively block stimulating lights. ;COPYRIGHT: (C)2005,JPO&NCIPI

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G02B 21/06	G02B 21/06		

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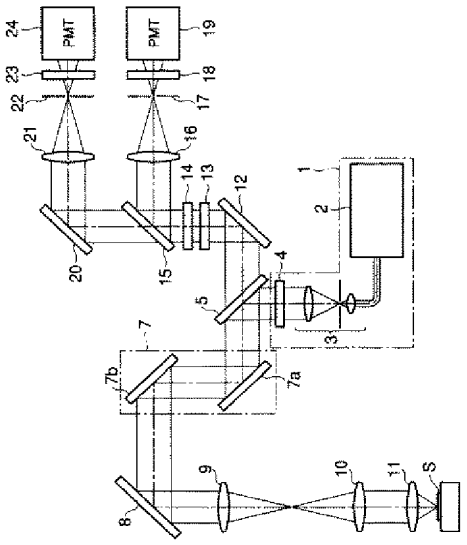
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最終頁に続く

(54)【発明の名称】蛍光顕微鏡

(57)【要約】
【課題】蛍光を検出する光検出器に到達する不所望な励起光の量を抑えつつも多く量の蛍光が光検出器に到達する蛍光顕微鏡を提供する。
【解決手段】走査型蛍光顕微鏡は、励起光を含む光ビームを射出する光源部１と、光源部１からの光ビームを試料Ｓに照射する励起光照射光学系と、試料Ｓから発生した蛍光を含む光ビームを光源部１から試料Ｓに向かう光ビームから分離する分離光学素子５と、分離光学素子５で分離された蛍光を含む光ビームを二本に分割する光ビーム分割部１５と、分割された二本の光ビームからそれぞれ特定の波長範囲内の蛍光を選択して透過する二つの蛍光選択透過部１８と２３と、蛍光選択透過部１８と２３を透過した蛍光をそれぞれ検出する二つの光検出器１９と２４と、分離光学素子５と光ビーム分割部１５の間に位置し、それぞれの励起光を選択的に遮断する二つの励起光遮断光学素子１３と１４とを有している。
【選択図】　図１



【特許請求の範囲】
【請求項1】
光ビームを試料に照射し試料から発する蛍光に基づいて試料を観察する蛍光顕微鏡であって、
少なくとも一種類の単一波長の励起光を含む光ビームを射出する光源部と、
光源部から射出された光ビームを試料に照射して試料から少なくとも一種類の蛍光を発生させる励起光照射光学系と、
試料から発生した少なくとも一種類の蛍光を含む光ビームを、光源部から試料に向かう光ビームから分離する分離光学素子と、
分離光学素子で分離された少なくとも一種類の蛍光を含む光ビームから特定の波長範囲内の蛍光を選択して透過する少なくとも一つの蛍光選択透過部を含む蛍光選択透過機構と、
蛍光選択透過機構を透過した蛍光を検出する少なくとも一つの光検出器を含む蛍光検出部と、
分離光学素子と蛍光選択透過部の間の光路上に位置し、光源部から射出された光ビームに含まれる少なくとも一種類の励起光を選択的に遮断する少なくとも一つの励起光遮断光学素子を含む励起光遮断部とを有している、蛍光顕微鏡。
【請求項2】
請求項１において、
光源部は、複数種類の単一波長の励起光を含む光ビームを射出し、
励起光照射光学系は、複数種類の単一波長の励起光を含む光ビームを試料に照射して試料から複数種類の蛍光を発生させ、
分離光学素子は、試料から発生した複数種類の蛍光を含む光ビームを光源部から試料に向かう光ビームから分離し、
蛍光顕微鏡は更に、分離光学素子で分離された複数種類の蛍光を含む光ビームを、複数種類の蛍光の波長帯域に基づいて複数に分割する光ビーム分割部を有しており、
蛍光選択透過機構は、光ビーム分割部で分割された複数の光ビームから、それぞれ、特定の波長範囲内の蛍光を選択して透過する複数の蛍光選択透過部を有しており、
蛍光検出部は、複数の蛍光選択透過部を透過した蛍光をそれぞれ検出する複数の光検出器を有しており、
励起光遮断部は、光源部から射出された光ビームに含まれる複数種類の励起光をそれぞれ選択的に遮断する複数の励起光遮断光学素子を有している、蛍光顕微鏡。
【請求項3】
請求項１または請求項２において、励起光遮断光学素子はホログラフィックノッチフィルターである、蛍光顕微鏡。
【請求項4】
請求項１ないし請求項３のいずれかひとつにおいて、励起光照射光学系は、励起光を含む光ビームを試料に収束させる対物レンズと、対物レンズにより試料に形成される励起光を含む光ビームのスポットを走査する走査光学系とを含んでいる、蛍光顕微鏡。
【請求項5】
請求項１ないし請求項４のいずれかひとつにおいて、蛍光選択透過部は、特定の波長範囲から外れた波長の光を吸収する吸収フィルターである、蛍光顕微鏡。
【請求項6】
請求項１ないし請求項４のいずれかひとつにおいて、蛍光選択透過部は、入射する光ビームを波長分散させる分散素子と、分散素子により分散された光ビームを収束させる収束レンズと、収束レンズにより収束された光ビームの一部の波長域を通す光学的開口とを有し、収束レンズは入射した光ビームを波長成分に応じて異なる位置に収束させ、光学的開口は特定の波長成分の光を選択的に透過する、蛍光顕微鏡。
【請求項7】
請求項６において、蛍光選択透過部は光学的開口に対する波長成分の収束位置を決め、分散素子の回転角度を変更可能とし、蛍光顕微鏡は、分散素子の回転角度を変えることによ

り、光学的開口を透過する光ビームの波長域を可変して、蛍光を細分化した波長帯域毎に検出し得る、蛍光顕微鏡。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】

本発明は、蛍光顕微鏡に関する。

【０００２】

【従来の技術】

蛍光顕微鏡は、一般に、染色された試料に、少なくとも一種類の波長の励起光を含む光ビームを照射して試料を励起し、試料から発生した少なくとも一種類の蛍光を、少なくとも一つの検出器をで検出し、検出された蛍光に基づいて試料の画像（蛍光画像）を取得する。

【０００３】

このような蛍光顕微鏡は、例えば特開平１１－２３１２２２号公報に開示されている。

【０００４】

【特許文献１】

特開平１１－２３１２２２号公報

【０００５】

【発明が解決しようとする課題】

蛍光顕微鏡においては、一般に、波長により分光し得るダイクロイックミラーを用いて、試料に照射される励起光から、試料から発生した蛍光が分離される。通常、試料からダイクロイックミラーに到達する光は、試料から発生した蛍光だけでなく、試料で反射した励起光も含まれている。

【０００６】

一般に、試料を励起する励起光と、試料から発生する蛍光は、比較的近い波長を有している。励起光と蛍光の分離のためには、ダイクロイックミラーは、特定の波長を境に二値的に変化する透過率特性を有していることが理想的であるが、実際の透過率特性は、比較的急峻に変化していても、その部分はある程度の幅を有している。また、その部分の両端における透過率も０と１ではない。このため、ダイクロイックミラーで分離された光には、蛍光だけでなく、試料で反射された励起光も含まれる。

【０００７】

また、蛍光を検出する光検出器の手前には、検出対象の蛍光だけを検出するために、検出対象の蛍光に合った波長帯域の光を選択的に透過するフィルター等の光学要素が配置される。前述したように、ダイクロイックミラーで分離された光には不所望な励起光も含まれるため、このようなフィルター等の光学要素は、単に検出対象の蛍光を透過するだけでなく、不所望な励起光を確実に遮断する機能も要求される。

【０００８】

通常、不所望な励起光を遮断することを優先すると、それに伴って光検出器に到達する検出対象の蛍光の量も減ってしまい、反対に、検出対象の蛍光を多く透過することを優先すると、光検出器に到達する不所望な励起光も増えてしまう。

【０００９】

このような事情は、複数種類の励起光を含む光ビームを試料に照射して複数種類の蛍光を発生させる場合、更に顕著である。

【００１０】

本発明は、このような実状を考慮して成されたものであり、その目的は、蛍光を検出する光検出器に到達する不所望な励起光の量を抑えつつ、多く量の蛍光が光検出器に到達する蛍光顕微鏡を提供することである。

【００１１】

【課題を解決するための手段】

本発明は、以下の各項に列記する蛍光顕微鏡を含んでいる。

【００１２】
１．本発明の蛍光顕微鏡は、少なくとも一種類の単一波長の励起光を含む光ビームを射出する光源部と、光源部から射出された光ビームを試料に照射して試料から少なくとも一種類の蛍光を発生させる励起光照射光学系と、試料から発生した少なくとも一種類の蛍光を含む光ビームを、光源部から試料に向かう光ビームから分離する分離光学素子と、分離光学素子で分離された少なくとも一種類の蛍光を含む光ビームから特定の波長範囲内の蛍光を選択して透過する少なくとも一つの蛍光選択透過部を含む蛍光選択透過機構と、蛍光選択透過機構を透過した蛍光を検出する少なくとも一つの光検出器を含む蛍光検出部と、分離光学素子と蛍光選択透過部の間の光路上に位置し、光源部から射出された光ビームに含まれる少なくとも一種類の励起光を選択的に遮断する少なくとも一つの励起光遮断光学素子を含む励起光遮断部とを有している。
【００１３】
この蛍光顕微鏡においては、不所望な励起光は、それ専用の励起光遮断光学素子によって、良好に遮断される。これにより、蛍光選択透過部に要求される光学的特性が緩和される。このため、蛍光選択透過部は、比較的広い波長範囲の光を透過してもよい。その結果、光検出器に、多くの量の蛍光が到達する。
【００１４】
２．本発明の別の蛍光顕微鏡は、第１項の蛍光顕微鏡において、光源部は、複数種類の単一波長の励起光を含む光ビームを射出し、励起光照射光学系は、複数種類の単一波長の励起光を含む光ビームを試料に照射して試料から複数種類の蛍光を発生させ、分離光学素子は、試料から発生した複数種類の蛍光を含む光ビームを光源部から試料に向かう光ビームから分離し、蛍光顕微鏡は更に、分離光学素子で分離された複数種類の蛍光を含む光ビームを、複数種類の蛍光の波長帯域に基づいて複数に分割する光ビーム分割部を有しており、蛍光選択透過機構は、光ビーム分割部で分割された複数の光ビームから、それぞれ、特定の波長範囲内の蛍光を選択して透過する複数の蛍光選択透過部を有しており、蛍光検出部は、複数の蛍光選択透過部を透過した蛍光をそれぞれ検出する複数の光検出器を有しており、励起光遮断部は、光源部から射出された光ビームに含まれる複数種類の励起光をそれぞれ選択的に遮断する複数の励起光遮断光学素子を有している。
【００１５】
この蛍光顕微鏡においては、複数種類の励起光を含む光ビームを試料に照射して複数種類の蛍光を発生させる場合においても、不所望な励起光が良好に遮断されつつも、光検出器に多くの量の蛍光が到達する。
【００１６】
３．本発明の別の蛍光顕微鏡は、第１項または第２項の蛍光顕微鏡において、励起光遮断光学素子はホログラフィックノッチフィルターである。
【００１７】
この蛍光顕微鏡は、不所望な励起光を良好に遮断する好適な励起光遮断光学素子の一態様を与える。
【００１８】
４．本発明の別の蛍光顕微鏡は、第１項ないし第３項のいずれかひとつの蛍光顕微鏡において、励起光照射光学系は、励起光を含む光ビームを試料に収束させる対物レンズと、対物レンズにより試料に形成される励起光を含む光ビームのスポットを走査する走査光学系とを含んでいる。
【００１９】
この蛍光顕微鏡は、走査型蛍光顕微鏡を提供する。
【００２０】
５．本発明の別の蛍光顕微鏡は、第１項ないし第４項のいずれかひとつの蛍光顕微鏡において、蛍光選択透過部は、特定の波長範囲から外れた波長の光を吸収する吸収フィルターである。
【００２１】

この蛍光顕微鏡は、蛍光選択透過部の一態様を与える。
【００２２】
６．本発明の別の蛍光顕微鏡は、第１項ないし第４項のいずれかひとつの蛍光顕微鏡において、蛍光選択透過部は、入射する光ビームを波長分散させる分散素子と、分散素子により分散された光ビームを収束させる収束レンズと、収束レンズにより収束された光ビームの一部の波長域を通す光学的開口とを有し、収束レンズは入射した光ビームを波長成分に応じて異なる位置に収束させ、光学的開口は特定の波長成分の光を選択的に透過する。
【００２３】
この蛍光顕微鏡は、蛍光選択透過部の別の一態様を与える。
【００２４】
７．本発明の別の蛍光顕微鏡は、第６項の蛍光顕微鏡において、蛍光選択透過部は光学的開口に対する波長成分の収束位置を決め、分散素子の回転角度を変更可能とし、蛍光顕微鏡は、分散素子の回転角度を変えることにより、光学的開口を透過する光ビームの波長域を可変して、蛍光を細分化した波長帯域毎に検出し得る。
【００２５】
この蛍光顕微鏡は、検出対象の蛍光のスペクトルデータを取得可能である。
【００２６】
【発明の実施の形態】
以下、図面を参照しながら本発明の実施の形態について説明する。
【００２７】
第一実施形態
本実施形態は、走査型蛍光顕微鏡に向けられている。以下、図１と図３～図８を参照しながら本実施形態について説明する。
【００２８】
図１に示されるように、本実施形態の走査型蛍光顕微鏡は、少なくとも一種類の単一波長の励起光を含む光ビームを射出する光源部１を有している。光源部１は、これに限定されないが、例えば、複数の単一波長の光を含む光ビームを発するレーザ光源２と、レーザ光源２から発せられた光ビームの径を拡大するビームエキスパンダ３と、試料Ｓの励起に必要な特定の波長の光を選択的に透過するレーザーラインフィルター４とを有している。
【００２９】
例えば、レーザ光源２は、４８８ｎｍと５６８ｎｍと６４７ｎｍの波長の光を同時発振するクリプトンアルゴンレーザであり、レーザーラインフィルター４は、レーザ光源２から発せられた光のうち、４８８ｎｍと５６８ｎｍの波長の光を選択的に透過する。
【００３０】
従って、光源部１は、二種類の単一波長の励起光を含む光ビームを射出する。しかし、光源部１は、これに限定されるものではなく、一種類の単一波長の励起光から成る光ビームを射出してもよく、あるいは、三種類以上の単一波長の励起光を含む光ビームを射出してもよい。
【００３１】
図１において、走査型蛍光顕微鏡は更に、光源部１から射出された二種類の励起光を含む光ビームを試料Ｓに照射する励起光照射光学系を有している。励起光照射光学系は、励起光を含む光ビームを試料Ｓに収束させる対物レンズ１１と、励起光を含む光ビームを対物レンズ１１に向けて偏向するミラー８と、ミラー８から対物レンズ１１に至る間の光路上に配置された瞳投影レンズ９と結像レンズ１０とを含んでいる。
【００３２】
励起光照射光学系は更に、対物レンズ１１により試料Ｓに形成される励起光を含む光ビームのスポットを二次元的に走査する走査光学系７を有している。走査光学系７は、光源部１とミラー８の間の光路上に配置された、ガルバノメータスキャナ７ａとガルバノメータスキャナ７ｂとで構成されている。
【００３３】

二つのガルバノメータスキャナ７ａと７ｂは、それぞれ、方向を変更可能な反射面を有しており、反射面の方向を変更することにより、反射面で反射された光ビームを一方向に沿って（つまり一次元的に）走査する。二つのガルバノメータスキャナ７ａと７ｂは、その反射面で反射された光ビームを互いに直交する方向（Ｘ方向とＹ方向）に走査する。このため、走査光学系７により、試料Ｓに形成される光ビームのスポットは、二次元的に走査される。

【００３４】

試料Ｓは光ビームの照射に反応して蛍光を発する。試料Ｓは、照射された光ビームに含まれる励起光の種類に対応した種類の蛍光を発する。本実施形態では、試料Ｓは二種類の試薬で染色されており、試料Ｓには二種類の励起光を含む光ビームが照射される。このため、試料Ｓからは二種類の蛍光が発生する。試料Ｓから発生する二種類の蛍光は、それぞれ、試料Ｓに照射される二種類の励起光の波長帯域に対応した波長帯域を有する。

【００３５】

走査型蛍光顕微鏡は更に、試料Ｓから発生した蛍光を含む光ビームを、光源部１から試料Ｓに向かう光ビームから分離する分離光学素子５を有している。簡単に言えば、分離光学素子５は励起光と蛍光とを分離する。分離光学素子５は、例えば、光源部１と試料Ｓの間の光路上に位置するダイクロイックミラーで構成される。

【００３６】

図１において、走査型蛍光顕微鏡は更に、分離光学素子５で分離された少なくとも一種類の蛍光を含む光ビームから特定の波長範囲内の蛍光を選択して透過する少なくとも一つの蛍光選択透過部を含む蛍光選択透過機構と、蛍光選択透過機構を透過した蛍光を検出する少なくとも一つの光検出器を含む蛍光検出部とを有している。

【００３７】

より詳しくは、試料Ｓからは二種類の蛍光が発生されることに対応して、走査型蛍光顕微鏡は更に、分離光学素子５で分離された蛍光を含む光ビームを、それに含まれる二種類の蛍光の波長帯域に基づいて二本に分割する光ビーム分割部１５を有している。蛍光選択透過機構は、光ビーム分割部１５で分割された二本の光ビームから、それぞれ、特定の波長範囲内の蛍光を選択して透過する二つの蛍光選択透過部１８と２３を有している。蛍光検出部は、二つの蛍光選択透過部１８と２３を透過した蛍光をそれぞれ検出する二つの光検出器１９と２４を有している。

【００３８】

光ビーム分割部１５は、これに限定されないが、例えば、ダイクロイックミラーで構成される。二つの蛍光選択透過部１８と２３は、それぞれ、これに限定されないが、例えば、特定の波長範囲から外れた波長の光を吸収する吸収フィルターで構成される。二つの光検出器１９と２４は、それぞれ、これに限定されないが、例えば、フォトマルチプライヤ（ＰＭＴ）で構成される。

【００３９】

走査型蛍光顕微鏡は更に、分離光学素子５で分離された蛍光を含む光ビームを光ビーム分割部１５に向けて偏向するミラー１２と、光ビーム分割部１５を透過した光ビームを光検出器２４に向けて偏向するミラー２０とを有している。

【００４０】

走査型蛍光顕微鏡は更に、光ビーム分割部１５と蛍光選択透過部１８の間の光路上に配置された集光レンズ１６およびピンホール１７と、ミラー２０と蛍光選択透過部２３の間の光路上に配置された集光レンズ２１およびピンホール２２とを有している。

【００４１】

ピンホール１７とピンホール２２は、それぞれ、対物レンズ１１により試料Ｓに形成される励起光を含む光ビームのスポットに対して共焦点の位置関係に配置されている。このため、ピンホール１７とピンホール２２は、共焦点効果により、試料Ｓに形成される光ビームのスポットから外れた位置、特に光軸に沿って外れた位置から発生された蛍光を効果的に遮断する。このため、光検出器１９と２４は、試料Ｓに形成される光ビームのスポット

の位置からの蛍光を選択的に検出し得る。

【００４２】

走査型蛍光顕微鏡は更に、光源部１から射出される光ビームに含まれる二種類の励起光をそれぞれ選択的に遮断する励起光遮断光学素子１３と励起光遮断光学素子１４とを有している。二つの励起光遮断光学素子１３と１４は共に分離光学素子５と光ビーム分割部１５の間の光路上に配置されている。二つの励起光遮断光学素子１３と１４は、好ましくは、それぞれ、ホログラフィックノッチフィルターで構成される。

【００４３】

走査型蛍光顕微鏡においては、光源部１から発せられた励起光を含む光ビームは対物レンズ１１により試料Ｓに収束される。対物レンズ１１により試料Ｓに形成された光ビームのスポットは、走査光学系７により試料Ｓの表面上あるいは内部を二次元的に走査される。試料Ｓに照射される光ビームは二種類の励起光を含んでおり、試料Ｓはそれに対応した二種類の試薬で染色されているため、試料Ｓからは二種類の蛍光が発生する。

【００４４】

試料Ｓから発生した二種類の蛍光は、それぞれ、光検出器１９と光検出器２４により検出される。特に、共焦点効果により、光ビームのスポットの位置から発生した蛍光が、光検出器１９と光検出器２４により選択的に検出される。光検出器１９と光検出器２４で得られる信号を、走査光学系７を制御する走査信号に同期させて処理することにより、それぞれの蛍光に基づく試料Ｓの蛍光画像が得られる。

【００４５】

前述したように、レーザ光源２は、４８８ｎｍと５６８ｎｍと６４７ｎｍの波長の光を発する。レーザーラインフィルター４は、図３に示される透過率特性を有しており、レーザ光源２で発せられた光のうち、４８８ｎｍと５６８ｎｍの光を選択的に透過する。従って、光源部１からは、４８８ｎｍと５６８ｎｍの励起光を含む光ビームが射出される。

【００４６】

光源部１から射出された４８８ｎｍと５６８ｎｍの励起光は試料Ｓに照射され、試料Ｓからは、４８８ｎｍの励起光に対応して、５００ｎｍ～５４０ｎｍの波長帯域の蛍光が発生され、５６８ｎｍの励起光に対応して、５８５ｎｍ～６５０ｎｍの波長帯域の蛍光が発生される。

【００４７】

励起光と蛍光とを分離する分離光学素子５を構成するダイクロイックミラーは、例えば図４に示される透過率特性を有しており、光源部１から射出される４８８ｎｍと５６８ｎｍの波長の励起光を反射する一方、試料Ｓから発生される５００～５４０の波長帯域の蛍光と５８５ｎｍ～６５０ｎｍの波長帯域の蛍光を良好に透過する。

【００４８】

図４から分かるように、分離光学素子５を構成するダイクロイックミラーは、４８８ｎｍと５６８ｎｍの波長の励起光をいくらかは透過する。このため、試料Ｓで反射された励起光のいくらかは、試料Ｓから発生された蛍光と一緒に分離光学素子５であるダイクロイックミラーを透過する。

【００４９】

励起光遮断光学素子１３を構成するホログラフィックノッチフィルターは、図５にＨＮＦ１３で示される透過率特性を有し、励起光遮断光学素子１４を構成するホログラフィックノッチフィルターは、図５にＨＮＦ１４で示される透過率特性を有している。

【００５０】

図５から分かるように、励起光遮断光学素子１３を構成するホログラフィックノッチフィルターは、４８８ｎｍの波長の近傍の帯域において、透過率がほとんど０である。従って、励起光遮断光学素子１３を構成するホログラフィックノッチフィルターは、４８８ｎｍの波長の励起光をほとんど完全に遮断する。

【００５１】

同様に、励起光遮断光学素子１４を構成するホログラフィックノッチフィルターは、５６

８ｎｍの波長の近傍の帯域において透過率がほとんど０である。従って、励起光遮断光学素子１４を構成するホログラフィックノッチフィルターは、５６８ｎｍの波長の励起光をほとんど完全に遮断する。

【００５２】

その結果、光ビーム分割部１５に到達した光ビームは、試料Ｓで反射された４８８ｎｍと５６８ｎｍの波長の励起光を殆ど含まない。つまり、光ビーム分割部１５に到達した光ビームは、実質的に、検出対象である試料Ｓから発生された５００ｎｍ～５４０ｎｍの波長帯域の蛍光と５８５ｎｍ～６５０ｎｍの波長帯域の蛍光で構成されている。

【００５３】

光ビーム分割部１５を構成するダイクロイックミラーは、例えば図６に示される透過率特性を有しており、５００ｎｍ～５４０ｎｍの波長帯域の蛍光を良好に反射する一方、５８５ｎｍ～６５０ｎｍの波長帯域の蛍光を透過する。

【００５４】

蛍光選択透過部１８を構成する吸収フィルターは、例えば図７にＢＡ１８で示される透過率特性を有し、蛍光選択透過部２３を構成する吸収フィルターは、図７にＢＡ２３で示される透過率特性を有している。

【００５５】

図７から分かるように、励起光遮断光学素子１３を構成するホログラフィックノッチフィルターの透過率特性ＨＮＦ１３と励起光遮断光学素子１４を構成するホログラフィックノッチフィルターの透過率特性ＨＮＦ１４に対応して、吸収フィルター１８と吸収フィルター２３は共に比較的広い透過帯域を有している。

【００５６】

比較例として、図１の構成から励起光遮断光学素子１３と励起光遮断光学素子１４とが省かれた構成（すなわち従来の走査型蛍光顕微鏡）において、蛍光選択透過部１８を構成する吸収フィルターに要求される透過率特性と、蛍光選択透過部２３を構成する吸収フィルターに要求される透過率特性を図８に示す。

【００５７】

図１の構成から励起光遮断光学素子１３と励起光遮断光学素子１４とが省かれた構成においては、蛍光選択透過部１８と蛍光選択透過部２３に到達した光は、検出対象の蛍光の他に、試料Ｓで反射された不所望な励起光も含んでいる。このため、蛍光選択透過部１８と蛍光選択透過部２３は、単にそれぞれの検出対象の蛍光を透過するだけでなく、不所望な励起光を良好に遮断することも要求される。

【００５８】

このため、蛍光選択透過部１８を構成する吸収フィルターの透過特性は、４８８ｎｍと５６８ｎｍの波長の励起光を良好に遮断するように、図８にＢＡ１８で示されるように、透過帯域が狭いものにならざるを得ない。同様に、蛍光選択透過部２３を構成する吸収フィルターの透過特性は、５６８ｎｍの波長の励起光を良好に遮断するように、図８にＢＡ２３で示されるように、透過帯域が狭いものものにならざるを得ない。

【００５９】

その結果、例えば、蛍光選択透過部１８は、５００ｎｍ～５４０ｎｍの透過帯域を有する吸収フィルターで構成され、蛍光選択透過部２３は、５８５ｎｍ～６５０ｎｍの透過帯域を有する吸収フィルターで構成される。図８において、「５８５ｎｍ（蛍光）」は蛍光のピーク波長を示しており、実際の蛍光はこの両側にある帯域を持って広がっている。従って、ＢＡ２３で示される特性の吸収フィルターであっても、蛍光を検出することは可能である。

【００６０】

このような透過帯域の狭い吸収フィルターは、好ましくないことに、検出対象である蛍光をも吸収してしまい、その後方に位置する光検出器１９や光検出器２４に到達する蛍光の量を減少させてしまう。

【００６１】

これに対して、本実施形態の走査型蛍光顕微鏡においては、不所望な４８８ｎｍと５６８ｎｍの波長の励起光は、分離光学素子５と光ビーム分割部１５の間の光路上に配置された二つの励起光遮断光学素子１３と１４によって遮断される。このため、蛍光選択透過部１８と蛍光選択透過部２３に到達した光は、不所望な励起光を殆ど含まない。

【００６２】

従って、蛍光選択透過部１８と蛍光選択透過部２３には、不所望な励起光を良好に遮断することは要求されない。このため、蛍光選択透過部１８と蛍光選択透過部２３に、透過帯域の広い吸収フィルターを使用することが可能になる。具体的には、本実施形態における吸収フィルター（図７）の励起光遮断レベルは、従来例における吸収フィルター（図８）の励起光遮断レベルに比べて１桁から３桁程度低くてよく、その分、蛍光の透過帯域を広げることができる。これにより、蛍光選択透過部１８と蛍光選択透過部２３の後方にそれぞれ位置する光検出器１９と光検出器２４に、より多くの量の蛍光を到達させることができる。

【００６３】

その結果、本実施形態の走査型蛍光顕微鏡は、励起光の波長に近い波長の蛍光も検出できる。これにより、例えば、より明るい蛍光画像を得ることができる。

【００６４】

第二実施形態

本実施形態は、走査型蛍光顕微鏡に向けられている。以下、図２を参照しながら本実施形態について説明する。

【００６５】

図２は、本発明の第二実施形態の走査型蛍光顕微鏡を示している。図２において、図１中の部材と同一の参照符号で指示された部材は同様の部材であり、その詳しい説明は省略する。

【００６６】

本実施形態の走査型蛍光顕微鏡は、主に蛍光選択透過機構の構成の点で、第一実施形態の走査型蛍光顕微鏡と相違している。

【００６７】

図２に示されるように、本実施形態の走査型蛍光顕微鏡は、分離光学素子５とミラー１２の間の光路上に、分離光学素子５で分離された蛍光を含む光ビームを収束させる結像レンズ２５と、結像レンズ２５の結像位置に配置された共焦点絞り２６と、共焦点絞り２６を通過し発散光ビームを平行光ビームに変えるレンズ２７とを有している。

【００６８】

共焦点絞り２６は、対物レンズ１１により試料Ｓに形成される励起光を含む光ビームのスポットに対して共焦点の位置関係に配置されている。このため、共焦点絞り２６は、共焦点効果により、試料Ｓに形成される光ビームのスポットから外れた位置、特に光軸に沿って外れた位置から発生された蛍光を効果的に遮断する。これにより、最終的に得られる蛍光画像の分解能の向上に貢献する。

【００６９】

本実施形態の走査型蛍光顕微鏡は、光ビーム分割部１５から光検出器１９に至る光路上に蛍光選択透過部２８を有し、光ビーム分割部１５からミラー２０を経て光検出器２４に至る光路上に蛍光選択透過部３２を有している。

【００７０】

別の観点から、図１に示される第一実施形態の走査型蛍光顕微鏡との相違に注目して述べれば、本実施形態の走査型蛍光顕微鏡は、分離光学素子５とミラー１２の間の光路上に結像レンズ２５と共焦点絞り２６とレンズ２７とが追加されており、第一実施形態の走査型蛍光顕微鏡の集光レンズ１６とピンホール１７と蛍光選択透過部１８が蛍光選択透過部２８に置き換えられ、集光レンズ２１とピンホール２２と蛍光選択透過部２３が蛍光選択透過部３２に置き換えられた構成をしている。

【００７１】

蛍光選択透過部２８は、入射する光ビームを波長分散させる分散素子（反射回折格子）２９と、分散素子２９により分散された光ビームを収束させる収束レンズ３０と、収束レンズ３０により収束された光ビームの一部の波長域を通す光学的開口すなわちスリット３１とを有している。

【００７２】

同様に、蛍光選択透過部３２は、入射する光ビームを波長分散させる分散素子（反射回折格子）３３と、分散素子３３により分散された光ビームを収束させる収束レンズ３４と、収束レンズ３４により収束された光ビームの一部の波長域を通す光学的開口すなわちスリット３５とを有している。

【００７３】

蛍光選択透過部２８と蛍光選択透過部３２は、例えば、グレーティングガルバノで構成される。蛍光選択透過部２８と蛍光選択透過部３２は全く同様の機能を有している。このため、以下では、代表的に、蛍光選択透過部２８の動作について述べる。

【００７４】

蛍光選択透過部２８において、分散素子２９に入射した光ビームは、波長に従って異なる方向に分散される。つまり、入射光ビームに含まれる光は、波長成分毎に異なる方向に変更される。このため、収束レンズ３０に入射した光は、光軸を横切る方向に関して、波長成分に応じて異なる位置に収束される。つまり、収束レンズ３０は入射した光ビームを波長成分に応じて異なる位置に収束させる。このため、蛍光選択透過部２８に入射した光のうち、特定の波長範囲の光だけがスリット３１を通過し得る。つまり、スリット３１は特定の波長成分の光だけを選択的に透過する。その結果、スリット３１を通過した特定の波長範囲の光だけが光検出器１９で検出される。

【００７５】

全く同様にして、蛍光選択透過部３２に入射した光ビームのうち、特定の波長範囲の光だけが、スリット３５を通過して光検出器２４で検出される。

【００７６】

本実施形態の走査型蛍光顕微鏡においては、二つの励起光遮断光学素子（好ましくはホログラフィックノッチフィルター）１３と１４によって、蛍光選択透過部２８と蛍光選択透過部３２に到達する前に、不所望な励起光が除去される。このため、蛍光選択透過部２８と蛍光選択透過部３２に入射した光は、不所望な励起光を殆ど含まない。このため、スリット３１とスリット３５は、開口の大きさを比較的大きくすることが可能である。これにより、スリット３１とスリット３５の後方にそれぞれ位置する光検出器１９と光検出器２４に、より多くの量の蛍光を到達させることができる。

【００７７】

その結果、本実施形態の走査型蛍光顕微鏡は、励起光の波長に近い波長の蛍光も検出できる。これにより、例えば、より明るい蛍光画像を得ることができる。

【００７８】

より好ましくは、蛍光選択透過部２８は分散素子２９の方向を調整可能であり、蛍光選択透過部３２は分散素子３３の方向を調整可能であるとよい。例えば、蛍光選択透過部２８において、分散素子２９の方向を変えることにより、蛍光を細分化した波長帯域毎に検出することも可能である。同様に、蛍光選択透過部３２において、分散素子３３の方向を変えることにより、蛍光を細分化した波長帯域毎に検出することも可能である。

【００７９】

つまり、本実施形態の走査型蛍光顕微鏡は、蛍光選択透過部２８と蛍光選択透過部３２をこの様に構成することにより、検出対象の蛍光のスペクトルデータを取得することも可能である。

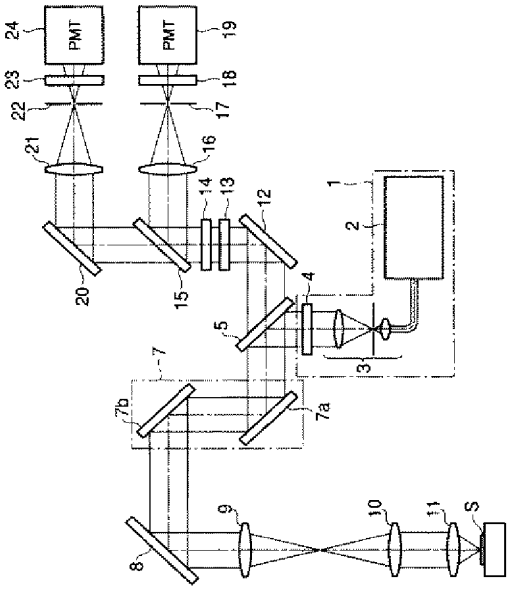
【００８０】

これまで、図面を参照しながら本発明の実施の形態を述べたが、本発明は、これらの実施の形態に限定されるものではなく、その要旨を逸脱しない範囲において様々な変形や変更が施されてもよい。

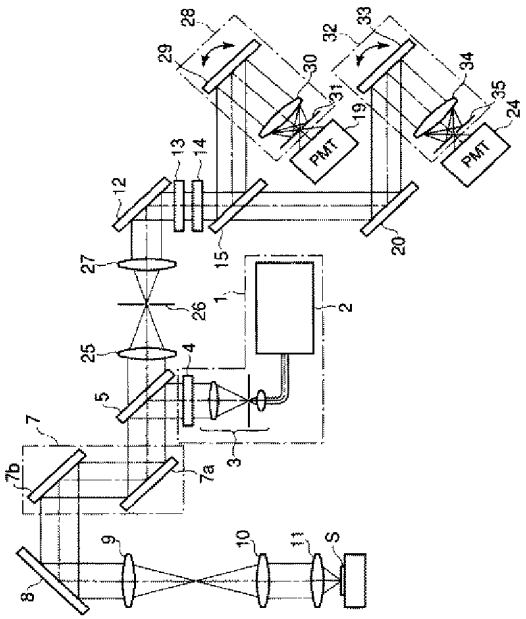
【００８１】
上述した実施形態では、光源部１は二種類の単一波長の励起光を含む光ビームを射出し、試料Ｓからは二種類の蛍光が発生されることに対応して、蛍光選択透過機構は二つの蛍光選択透過部を有し、蛍光検出部は二つの光検出器１９と２４を有し、励起光遮断部は二つの励起光遮断光学素子１３と１４を有している。
【００８２】
しかし、蛍光選択透過部と光検出器と励起光遮断光学素子の個数は、これに限定されるものではなく、光源部１から射出される光ビームに含まれる単一波長の励起光の種類、あるいは、試料Ｓから発生される蛍光の種類に対応して、適宜変更されてよい。
【００８３】
前述したように、光源部１は一種類の単一波長の励起光を含む光ビームを射出してもよい。その場合、蛍光選択透過機構は一つの蛍光選択透過部を有し、蛍光検出部は一つの光検出器を有し、励起光遮断部は一つの励起光遮断光学素子を有していればよい。
【００８４】
また、光源部１は三種類以上の単一波長の励起光を含む光ビームを射出してもよい。その場合、蛍光選択透過機構は対応する個数の蛍光選択透過部を有し、蛍光検出部は対応する個数の光検出器を有し、励起光遮断部は対応する個数の励起光遮断光学素子を有しているとよい。
【００８５】
結局、光源部１は少なくとも一種類以上の単一波長の励起光を含む光ビームを射出し、それに対応して、蛍光選択透過機構は少なくとも一つの蛍光選択透過部を有し、蛍光検出部は少なくとも一つの光検出器を有し、励起光遮断部は少なくとも一つの励起光遮断光学素子を有していればよい。
【００８６】
また、光源部１から射出される光は、レーザー光のようなほぼ単一波長の光が最も好ましいが、レーザー光でなくとも帯域の狭い光であってもよい。帯域の狭い光は、それ自体が光源から射出されるものに限らず、光源から射出された光からフィルター等の波長選択手段によって作られたものであってもよい。
【００８７】
【発明の効果】
本発明によれば、蛍光を検出する光検出器に到達する不所望な励起光の量を抑えつつ、多く量の蛍光が光検出器に到達する蛍光顕微鏡が提供される。
【図面の簡単な説明】
【図１】本発明の第一実施形態の走査型蛍光顕微鏡の構成を概略的に示している。
【図２】本発明の第二実施形態の走査型蛍光顕微鏡の構成を概略的に示している。
【図３】図１と図２に示された光源部内のレーザーラインフィルターの透過率特性を示している。
【図４】図１と図２に示された分離光学素子であるダイクロイックミラーの透過率特性を示している。
【図５】図１と図２に示された励起光遮断素子であるホログラフィックノッチフィルターの透過率特性を示している。
【図６】図１と図２に示された光ビーム分割部であるダイクロイックミラーの透過率特性を示している。
【図７】図１と図２に示された蛍光選択透過部である吸収フィルターの透過率特性を示している。
【図８】図１の構成からホログラフィックノッチフィルターが省かれた構成である従来の走査型蛍光顕微鏡において要求される吸収フィルターの透過率特性を示している。
【符号の説明】
１…光源部、２…レーザ光源、３…ビームエキスパンダ、４…レーザーラインフィルター、５…分離光学素子、７…走査光学系、７ａ…ガルバノメータスキャナ、７ｂ…ガルバノ

メータスキャナ、８…ミラー、９…瞳投影レンズ、１０…結像レンズ、１１…対物レンズ、１２…ミラー、１３…励起光遮断光学素子、１４…励起光遮断光学素子、１５…光ビーム分割部、１８…蛍光選択透過部、１９…光検出器、２３…蛍光選択透過部、２４…光検出器、２８…蛍光選択透過部、３２…蛍光選択透過部。

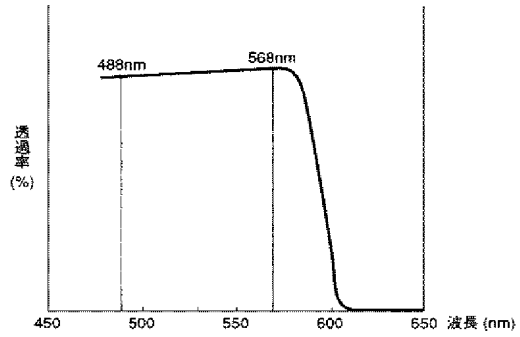
【図1】



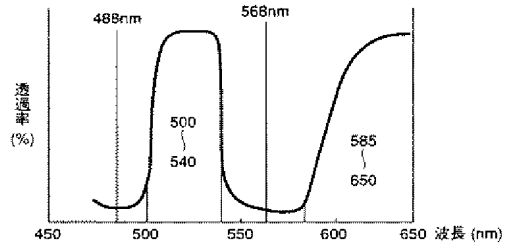
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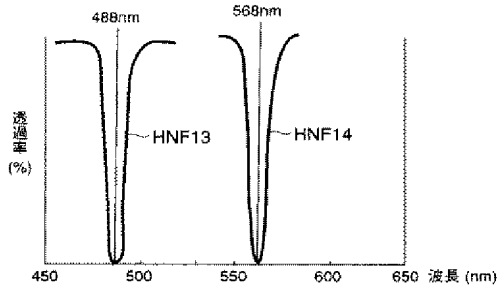
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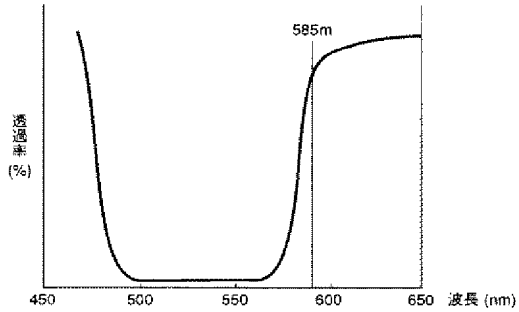
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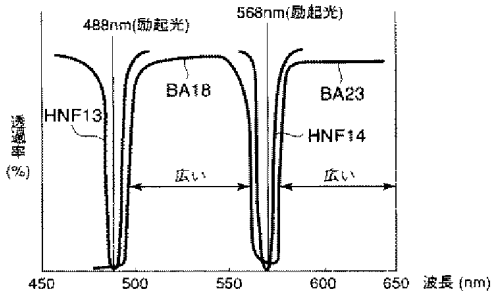
【図5】



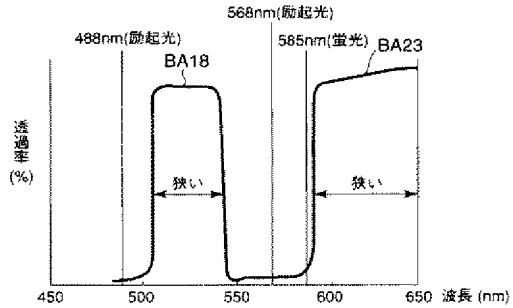
【図6】



【図7】



【図8】



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FINE PARTICLE MEASUREMENT INSTRUMENT

Inventor(s): TOISHI MITSURU; SEO KATSUHIRO; TAKASAKI KOJI; YAMADA SHINJI; FUKUMOTO ATSUSHI ± (TOISHI MITSURU, ; SEO KATSUHIRO, ; TAKASAKI KOJI, ; YAMADA SHINJI, ; FUKUMOTO ATSUSHI)

Applicant(s): SONY CORP ± (SONY CORP)

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Abstract of JP2012026837 (A)

PROBLEM TO BE SOLVED: To provide a fine particle analyzer which efficiently acquires necessary light components, in particular, back-scattered light and fluorescent light. ;SOLUTION: In a fine particle measurement instrument, an optical filter divided into a plurality of areas is provided on an optical path through which light generated from a fine particle irradiated with light is guided to an optical detector, and the optical filter has a first area having a wavelength selectivity to block reflected light from the fine particle and unnecessary scattered light components but transmit fluorescent light, and a second area disposed at least around the first area and having no wavelength selectivities to transmit the necessary scattered light components. ;COPYRIGHT: (C) 2012,JPO&INPIT;PROBLEM TO BE SOLVED: To provide a fine particle analyzer which efficiently acquires necessary light components, in particular, back-scattered light and fluorescent light.SOLUTION: In a fine particle measurement instrument, an optical filter divided into a plurality of areas is provided on an optical path through which light generated from a fine particle irradiated with light is guided to an optical detector, and the optical filter has a first area having a wavelength selectivity to block reflected light from the fine particle and unnecessary scattered light components but transmit

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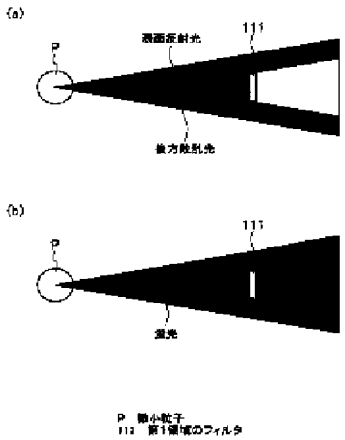
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最終頁に続く

(54)【発明の名称】微小粒子測定装置

(57)【要約】
【課題】　必要とする光成分、特に後方散乱光と蛍光を高効率に取得する微小粒子分析装置を提供すること。
【解決手段】　光を照射された微小粒子から発生する光を光検出器に導光する光学経路上に、複数の領域に分割された光学フィルタを備え、該光学フィルタは、微小粒子からの反射光と不要な散乱光成分を遮断し、蛍光を透過する波長選択性を有する第１領域と、少なくとも該領域の周囲に配され、必要な散乱光成分を透過する波長選択性のない第２領域と、を有する、微小粒子測定装置。
【選択図】図２



【特許請求の範囲】
【請求項１】
光を照射された微小粒子から発生する光を光検出器に導光する光学経路上に、複数の領域に分割された光学フィルタを備え、
該光学フィルタは、微小粒子からの反射光と不要な散乱光成分を遮断し、蛍光を透過する波長選択性を有する第１領域と、
少なくとも該領域の周囲に配され、必要な散乱光成分を透過する波長選択性のない第２領域と、を有する、微小粒子測定装置。
【請求項２】
前記波長選択性のない第２領域の貫通部分に前記波長選択性を有する第１領域を配する、請求項１記載の微小粒子測定装置。
【請求項３】
前記波長選択性を有する第１領域の外周に該第１領域を支持するフレームを備えるとともに、前記波長選択性のない第２領域が中空である、請求項１又は２記載の微小粒子測定装置。
【請求項４】
前記光学フィルタは、前記波長選択性を有する第１領域の周囲に配された波長選択性のない第２領域の周囲に、さらに波長選択性を有する第３領域を有する、請求項１～３のいずれか１項記載の微小粒子測定装置。
【請求項５】
前記光学経路上に、蛍光・後方散乱光検出器に蛍光および後方散乱光を導光する光ファイバーを備え、
前記複数の領域に分割された光学フィルタを、該光ファイバーによって前記光学フィルタを透過した光が受光されるように配置する、請求項１～４のいずれか１項記載の微小粒子測定装置。
【発明の詳細な説明】
【技術分野】
【０００１】
本発明は、微小粒子測定装置に関する。より詳しくは、微小粒子の特性を光学的に分析する微小粒子分析装置に関する。
【背景技術】
【０００２】
従来、フローセル内やマイクロチップ上に形成された流路内を通流する微小粒子に光を照射し、微小粒子からの散乱光や、微小粒子そのもの或いは微小粒子に標識された蛍光物質から発生する蛍光を検出して、微小粒子の光学特性を測定する微小粒子測定装置が用いられている。
先行技術文献１には、より高感度に計測するため、検出光受光用光ファイバを有する検体識別装置が開示されている。この装置では、検体からの後方散乱光の受光量変動を測定することにより、検体の状態（透過率、吸収等）を検出することができる。
【先行技術文献】
【特許文献】
【０００３】
【特許文献１】国際公開第２００５／１０３６４２号パンフレット
【発明の概要】
【発明が解決しようとする課題】
【０００４】
従来の微小粒子検出装置において、最適な散乱取得角度を得るためには、蛍光と散乱光を検出する光学検出系内に散乱光用電子倍増管や蛍光用電子倍増管に反射光成分が入射しないように、反射光を遮光するマスクを入れる必要がある。しかしながら、そのマスクによって蛍光などの必要な光成分も必要以上に遮断され、必要とする光成分の感度が劣化す

るという問題があった。尚、このような従来使用されていたマスクを、以下、「通常（従来）のマスク」という。

【０００５】

そこで、本発明は、必要な光成分、特に後方散乱光と蛍光をより効率的に取得する微小粒子分析装置を提供することを主な目的とする。

【課題を解決するための手段】

【０００６】

本発明は、光を照射された微小粒子から発生する光を光検出器に導光する光学経路上に、複数の領域に分割された光学フィルタを備え、該光学フィルタは、微小粒子からの反射光と不要な散乱光成分を遮断し、蛍光を透過する波長選択性を有する第１領域と、少なくとも該領域の周囲に配され、必要な散乱光成分を透過する波長選択性のない第２領域と、を有する、微小粒子測定装置を提供するものである。

これにより、前記第１領域で、微小粒子からの反射光や不要な散乱光成分をカットしつつ、必要な光成分の一つである蛍光を透過させることが可能となる。かつ、前記第２領域で、蛍光および必要な散乱光成分（例えば、後方散乱光）を通過させることも可能となる。

【０００７】

また、本発明は、前記波長選択性のない第２領域の貫通部分に前記波長選択性を有する第１領域を配するのが好適である。

また、本発明は、前記波長選択性を有する第１領域の外周に該第１領域を支持するフレームを備えるとともに、前記波長選択性のない第２領域が中空であるのが好適である。

これにより、第１領域と第２領域との界面での屈折率が異なるのを低減できる。

【０００８】

また、本発明は、前記光学フィルタは、前記波長選択性を有する第１領域の周囲に配された波長選択性のない第２領域の周囲に、さらに波長選択性を有する第３領域を有するのが好適である。前記第１領域でビーム系の中心をカットするのに加えて、この第３領域により、ビーム系の外側も必要に応じてカットすることが可能となる。よって、所望の角度からの後方散乱光を得ることが可能となる。

【０００９】

また、本発明は、前記光学経路上に、蛍光・後方散乱光検出器に蛍光および後方散乱光を導光する光ファイバーを備え、前記複数の領域に分割された光学フィルタを、該光ファイバーによって前記光学フィルタを透過した光が受光されるように配置するのが好適である。これにより、後方散乱光及び蛍光を高感度に検出できる。

【発明の効果】

【００１０】

本発明によれば、後方散乱光と蛍光とを効率的に取得する微小粒子分析装置が提供される。

【図面の簡単な説明】

【００１１】

【図１】本発明に係わる微小粒子分析装置の光学経路を説明するための模式図である。

【図２】本発明に係わる光学フィルタを備えた微小粒子分析装置において、レーザーを照射した微小粒子から発せられる反射光、後方散乱光及び蛍光を示した模式図である。

【図３】本発明に係わる微小粒子分析装置に備える光学フィルタの第１領域の透過スペクトラムを示す図である。

【図４】本発明に係わる微小粒子分析装置に備える光学フィルタの実施形態（上面方向）の例示を示す。（ａ）第１領域が円形状である場合、（ｂ）第１領域が四角形状である場合、（ｃ）第１～第３の各領域が円形状である場合、（ｄ）第１～第３の各領域が四角形状である場合。

【図５】本発明に係わる微小粒子分析装置に備える光学フィルタの左右方向の断面図の例示を示す。（ａ）第２領域の（平面）フィルタの上部に第１領域の（平面）フィルタの下

部が接合する場合、（ｂ）第２領域の（平面）フィルタの貫通部分に第１領域の（平面）フィルタが配され、第２領域の（平面）フィルタの上面よりも第１領域の（平面）フィルタの上面が突出する場合、（ｃ）第２領域の（平面）フィルタの貫通部分に第１領域が配され、第１領域と第２領域の上下面が面一である場合。
【図６】本発明に係わる微小粒子分析装置に備える光学フィルタの例示を示す。第１領域の（平面）フィルタが円筒状のフレームによって支持され、第２領域が中空である場合。
【図７】本発明の光学フィルタの変形実施形態の一例（反射型光学フィルタ）を示す図である。（ａ）反射型光学フィルタによる反射光、後方散乱光及び蛍光の流路を示す図である。（ｂ）本発明の反射型光学フィルタの第１領域の透過スペクトラムを示す図である。
【図８】（ａ）通常（従来）のマスクを備える微小粒子分析装置；（ｂ）本発明に係わる異なる透過波長の領域を有するマスクを備える微小粒子分析装置；（ｃ）マスクを備えない微小粒子分析装置による蛍光感度の測定結果を示す図である。
【図９】（ａ）通常（従来）のマスクを備える微小粒子分析装置；（ｂ）本発明に係わる異なる透過波長の領域を有するマスクを備える微小粒子分析装置による後方散乱の測定結果を示す図である。
【図１０】通常（従来）のマスクを備えた微小粒子分析装置において、レーザーを照射した微小粒子から発せられる反射光、後方散乱光及び蛍光を示した模式図である。
【発明を実施するための形態】
【００１２】
以下、本発明を実施するための好適な形態について図面を参照しながら説明する。なお、以下に説明する実施形態は、本発明の代表的な実施形態の一例を示したものであり、これにより本発明の範囲が狭く解釈されることはない。尚、説明は以下の順に行う。
１．微小粒子測定装置の装置構成
（１）光学検出手段
（１－１）ＦＬ・ＢＳ集光系－光学フィルタ
２．微小粒子測定装置の動作
３．変形例
（１）反射型光学フィルタ
（２）反射型光学フィルタを備えた場合の動作
【００１３】
＜１．微小粒子測定装置の構成＞
図１は、本発明に係わる微小粒子測定装置の概略構成、特に光学検出系（光学流路）を模式的に示す図である。
当該微小粒子装置は、フローセルやマイクロチップなどの流路系、このフローセル内やマイクロチップ上に形成された流路内を通過する微小粒子Ｐからの複数の蛍光、後方散乱光、前方散乱光などの光成分を検出する光学検出手段などを備えている。尚、当該微小粒子測定装置は、必要に応じて測定後の微小粒子Ｐを回収や分離するソート手段を備えていてもよい。
【００１４】
（１）光学検出手段
図１に示すように、前記光学検出手段は、光学照射系１０１、蛍光・後方散乱光集光系１０２、前方散乱光検出系１０３、および蛍光・後方散乱光検出系１０４から構成されている。これによって、光が照射された微小粒子から発生する光成分を各光検出器に導入し、所望の光成分を検出、測定することが可能となる。
【００１５】
前記光学照射系１０１は、光（励起光）を照射する光源（λ１～４）１、この光源１からの光を平行光とする集光レンズ２、更に光を同軸上に配置するダイクロイックミラー３などを備えている。
前記光学照射系１０１より照射された光（励起光）は、蛍光・後方散乱光（以下、「ＦＬ・ＢＳ」ともいう）集光系内にあるハーフミラー４及び対物レンズ５を透過し、流路６

内を通流する微小粒子Ｐに照射される。このとき、微小粒子Ｐから蛍光および必要な散乱光成分と共に反射光が発せられ、また不要な散乱光成分も発せられる。

ここで、蛍光および必要な散乱光成分は、微小粒子Ｐの光学的情報（特性）を得る上で重要な光成分である。当該必要な散乱光成分としては、例えば、後方散乱光、側方散乱光などが挙げられる。また、不要な散乱光成分は、照射された光（励起光）が流路系や基板又は細胞などに当たることにより生じるものである。

【００１６】

尚、本発明において、「微小粒子」には、細胞や微生物、リポソーム等の生体関連微小粒子、およびラテックス粒子やゲル粒子、工業用粒子等の合成粒子などが広く含まれているものとする。

生体関連微小粒子には、各種細胞を構成する染色体、リポソーム、ミトコンドリア、オルガネラ（細胞小器官）などが含まれる。対象とする細胞には、動物細胞（血球系細胞など）および植物細胞が含まれる。対象とする微生物には、大腸菌等の細菌類、タバコモザイクウイルス等のウイルス類、イースト菌等の菌類などが含まれる。さらに、生体関連微小粒子には、核酸やタンパク質、これらの複合体などの生体関連高分子も包含され得るものとする。

また、工業用粒子は、例えば有機もしくは無機高分子材料や金属等であってもよい。有機高分子材料には、ポリスチレン、スチレン・ジビニルベンゼン、ポリメチルメタクリルレートなどが含まれる。無機高分子材料には、ガラス、シリカ、磁性体材料などが含まれる。金属には、金コロイド、アルミ等が含まれる。

上記微小粒子の形状は、一般には球形であるのが普通であるが、非球形であってもよく、また大きさや質量なども特に限定されない。

【００１７】

前記前方散乱（以下、「ＦＳ」ともいう）検出系１０３は、ＦＳ集光レンズ７、前方散乱光を所望の角度成分でカットするＦＳマスク８、所望の波長成分のみを透過するバンドパスフィルタ９、透過した光を検出するＦＳ光学検出器１０を備えている。

【００１８】

前記蛍光・後方散乱光集光系１０２は、微小粒子Ｐからの光成分を集光する対物レンズ５、これら光成分を表面で反射させるハーフミラー４、この光成分が導光される複数の領域に分割された光学フィルタ１１、およびＦＬ・ＢＳ集光レンズ１２を備えている。

ここで、当該複数の領域に分割された光学フィルタ（以下、「光学フィルタ」や「ノッチフィルタマスク」ともいう）１１は、ハーフミラー４で反射された光成分から反射光および不要な散乱光成分を遮断（低減）し、必要な光成分（例えば、蛍光および後方散乱光）を透過させるためのものである。また、ＦＬ・ＢＳ集光レンズ１２は、必要な光成分を光ファイバー１３に導光するためのものである。

【００１９】

前記蛍光・後方散乱検出系１０４は、光ファイバー１３から出た光成分の蛍光および後方散乱光を検出するように構成されている。

具体的には、光ファイバー１３からの光成分を平行光とするＦＬ・ＢＳ集光レンズ１４、この平行光のうちの後方散乱光を反射し、蛍光を透過するノッチフィルタ１５と、後方散乱光を検出するＢＳ光学検出器１６が備えられている。さらに、ノッチフィルタ１５で透過した蛍光成分のうち所望の波長成分を透過し、その他の波長成分を反射させるＦＬダイクロックミラー１７、この所望の波長成分をさらに選択するＦＬバンドパスフィルタ１８，２０、および所望の波長成分を検知する光学電子倍増管１９，２１が備えられている。

なお、必要に応じて、ノッチフィルタマスク、ダイクロイックミラー、バンドパスフィルタ、光学電子倍増管および光学検出器を増減してもよい。

【００２０】

尚、上述のした微小粒子測定装置の光学照射系１０１、前方散乱光検出系１０３、および蛍光・後方散乱検出系１０４は、従来公知の微小粒子測定装置と同様の構成とすること

ができる。

【００２１】

（１－１）ＦＬ・ＢＳ集光系－光学フィルタ

図２～６を参照して、以下に、本発明に係わる微小粒子測定装置におけるＦＬ・ＢＳ集光系１０２およびこれに備えられている光学フィルタ１１について説明する。

【００２２】

まず、前記光学フィルタ１１について説明する。

前記光学フィルタ１１は、微小粒子Ｐよりの光成分からの反射光および不要な散乱光成分を低減すると共に、蛍光成分および必要な散乱光成分（特に後方散乱光成分）を高効率に得るためのものである。このため、当該光学フィルタ１１の略中心部分には、微小粒子Ｐよりの（表面）反射光および不要な散乱光成分が入射する第１領域１１１のフィルタが配されており（例えば、図２参照）、当該第１領域１１１のフィルタは特定の波長選択性を有する。この特定の波長選択性によって、反射光および不要な散乱光成分を遮断（低減）しつつ、かつ蛍光成分を透過させることが可能となる。

前記第１領域１１１のフィルタは、不必要な所望の光成分のみを高精度に反射させ、必要な光成分を透過させることが可能となるような透過スペクトラムを有していればよい。より具体的には、一般的に入射光や反射光の波長は蛍光波長よりも低いことから、低い波長を透過させないフィルタが好ましい。

例えば、図３に示すように、前記第１領域１１１の特定の波長選択性を有するフィルタは、４８８ｎｍ付近、具体的には４８０～５００ｎｍという特定の波長成分のみを透過させないものが好適である。当該波長選択性を有するフィルタは、当該特定の波長成分のみを他の透過成分に対して５桁透過率が低い特性を有するように調製するのが好適である。これにより、反射光および不要な散乱光成分を遮断（低減）しつつ、かつ蛍光成分を高精度に透過させるので、蛍光の光量を高効率に取得することが可能となる。

【００２３】

ここで、従来のマスクを用いた場合の微小粒子Ｐから発せられる反射光、後方散乱光および蛍光を示した模式図を図１０に示す。このように、通常のマスクによって、表面反射光と共に蛍光も遮断されることとなる。これにより光検出器に入る蛍光の光量が少なくなり、この蛍光感度が劣化してしまう。

これに対し、図２（ａ）に示すように、前記第１領域１１１のフィルタは、上述のように特定の波長選択性を有するので、微小粒子Ｐからの伝播角の小さい反射光と不要な散乱光成分とを遮断しつつ、蛍光を透過させる。しかも、図２（ｂ）に示すように、後方散乱光や蛍光は原理的に反射光成分よりも伝播角が大きくなるので、前記第１領域１１１のフィルタの周囲を、必要な光成分（後方散乱光成分や蛍光成分）が通過することとなる。これにより、光学検出系に入る蛍光の光量のロスが低減できる。さらに、後方散乱光も取得することができるので、後方散乱光と蛍光とを同時に高効率に取得することも可能となる。加えて、微小粒子測定装置に前記光学フィルタをセットするだけでよく、複雑な構成も必要としない。これにより、後方散乱光および蛍光を高効率に取得できる光学検出系および微小粒子測定装置をシンプルかつコンパクトに実現することも可能となる。

【００２４】

図４および図５に、前記特定の波長選択性を有する第１領域と、少なくとも該領域の周囲に配された前記波長選択性のない第２領域を有する前記光学フィルタ１１を例示するが、前記光学フィルタ１１の形状や構造はこれに限定されるものではない。

まず、図４（ａ）および（ｂ）に示すような前記特定の波長選択性を有する第１領域１１１のフィルタと、該領域の周囲に配された前記波長選択性のない第２領域１１２のフィルタを有する前記光学フィルタ１１について説明する。

前記光学フィルタ１１は、第１領域１１１のフィルタと第２領域１１２のフィルタを有し、前記光学フィルタの略中央付近に、第１領域１１１のフィルタが配置されている。当該略中央付近とは、反射光や不要な散乱光成分などの光成分が入射される部分である。

前記第１領域１１１のフィルタは、微小粒子Ｐからの反射光と不要な散乱光成分とを遮

断し、蛍光を透過するという特定の波長選択性を有する。
また、第２領域１１２のフィルタは、特定の波長選択性のないものであり、蛍光および必要な散乱光成分を透過する。ここで、特定の波長選択性のないフィルタとは、上記第１領域１１１に使用されるフィルタ（光学材料）ではなく、後方散乱光や蛍光が透過するもの（例えばガラスなど）であればよい。

【００２５】

さらに、図５に、上記図４（ａ）の前記光学フィルタの左右方向の断面図の例示を示すが、これに限定されるものではない。
図５（ａ）に示すような前記光学フィルタ１１は、第２領域１１２のフィルタの上面かつ略中央付近で、第１領域１１１のフィルタが接合しているものである。当該接合の際には、第１領域１１１や第２領域１１２のフィルタの光学的な特性を阻害しないような接着剤や光硬化樹脂を用いるのが好適である。さらに、可視域においての反射を防ぐため、図５（ａ）に示すような接合するタイプの前記光学フィルタにおいて、第２領域１１２のフィルタ表面にＡＲコート（Anti Refledion Coating）を施すのが好適である。

【００２６】

また、図５（ｂ）および（ｃ）に示すような前記光学フィルタ１１は、第２領域１１２のフィルタの略中央付近に貫通穴又は未貫通穴を形成し、当該貫通穴又は未貫通穴に第１領域１１１フィルタを嵌合や挿入などによって配する。さらに、図５（ｂ）に示すように第１領域１１１のフィルタが、第２領域１１２のフィルタの上面及び／又は下面より突出してもよい。また、図５（ｃ）に示すように第１領域１１１のフィルタが、第２領域１１２のフィルタの上面及び／又は下面と、面一になるようにしてもよい。なお、第１領域１１１のフィルタと第２領域フィルタとを左右方向や高さ方向で積層構造としてもよい。
図５（ｂ）や（ｃ）に示すような一方の領域の貫通穴に他方の領域を配する光学フィルタを形成することによって、図５（ａ）に示すような光学フィルタの場合に界面の屈折率が異なることによって生じ易いフィルタの性能劣化を低減することが可能となる。

【００２７】

さらに、図４（ｃ）や（ｄ）に示すような前記光学フィルタ１１は、前記特定の波長選択性を有する第１領域の周囲に配された特定の波長選択性のない第２領域の周囲に、さらに波長選択性を有する第３領域を有するものである。

【００２８】

図４（ｃ）および（ｄ）に示すような前記光学フィルタ１１は、上記第１領域１１１のフィルタの周囲に配された上記第２領域１１２のフィルタの周囲に、更に特定の波長特性を有する第３領域１１３のフィルタを配するものである。一般的に後方散乱光は粒子サイズに対する角度依存性があり、所望の角度成分のみの散乱光を取得する必要がある場合もある。このような場合に、図４（ｃ）および（ｄ）に示すような光学フィルタ１１の構造を採用することにより、第１領域１１１のフィルタでビーム系の中心をカットするだけでなく、その外側も第３領域１１３のフィルタでカットすることが可能となる。これにより、所望の角度からの後方散乱光を得ることが可能となる。
このとき、第１領域１１１の幅Ｌと、第２領域１１２の幅Ｍと、第３領域１１３の幅Ｎとの各幅は、それぞれ、中心から０～４ｍｍ：４～６ｍｍ：６～８ｍｍとするのが、所望の後方散乱光を得るため好適である。
ここで、幅Ｌは、第１領域１１１の半径の距離である。幅Ｍは、第１領域１１１の中心から第２領域１１２の外周までの距離－幅Ｌである。幅Ｎは、第１領域１１１の中心から第３領域１１３の外周までの距離－（幅Ｌ＋幅Ｍ）である。尚、多角形の場合には、中心から外辺までの距離（垂線の長さ）を基準とする。
尚、図４（ｃ）および（ｄ）に示すような光学フィルタ１１は、上述のように各領域のフィルタを接合や嵌合などを行うによって形成することが可能である。

【００２９】

上述のような前記光学フィルタは、特定の波長特性を有する光学材料や波長特性のない光学材料などといった異なる波長特性を有する光学材料（フィルタ）を少なくとも２種以

上組み合わせて調製すればよい。
異なる光学特性の光学材料を２種以上用いる光学フィルタの形成方法としては、特に限定されないので、上述の形成方法以外に、フォトニック結晶を形成する方法、例えばエッチング法（例、半導体リソグラフィ法）を用いればよい。フォトニック結晶を形成する方法であれば、各領域が積層状となった光学フィルタ１１も形成しやすいので、好適である。
【００３０】
また、図６に示すように、第１領域１１１のフィルタと、これを単数又は複数のワイヤー１２１を介して支持する円筒状のフレーム１２０とを備える光学フィルタ１１であってもよい。さらに、当該フレーム１２０の略中央付近に第１領域１１１のフィルタが配置され、当該第１領域の周囲で、かつ中空である空間１１５を第２領域とするのが好適である。このような簡単な構造を採用することにより、フィルタの材質の相違によって生じ易いフィルタの性能劣化を低減することが可能である。
【００３１】
前記光学フィルタ１１は、上述のとおり、ＦＬ・ＢＳ集光系に備えられている。前記光学フィルタ１１の配置は、前記ＦＬ・ＢＳ光学検出系（前記蛍光・後方散乱光検出器）に蛍光および後方散乱光を導光する光ファイバー１３の前に配置するのが好適である。具体的には、前記光学フィルタを、該光ファイバーによって前記光学フィルタを透過した光が受光されるように配置するのが好適である。
このような構成を採用することにより、前記光学フィルタ１１をＦＬ・ＢＳ光学検出系内に配置した場合よりも、蛍光の光量のロスが少なくなるので好適である。
【００３２】
＜２．微小粒子測定装置の動作＞
以下に、上述した前記光学フィルタを備えた微小粒子測定装置の動作について、図１を参照して説明する。
前記光学照射系１０１内の複数の波長の異なる各レーザー光源１からの各光（励起光）が、それぞれの集光レンズ２によって平行光となり、ダイクロイックミラー３によって同軸上に配置される。この光（励起光）は、ＦＬ・ＢＳ集光系１０２のハーフミラー４および対物レンズ５を透過し、流路６内の微小粒子Ｐに照射される。そして、当該微小粒子Ｐから、蛍光および必要な散乱光成分（前方散乱光、後方散乱光など）や反射光が発せられると共に不要な散乱光成分や反射光も生じる。
このときの微小粒子Ｐの前方散乱光は、ＦＳ検出系１０３内のＦＳ集光レンズ７、ＦＳマスク８、ＦＳバンドパスフィルタ９を経てＦＳ光学検出器１０に導光される。
また、微小粒子Ｐからの反射光、蛍光や必要な散乱光成分（後方散乱光など）並びに不要な散乱光成分は、ＦＬ・ＢＳ集光系１０２の集光レンズ５を通過してハーフミラー４で反射し、ノッチフィルタマスク（前記光学マスク）１１を通過する。このときノッチフィルタマスク１１の略中央部分にある第１領域１１１のフィルタにより、光成分の中心にある反射光や不要な散乱光成分および後方散乱光を遮断し、かつ蛍光成分が透過する。また、後方散乱光および蛍光は反射光成分よりも広い伝搬角であるため、後方散乱光および蛍光が第１領域１１１のフィルタの周囲に配された第２領域１１２のフィルタを透過する。このようにして、ノッチフィルタマスク１１を透過した後方散乱光および蛍光は、ＦＬ・ＢＳ集光レンズ１２を介して光ファイバー１３にカップリングされる。
この光ファイバー１３を出た後方散乱光および蛍光は、ＦＬ・ＢＳ集光レンズ１４によって平行光とされ、後方散乱光のみを反射するノッチフィルタ１５によって後方散乱光はＢＳ検出器１６に導光され、このＢＳ検出器１６で後方散乱光が検出される。また、蛍光は、ノッチフィルタ１５、ＦＬダイクロイックミラー１７、次いでＦＬバンドパスフィルタ１７を経ることによって、蛍光成分の中でも所望の波長成分が選択され、その選択された成分が光電子倍増管１９によって検出される。また、ノッチフィルタ１５、ＦＬダイクロイックミラー１７、次いでＦＬバンドパスフィルタ２０を経ることによって、蛍光成分の所望の波長成分がさらに選択され、この成分も同様にして光学電子倍増管２１によって

検出される。

【００３３】

＜３．変形例＞

（１）反射型光学フィルタ

ＦＬ・ＢＳ集光系１０２に備えられている前記光学フィルタ１１を、図７に示す反射型光学フィルタ４０に変更してもよい。

当該反射型光学フィルタ４０は、特定の波長選択性を有する第１領域３０と、該領域の周囲に配され、波長選択性のない第２領域３１とを有する。第１領域３０は、微小粒子Ｐからの反射光と不要な散乱光成分を透過し、蛍光を反射させるような特定の波長選択性を有する。また、第２領域３１は、必要な光成分（例えば、微小粒子Ｐからの蛍光や後方散乱光などの必要な散乱光成分）を反射させる波長選択性のない第２領域を有する。

より詳細に説明すると、前記反射型光学フィルタ４０は、その略中央部分にバンドパスフィルタ３０、かつそのバンドパスフィルタ３０の両端にミラー３１を有する。当該バンドパスフィルタ３０は、微小粒子Ｐからの反射光、また不要な散乱光成分を透過させ、かつ蛍光を反射させるものである。

当該バンドパスフィルタ３０の透過スペクトラムの一例を、図７（ｂ）に示す。

前記反射型光学フィルタの第１領域のフィルタは、上述した前記光学フィルタの第１領域１１１のフィルタの特性とは逆に、所望の不必要な光成分のみを高精度に透過させ、必要な光成分を反射させることが可能となるようなスペクトラムを有する。具体的には、バンドパスフィルタ３０（第１領域のフィルタ）は、前記光学フィルタ１１の第１領域１１１のフィルタの透過スペクトラムとは逆の波長選択性を有しており、例えば、４８８付近、具体的には４８０～５００ｎｍが透過するのみでこれ以外の波長は反射する。

一方、当該ミラー３１は、全波長域の光成分が反射するものであり、いわゆる波長選択性のないものである。

前記反射型光学フィルタ４０を採用することにより、蛍光成分などは反射成分を使うため、前記光学フィルタ１１よりも光の利用率がよくなる。

【００３４】

そして、前記反射型光学フィルタ４０を備えたＦＬ・ＢＳ集光系１０２は、前記反射型光学フィルタ４０にて反射された光成分（例えば、後方散乱光および蛍光成分など）が光ファイバー１３に受光されるように、構成されていればよい。例えば、ＦＬ・ＢＳ集光系１０２内に、後方散乱光および蛍光を反射するミラーを集光レンズ１２の前に設けてもよいし、或いは、光ファイバー１３の受光部の配置を変更してもよい。

【００３５】

（２）反射型光学フィルタを備えた場合の動作

図７に示すように、微小粒子Ｐからの光成分（反射光、後方散乱光、蛍光）や不要な散乱光成分は、反射型光学フィルタ４０に導光される。このうちの反射光は、伝搬角が小さいので、前記反射型光学フィルタ４０の略中央部分にあるバンドパスフィルタ３０（第１領域）を透過する。後方散乱光は、伝播角が広いので、前記反射型光学フィルタ４０のミラー全面に当たり、このとき略中央部分のバンドパスフィルタ３０（第１領域）を透過し、この周囲にあるミラー３１（第２領域）によって反射する。蛍光は、伝播角が広いので、前記反射型光学フィルタ４０のミラー全面に当たり、このとき略中央部分のバンドパスフィルタ３０（第１領域）およびこの周囲にあるミラー３１（第２領域）によって反射する。

反射した蛍光および後方散乱光は、反射ミラーなど（図示せず）を経て、光ファイバー１３に導光される。

【００３６】

なお、前記反射型光学フィルタ４０の形状や構造は、必要な光成分（例えば、蛍光および後方散乱光などの必要な散乱光成分）を反射させることができれば特に限定されないが、前記光学フィルタ１１と同じ形状や構造とするのが好適である。例えば、上述した図４および５に示すようなものが挙げられる。このとき、前記反射型光学フィルタ４０の微小

粒子Ｐからの光成分や不要な散乱光成分が当たる面は、蛍光および後方散乱光を効率よく反射させるため、面一とするのが好適である。

【実施例】

【００３７】

前記光学フィルタ１１を用いたときの効果を下記に示す。

製造例１：本発明の微小粒子測定装置に備える光学フィルタ

波長特性を有する第１領域のフィルタは、図３に示すようなスペクトラムを有していた。この第１領域のフィルタは、所望の４８８ｎｍの波長成分のみを他の透過成分に対して５桁透過率の低い特性を有していた。

また、波長特性のない第２領域のフィルタは、市販のカバーガラスを用い、可視域において反射を防ぐＡＲコートをした。

上記第１領域のフィルタを半径４～５ｍｍにカットし、上記第２領域のフィルタの上に貼り付けて、図４（ａ）および図５（ａ）のような形状および構造を有するノッチフィルタマスクを作製した。

【００３８】

試験例１：各微小粒子測定装置による蛍光感度の測定

下記の実験は、８種類の異なる明るさを持ったビーズを用いて、４８８ｎｍの励起光で蛍光を取得し、そのときのピークを、図８（ａ）通常のマスク、（ｂ）ノッチフィルタマスク、（ｃ）マスクを用いない時を比較した。

ここで、本実験では、８つのビーズでのヒストグラムのピークが全て独立して存在する状態であれば感度が高い状態といえる。強度の一番弱いピーク（一番左側）は蛍光体が付着していないビーズのため、この成分はノイズ成分となる。よってこのノイズ成分と他のピークとが完全に離れている状態が感度の高い状態で望ましい。

上記（ａ）と（ｂ）の実験では、同じ一辺４．５ｍｍのサイズのマスクを用いた。

（ａ）の通常のマスクを用いたときには、右から６，７，８番目のピークが繋がっていたのに対して、（ｂ）のノッチフィルタマスクを用いたときには、７－８個目のピークが分離していた。（ｃ）のマスクなしの時とほぼ変わらない特性であることから、ノッチフィルタマスクを用いたときは、蛍光の光量のロスがないことが確認できた。

【００３９】

試験例２：各微小粒子測定装置による散乱の測定

血液のサンプルを３種類の細胞に分けたもので、そのサイズと、細胞の複雑さによって前方散乱光と後方散乱光の信号が変化する。当然後方散乱光のマスクのサイズが最適化されていなかったり、マスクの消光比が悪いと、特性は劣化した。

上述のような（ａ）と（ｂ）の各マスクを用いた実験では、同じ一辺４．５ｍｍのサイズのマスクを用いた。

（ａ）の通常のマスクを用いたときのような、完全に中央の光をブロックしたときとほぼ同じ結果が、（ｂ）のノッチフィルタマスクを用いたときにも得られている。

【００４０】

以上のこれら２つの実験から、本発明の装置に用いる前記光学フィルタ（領域分割フィルタ）が、蛍光などの光量のロスがないにも拘らず、所定の波長に関してはフィルタとして機能して遮断していることが確認できた。

【産業上の利用可能性】

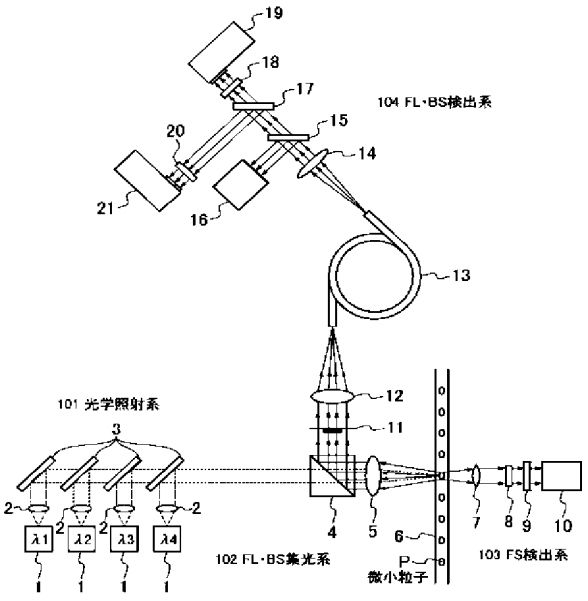
【００４１】

本発明に係わる微小粒子測定装置は、特定の波長選択性を有する領域と波長選択性のない領域とを組み合わせた領域分割の光学フィルタを用いることにより、微小粒子から発生する反射光や不要な散乱光成分を遮断し、後方散乱光の光量を低減させることなく、かつ蛍光の光量のロスを低減する。よって、本発明に係わる微小粒子測定装置は、散乱光と蛍光の何れをも高効率で取得することができる。さらに後方散乱光および蛍光を高効率に取得できる光学検出系をシンプルかつコンパクトに形成することも可能となる。

【符号の説明】

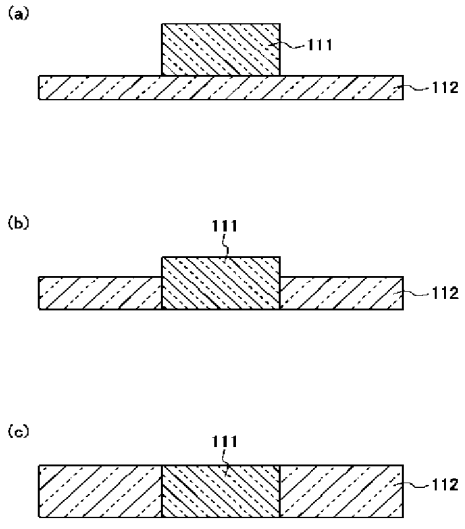
【００４２】
１１　光学フィルタ
１１１　第１領域
１１２　第２領域
１１３　第３領域
１１４　第４領域
３０　バンドパスフィルタ
３１　ミラー
４０　反射型光学フィルタ
１０１　光学照射系
１０２　蛍光・後方散乱光集光系
１０３　前方散乱検出系
１０４　蛍光・後方散乱光検出系

【図１】



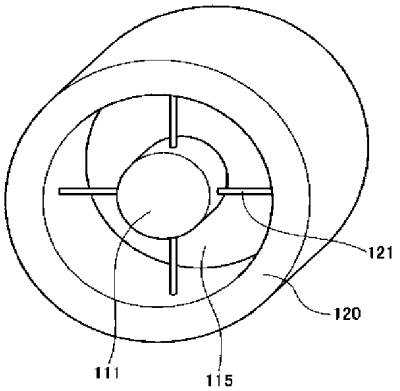
1　光源
2　集光レンズ
3　ダイクロイックミラー
4　ハーフミラー
5　対物レンズ
6　流路
7　FS集光レンズ
8　FSマスク
9　FSバンドパスフィルタ
10　FS光学検出器
11　ノッチフィルタマスク
12　FL･BS集光レンズa
13　光ファイバー
14　FL･BS集光レンズb
15　ノッチフィルタ
16　BS光学検出器
17　FLダイクロイックミラー
18　FLバンドパスフィルタa
19　光学電子倍増管a
20　FLバンドパスフィルタb
21　光学電子倍増管b

【図５】



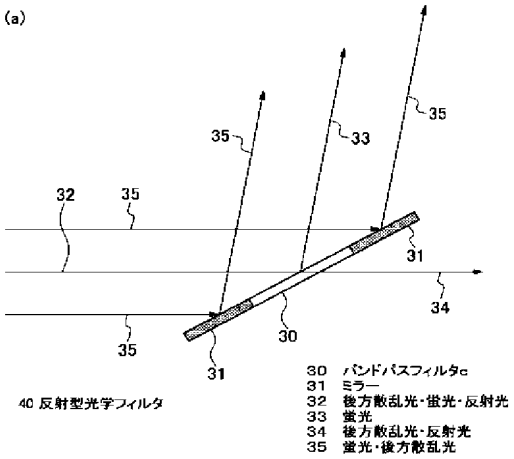
111　第1領域
112　第2領域

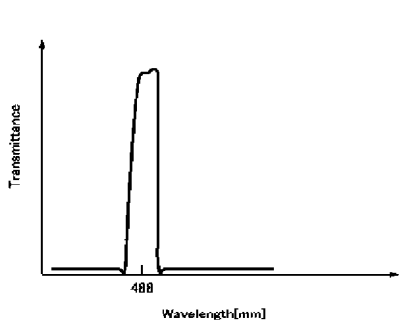
【図６】



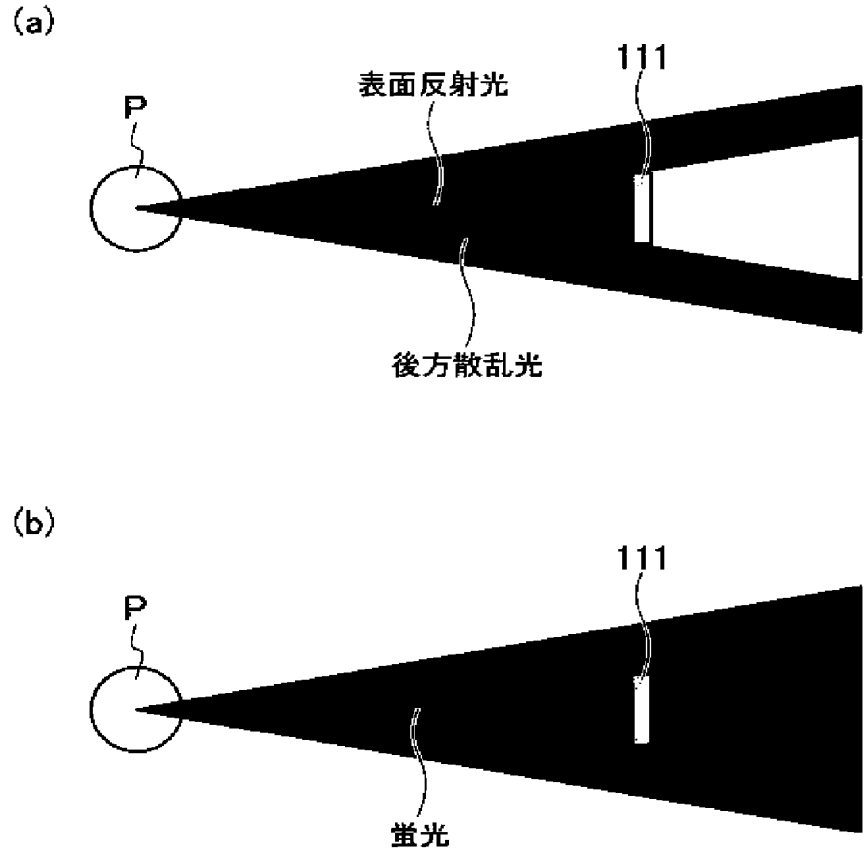
111 第1領域
115 空間
120 フレーム
121 ワイヤー

【図７】



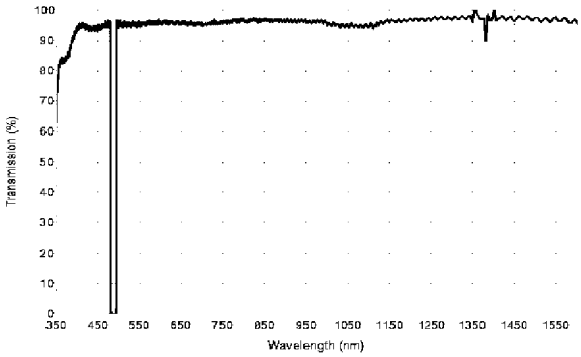


【図２】

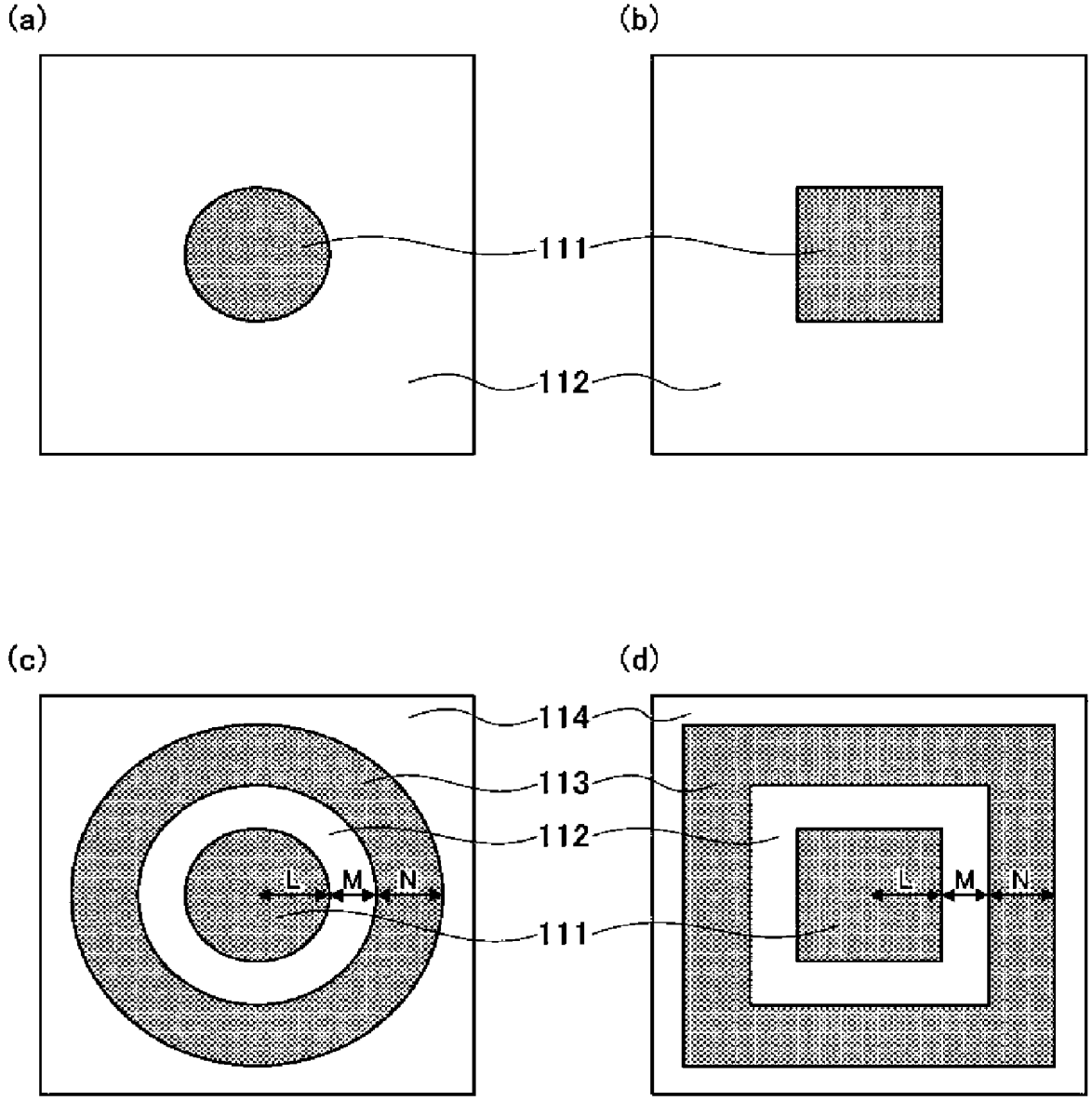


P　微小粒子
111　第１領域のフィルタ

【図３】


100
90
80
70
60
50
40
30
20
10
0
Transmission (%)
350
450
550
650
750
850
950
1050
1150
1250
1350
1450
1550
Wavelength (nm)

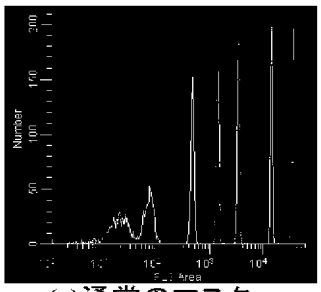
【図４】



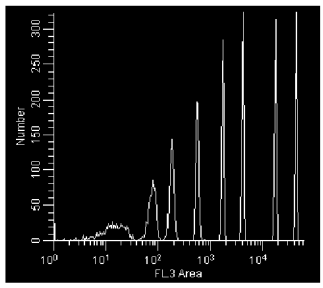
111　第1領域
112　第2領域
113　第3領域
114　第4領域

L,M,N　各領域の幅

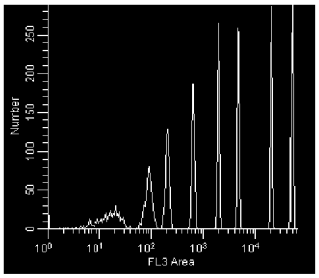
【図８】



(a)通常のマスク

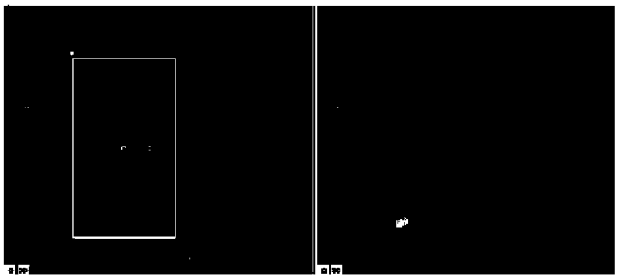


(b)ノッチフィルタマスク

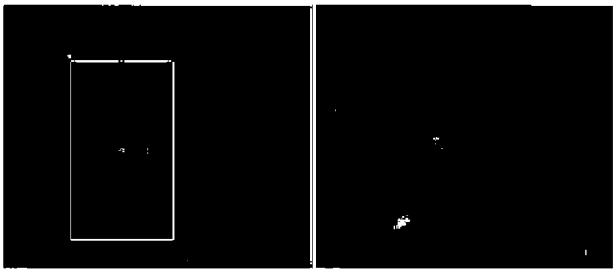


(c)マスクなし

【図９】

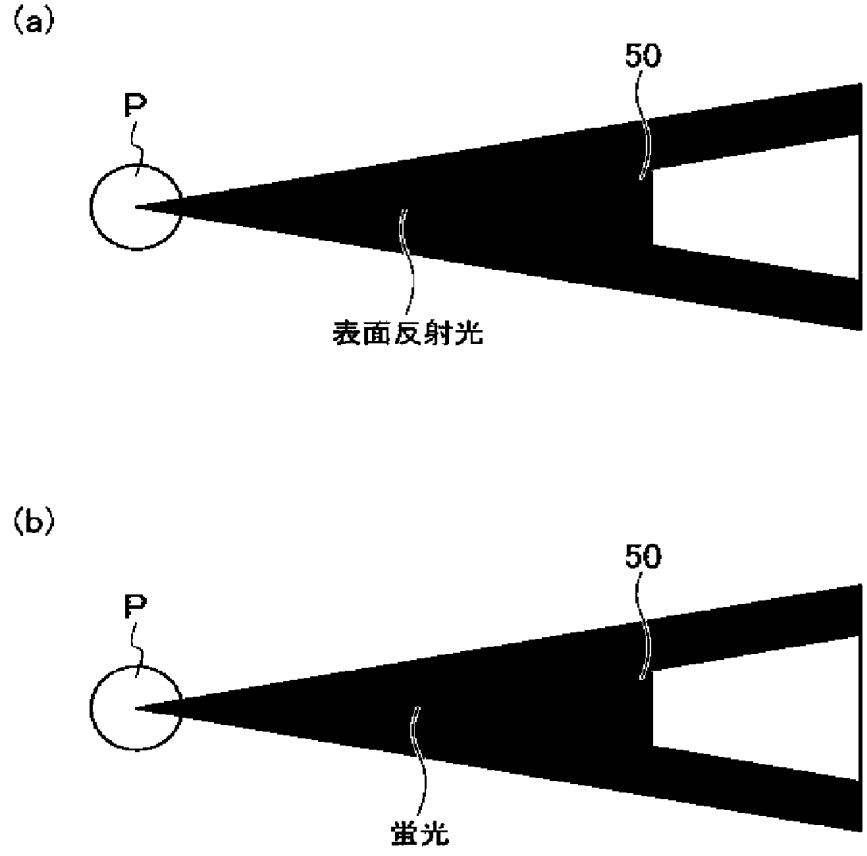


(a)通常のマスク



(b)ノッチフィルタマスク

【図１０】



P　微小粒子
50　マスク

フロントページの続き

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Espacenet

Bibliographic data: JPH1073528 (A) — 1998-03-17

FLOW CYTOMETER EQUIPPED WITH IMAGING FUNCTION

Inventor(s): KUSUSAWA HIDEO; KUBOTA FUMIO ± (KUSUSAWA HIDEO, ; KUBOTA FUMIO)

Applicant(s): TOA MEDICAL ELECTRONICS ± (TOA MEDICAL ELECTRONICS CO LTD)

Classification: **- international:** ***G01N15/14; G01N33/483;*** (IPC1-7): G01N15/14; G01N33/483

- cooperative:

Application number: JP19960248875 19960830 Global Dossier

Priority number (s): JP19960248875 19960830

Abstract of JPH1073528 (A)

PROBLEM TO BE SOLVED: To obtain a flow cytometer by which a cell in an intrinsically three- dimensional shape is analyzed with high accuracy by a method wherein a sample which contains particles is changed into a sample flow by using a sheath flow cell and the sample is irradiated with near-infrared rays so as to be imaged. SOLUTION: A pulse light source 2 is a light source of a type which emits light only for an instant by a light-emitting tigger signal, and flowing particles can be imaged without any blur even when a sample flow is fast at a high speed by several m/sec. Pulsed light L2 is guided to a collimating lens 12 by a kaleidoscope 2a, and it is narrowed down to be fine by a condensing lens 14 via a cold mirror 13 so as to irradiate the sample flow in a sheath flow cell 3. The pulsed light which is transmitted through the sample flow is image- formed by a video camera 17 via a collimating lens 8, dischroic mirror 9, a condensing lens 15 and a band-pass filter 16, and the transmitted-light image of a cell is imaged. In addition, the pulsed light L2 is irradiated via an optical fiber 2a, its coherency is dropped, and the image of the nucleus part of the cell can be imaged with good contrast. An image signal from the video camera 17 is transferred to an image processing part so as to be analyzed and processed.

(19)日本国特許庁（ＪＰ）　(12) **公開特許公報（Ａ）**　(11)特許出願公開番号

特開平10－73528

(43)公開日　平成10年(1998)３月17日

(51)Int.Cl.[6]	識別記号	庁内整理番号	ＦＩ		技術表示箇所
Ｇ０１Ｎ　15/14			Ｇ０１Ｎ　15/14	Ｄ	
33/483			33/483	Ｃ	

審査請求　未請求　請求項の数６　ＦＤ　（全　６　頁）

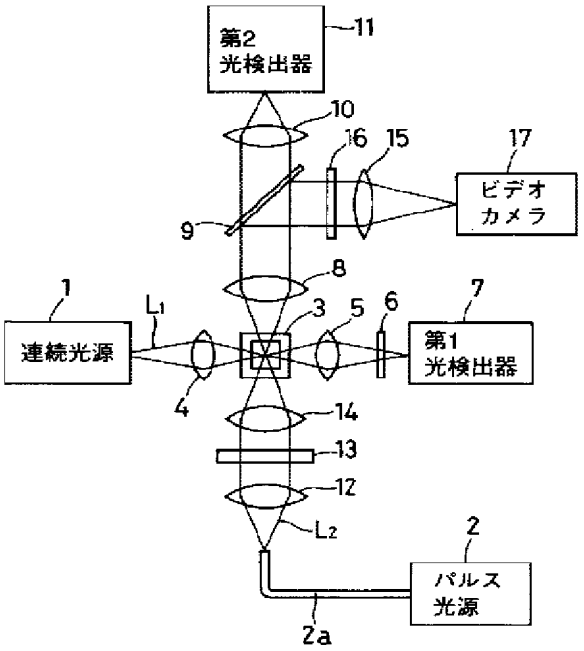
(21)出願番号　特願平8－248875

(22)出願日　平成８年(1996)８月30日

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(54)【発明の名称】　撮像機能付きフローサイトメータ

(57)【要約】
【課題】　細胞を変形させることなく、その核部分をコントラストよく撮像する。
【解決手段】　粒子含有試料を試料流に変換するシースフローセルと、近赤外光で前記試料流を照明する近赤外光源と、近赤外光で照明された試料流中の粒子を撮像する撮像器と、撮像された粒子像を画像処理する画像処理部を備える。



【特許請求の範囲】
【請求項１】　粒子含有試料を試料流に変換するシースフローセルと、近赤外光で前記試料流を照明する近赤外光源と、近赤外光で照明された試料流中の粒子を撮像する撮像器と、撮像された粒子像を画像処理する画像処理部を備えた撮像機能付きフローサイトメータ。
【請求項２】　近赤外光源は、７２０～１５００ｎｍの波長の光を試料流に照明するものである請求項１記載のフローサイトメータ。
【請求項３】　試料流を照明する近赤外線は、コヒーレンス長が粒子径と同程度、又はそれ以下である請求項１記載のフローサイトメータ。
【請求項４】　近赤外光源が、パルスレーザーからなる請求項１記載のフローサイトメータ。
【請求項５】　試料流を可視光で照明する可視光源と、可視光で照明された試料流中の粒子からの光を検出する光検出素子と、試料流から光検出素子までの光路中に設けられ可視光を光検出素子に入射せしめて近赤外光を排除する第１光選択器と、試料流から撮像器までの光路中に設けられ近赤外光を撮像器に入射せしめ可視光を排除する第２光選択器と、光検出素子から得られた粒子信号に基づいて粒子を分析する粒子分析部とをさらに備えた請求項１記載のフローサイトメータ。
【請求項６】　可視光源は、４００～７００ｎｍの波長の光を試料流に照明するものである請求項５記載のフローサイトメータ。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】この発明は、撮像機能付きフローサイトメータに関し、特に細胞などの粒子撮像するための撮像系を備えるフローサイトメータに関する。
【０００２】
【従来の技術】従来、細胞の核部分を細胞質部分から弁別して分析する方法として次のような方法が知られている（例えば、特開平６－５８９２６号公報参照）。つまり、細胞をスライド上に定着して洗浄した後、特別な染料で染色し、更に洗浄して余分な染料を除去し、これにカバーグラスをかけて細胞標本を作成し、そして、作成した標本に赤外線を照射して、細胞を撮像し、細胞像をデジタル化してコンピュータで分析を行うようにしている。
【０００３】
【発明が解決しようとする課題】しかしながら、従来のこのような分析方法においては、細胞標本の作成に手間を要するばかりでなく、細胞がスライドとカバーグラスとに挟まれて偏平化し、立体的な形状を有する細胞本来の像を撮像することができないため、例えば、核部分の直径やプロフィールのような情報を正しく得ることができないという問題点がある。
【０００４】この発明は、このような事情を考慮してなされたもので、粒子を含有する試料流に近赤外光を照射して撮像することにより、本来の立体的な形をした細胞の核部分をコントラストよく撮像することが可能で、またそれによって細胞の分析を高精度に行うことができる撮像機能付きフローサイトメータを提供するものである。
【０００５】
【課題を解決するための手段】この発明は、粒子含有試料を試料流に変換するシースフローセルと、近赤外光で前記試料流を照明する近赤外光源と、近赤外光で照明された試料流中の粒子を撮像する撮像器と、撮像された粒子像を画像処理する画像処理部を備えた撮像機能付きフローサイトメータを提供するものである。
【０００６】
【発明の実施の形態】この発明のシースフローセルは、粒子（細胞）を含む試料をシース液で包んで流すことにより流体力学的効果によって細い試料液の流れを形成させることのできるフローセルであり、これには従来公知のものを用いることができる。
【０００７】この発明の対象とする粒子は、血液や尿に含まれる血球や細胞を主としているが、特に限定されない。
【０００８】粒子を撮像するについて、粒子を照明する光の波長が短いと、光が粒子表面及び内部において散乱や吸収による減光が大きくなり、粒子内部を画像化することができない。
【０００９】逆に光の波長が長いと、粒子による減光が少なくなる（粒子透過性が大きくなる）。水の吸光度が増大する。画像分解能が低下する。これらのことにより良好な画像が得られない。
【００１０】よって、粒子内部をコントラストよく撮像するために近赤外光源は、波長が７２０～１５００ｎｍの範囲にある近赤外光を試料流に照明するものであることが好ましい。具体的には、近赤外パルス半導体レーザ（７２０～９８０ｎｍ）、カラーセンターレーザ（６００～９００ｎｍ）、色素レーザ（６００～１２００ｎｍ）、ＹＡＧレーザ（１．０６４μｍ）、ＹＬＦレーザ（１．０４８μ）、又はＴｉサファイアレーザー（モードロックタイプでキャビテイダンパー付）などにより構成される。
【００１１】粒子を撮像する撮像器には、一般的な２次元画像を撮像するビデオカメラを使用してもよいが、その場合には微弱な蛍光増を増幅するイメージインテンシファイアを備えたものを用いることが好ましい。さらに、そのイメージインテンシファイアにはシャッター手段を備えてもよい。
【００１２】画像処理部は、ＣＰＵ、ＲＯＭおよびＲＡＭからなるマイクロコンピュータによって構成することができる。さらに、近赤外光源から試料流への光路中に設けられ試料流を照明する近赤外光のコヒーレンシー

（可干渉性）を低下させる光学素子をさらに備え、コヒーレンス長を粒子径と同程度又はそれ以下にすることが好ましい。

【００１３】コヒーレンシーを低下させる光学素子としては、特開平７－１３４０９３号公報に記載されたものを使用することができる。

【００１４】この発明は、試料流を可視光で照明する可視光源と、可視光で照明された試料流中の粒子からの光を検出する光検出素子と、試料流から光検出素子までの光路中に設けられ可視光を光検出素子に入射せしめ近赤外光を排除する第１光選択器と、試料流から撮像器までの光路中に設けられ近赤外光を撮像器に入射せしめ可視光を排除する第２光選択器と、光検出素子から得られた粒子信号に基づいて粒子を分析する粒子分析部をさらに備えてもよい。第１光選択器と第２光選択器は１つの光学素子で実現されていてもよく、別々の光学素子で実現されていてもよい。

【００１５】可視光源は、試料流に照明する光が４００～７００ｎｍの波長を有することが好ましい。

【００１６】また、近赤外光の波長が７２０～１５００ｎｍ、可視光の波長が４００～７００ｎｍの場合には、第１光選択器として７００ｎｍより短い波長を光検出素子に入射せしめ、第２光選択器として７００ｎｍあるいは７２０ｎｍよりも長い波長を撮像器に入射せしめる光学素子をそれぞれ使用すればよい。

【００１７】

【実施例】この発明の実施例における光学系を図１に示す。この実施例では、散乱光や蛍光を検出するための連続発光光源１と、細胞像を撮像するためのパルス光源２との２つの光源を設けている。光源１は可視光源、光源２は近赤外光源である。

【００１８】なお、光源１には、中心波長が４８８ｎｍの可視光を発するＡｒレーザーを使用し、光源２には、中心波長が７８０ｎｍで、スペクトル幅が７ｎｍの図３に示すスペクトル特性を備えた近赤外光を発するパルスレーザダイオードを使用している。

【００１９】この２つの光源１、２からの連続可視光Ｌ１とパルス近赤外光Ｌ２は、角型のシースフローセル３（図１では紙面に垂直方向に試料流が流れる）に対して互いに直交するように照射している。

【００２０】この照射領域に細胞が流れてくると、その細胞による前方散乱光が集光レンズ５によって集められ、バンドパスフィルター６を介してフォトダイオードからなる第１光検出器７で受光される（ビームストッパは図示しない）。細胞の側方散乱光は、コリメートレンズ８、ダイクロイックミラー９およびコンデンサレンズ１０を介して光電子増倍管からなる第２光検出器１１で受光、増倍される。

【００２１】なお、第１光検出器７は波長選択された複数の光をそれぞれ検出する複数の光検出素子を含んでもよい。この場合、各光検出素子により、前方散乱光や前方蛍光などを検出することができる。

【００２２】同様に、第２光検出器１０は波長選択された複数の光をそれぞれ検出する複数の光検出素子を含んでもよい。この場合、各光検出素子により、側方散乱光や側方蛍光を検出することができる。

【００２３】図２はこの実施形態の信号処理系の構成を示し、第１および第２光検出器７、１１によってそれぞれ検出された粒子信号、即ち、前方散乱光強度信号Ｓ１および側方蛍光強度信号Ｓ２は、データ処理部１００の粒子分析部１００ａに入力され、各検出信号パルスの高さ情報がＡ／Ｄ変換され、散乱光強度と蛍光強度の２つの特徴パラメータが生成される。

【００２４】撮像制御部１００ｃは信号Ｓ１、Ｓ２がデータ処理部１００に入力されると同時又は所定時間後に細胞を撮像するための発光トリガ信号Ｔｓをパルス光源２に対して供給する。

【００２５】パルス光源２は、発光トリガ信号Ｔｓによって一瞬だけ（２５ナノ秒程度）発光するタイプの光源であり、試料流の流速が数ｍ／秒と高速であっても、流れる粒子をブレ無く撮像することができる。

【００２６】パルス光Ｌ２は図１に示すように、コヒーレンス低下手段の一例であるカライドスコープ（マルチモード光ファイバーでも可能である）２ａによってコリメートレンズ１２へ導かれ、コールドミラー１３を通過してコンデンサレンズ１４によって細く絞られて試料流に照射される。

【００２７】光ファイバー２ａを介して照射することにより、パルス光Ｌ２のコヒーレンシーが落ち、後述するように細胞の核部分の像をコントラスト良く撮像することができる。なお、光ファイバー２ａには、コア一径８００μｍ、長さ２ｍ（住友電工株式会社製ＭＫＨ－８００）のものを用いている。

【００２８】試料流を透過したパルス光は、コリメートレンズ８によって平行光に変換されダイクロイックミラー９に反射されて、コンデンサレンズ１５とバンドパスフィルター１６を介してビデオカメラ１７の受光面に結像され、細胞の透過光像が撮像される。ビデオカメラ１７からの画像信号Ｖｓは図２に示す画像処理部１００ｂに渡され、ディジタル画像として記憶、保存される。

【００２９】粒子分析部１００ａは、前方散乱光および側方蛍光強度の特徴パラメータに基づいてスキャッタダラム（２次元散布図）を生成し、その解析を行う。

【００３０】なお、入力部２００はキーボードやマウスからなり、データ処理部１００に対して各種の指令や条件の設定などを行う。また、出力部３００はＣＲＴやプリンタからなり粒子分析部１００ａと画像分析部１００ｂで得られたスキャッタダラム、粒子像および解析結果などを出力する。

【００３１】ここで、波長４８８ｎｍの光で励起され波

長５００～７００ｎｍの光として発せられる蛍光を検出するために、５００～７００ｎｍの波長の光を透過させ７００ｎｍ以上の波長の光を反射するダイクロイークミラー９を使用している。そして、波長７８０ｎｍ以上の近赤外光画像を得るために、さらに、７８０ｎｍの波長の光を透過させるバンドパスフィルター１６を使用している。

【００３２】従って、ビデオカメラ１７には、可視光Ｌ１（４８８ｎｍ）によって細胞から生じた側方の光は入射しないので、ビデオカメラ１７は光源１からの光の影響を受けない良好な細胞画像を得ることができる。

【００３３】同様に、第２検出器１１にはダイクロイックミラー９の作用により、可視光Ｌ１によって生じた側方の光のみが入射するので、第２検出器１１は、光源２からの光の影響を受けない良好な粒子信号を得ることができる。

【００３４】また、バンドパフルター６には、４８８ｎｍの波長の光を透過させるものを使用しているので、第１光検出器７には、可視光Ｌ１によって生じた前方散乱光のみが入射し、近赤外光Ｌ２によって生じた側方の光は全く入射しないので、第１光検出器は光源２からの光の影響を受けない良好な粒子信号を得ることができる。

【００３５】試料流に含まれる細胞は球形に近い形状を有するので、可視光を用いると、細胞表面及び細胞質部分において散乱・吸収による減光により細胞内部を鮮明に画像化することは難しい。しかし、近赤外領域の波長の光については、細胞表面及び細胞質部分での減光が小さいために内部を鮮明に画像化しやすい。

【００３６】図４は、この実施例によって得られた細胞画像の７つの例を示し、図５はその比較例（撮像用のパルス光源２として波長が５２３ｎｍの可視光源を使用したもの）として２つの細胞画像例を示している。

【００３７】なお、この実施形態では、近赤外線を照射する光源として赤外光パルスレーザーのような単色性の高い光源を使用し、光学素子により、その光のコヒーレンシーを低下させている。そのことにより、核部分の細胞質部分に対する減光比を最適に調整し、それによって核部分が鮮明に写った画像を得るようにしている（この実施形態では、コヒーレンス長さを５～６μｍに設定している）。

【００３８】また、近赤外線光源を、波長帯域の広い赤外線ランプと、波長幅を制限する狭帯域フィルターとで代用することも可能である。

【００３９】この実施形態では、図１に示すように、レンズ１２とレンズ１４との間の光のコリメート領域には、赤外光を透過し可視光を反射するコールドミラー１３が設けられている。従って、可視光Ｌ１によって照射された細胞から生じる側方散乱光や側方蛍光の内、レンズ１４側に入射したものは、コールドミラー１３で反射されて第２光検出器１へ導かれる。これによって、検出される光の強度が増大し、とくに第２光検出器１１の収集効率が向上する。

【００４０】なお、このようなコールドミラーをシースフローセル３とレンズ１４の間に設置することも可能であるが、この場合には、コールドミラーを凹面ミラーとする必要がある上、その位置の設定が容易でない。

【００４１】これに対して、この実施形態におけるコールドミラー１３は光路コリメート領域に設けられているので、平面ミラーを用いることができると共に、その位置設定が容易である。

【００４２】

【発明の効果】この発明によれば、細胞を変形させることなくその核部分をコントラストよく撮像することができる。

【図面の簡単な説明】

【図１】この発明の実施例の光学系を示す構成図である。

【図２】この発明の実施例の信号処理系を示す構成図である。

【図３】この発明の実施例の光源のスペクトル特性図である。

【図４】この発明の実施例によって得られた細胞画像例（顕微鏡写真）である。

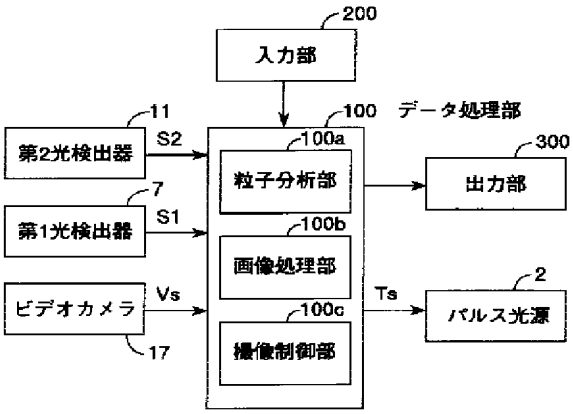
【図５】図４の画像に対する比較例を示す画像例（顕微鏡写真）である。

【符号の説明】

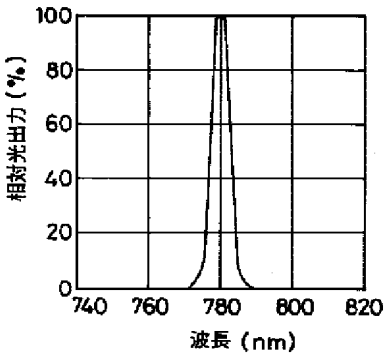
１　連続発光光源
２　パルス光源
２ａ　光ファイバー
３　シースフローセル
４　コンデンサレンズ
５　集光レンズ
６　バンドパスフィルター
７　第１光検出器
８　コリメートレンズ
９　ダイクロイックミラー
１０　コンデンサレンズ１０
１１　第２光検出器
１２　コリメートレンズ
１３　コールドミラー
１４　コンデンサレンズ
１５　コンデンサレンズ
１６　バンドパスフィルター
１７　ビデオカメラ

【図１】

【図２】

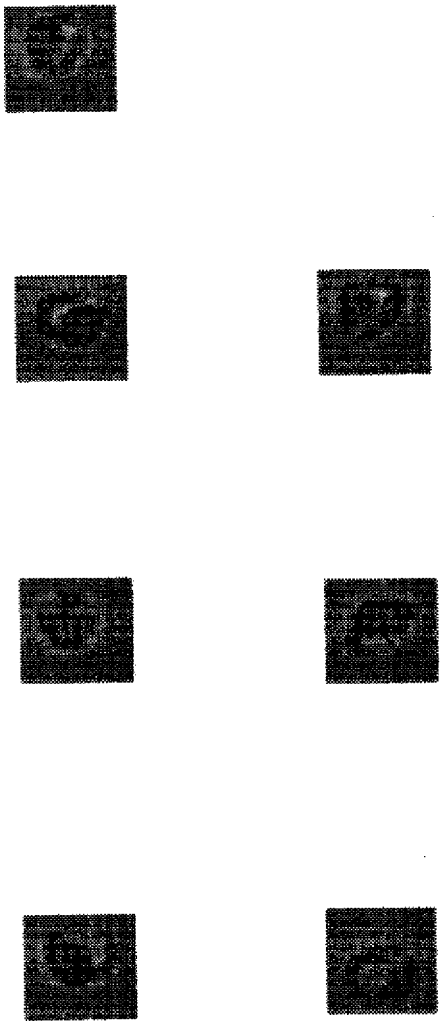


【図３】



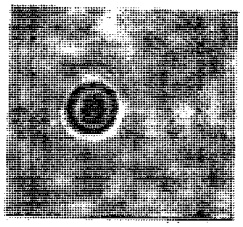
【図４】

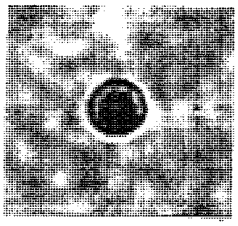
図面代用写真



【図５】

図面代用写真





Espacenet

Bibliographic data: JPH09502794 (A) — 1997-03-18

LIQUID FLOW CYTOMETER

Inventor(s):

Applicant(s):

Classification: - international: ***G01N15/14; G01N21/53; G01N21/64; G01N33/49;*** (IPC1-7): G01N15/14; G01N21/53; G01N21/64; G01N33/49
- cooperative: **G01N15/14**

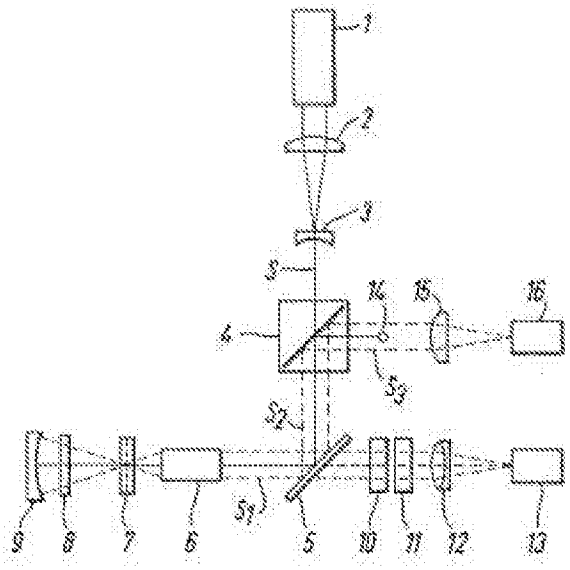
Application number: JP19950501624 19940608 Global Dossier

Priority number (s): WO1994NO00106 19940608 ; NO19930002088 19930608

Also published as: AU6986094 (A) DE69412646 (T2) DK0737307 (T3) EP0737307 (A1) EP0737307 (B1) more

Abstract not available for JPH09502794 (A)
Abstract of corresponding document: WO9429695 (A1)

A liquid flow cytometer for counting and classification of particles such as biological cells and other microscopic particles in liquids, where the cytometer comprises an optical system for irradiation and detection in a metering zone (7), thus enabling optical dispersion and fluorescence caused by the particles to be simultaneously detected when the particles pass the metering zone. The optical system comprises in a first beam path (S1) a mirror reflector (9) with its focal point in the metering zone (7), a quarter-wave plate (8) between the metering zone (7) and the mirror reflector (9), a lens (6) with its focal point in the metering zone (7) and located on the opposite side of it together with a dichroic mirror (5) behind the lens and provided in



【公報種別】特許法第１７条第１項及び特許法第１７条の２の規定による補正の掲載
【部門区分】第６部門第１区分
【発行日】平成１３年１１月１３日（２００１．１１．１３）

【公表番号】特表平９－５０２７９４
【公表日】平成９年３月１８日（１９９７．３．１８）
【年通号数】
【出願番号】特願平７－５０１６２４
【国際特許分類第７版】
G01N 15/14
21/53
21/64
33/49
【ＦＩ】
G01N 15/14 D
21/53 Z
21/64 Z
33/49 A

手続補正書

平成１３年６月６日

特許庁長官　殿

１．事件の表示
平成７年特許願第５０１６２４号

２．補正をする者
住所　ノルウェー国　オスロ　エヌ　１１８７，スペインバイエン　２９エイ
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国籍　ノルウェー国

住所　ノルウェー国　オスロ　エヌ－１１５８，ヘラゲルドバイエン
５７ビィ
名称　タンゲン，　ライダー
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クリスタルタワー１５階
氏名　（７８２８）　弁理士　山本　秀策
電話（大阪）０６－６９４１－３９１０

４．補正対象書類名
請求の範囲および明細書

５．補正対象項目名
請求の範囲および明細書

６．補正の内容
6.1　請求の範囲を別紙のとおり補正します。
6.2　明細書を以下のとおり補正します。
6.2.1　発明の名称を「フローサイトメトリー装置」に補正します。
6.2.2　明細書第１頁下から第４行～最下行の「その反対側に設けられたレンズと、レンズの背後に位置し、かつレンズ軸に対して実質的に横線方向に部分反射を行うように設けられたダイクロイックミラーとを有し、」を「この測定ゾーンとダイクロイックミラーとの間に設けられたレンズとを有し、このダイクロイックミラーが、レンズの軸に対して法線方向の部分反射を行うように設けられ、」に補正します。
6.2.3　明細書第５頁第１行～第２行の「それ自体公知の光源」を「光源」に補正します。
6.2.4　明細書第５頁第３行～第４行の「実質的に法線方向に」を「伝播方向の」に補正します。
6.2.5　明細書第５頁第５行の「背後に」を「次に」に補正します。
6.2.6　明細書第９頁第18行の２つの「pc/ml.」を「粒子/ml.」に補正します。

「
米国特許第4,662,742号は、蛍光を透過し、そして散乱光を反射するためのビームスプリッタを有する光路を含む、フローサイトメトリー装置を開示する。
EP-A-0 539 351は、蛍光のみを放出するためのフローサイトメーターを開示する。
」

請求の範囲

１．液体中の生物細胞または他の微粒子のような粒子を計測し、そして分類するための液体フローサイトメーターであって、ここで該サイトメーターは、測定ゾーン（７）における反射および検出のための光学系を有することによって、該粒子が該測定ゾーンを通過するときに該粒子によって発生する光散乱および蛍光を同時に検出することを可能にし、

該光学系が、第１のビーム路（S_1）内に、該測定ゾーン（７）内に焦点を有する反射鏡（９）と；該測定ゾーン（７）と該反射鏡（９）との間に位置する1/4波板（８）と；該測定ゾーン（７）内に焦点を有し、かつ該測定ゾーン（７）とダイクロイックミラー（５）との間に設けられたレンズ（６）とを有し；

該ダイクロイックミラー（５）が、該レンズ（６）の軸に対して法線方向の部分反射を行うように設けられ、

該反射の方向が該光学系の第２のビーム路（S_2）内にあり、そして該第２のビーム路（S_2）内で、該ダイクロイックミラー（５）と光源（１）との間に位置するビームスプリッタ（４）を有しており、

該ビームスプリッタ（４）が該第２のビーム路（S_2）に対して法線方向の第３のビーム路（S_3）を創出し、そして該サイトメーターがさらに、該第１のビーム路（S_1）内で、該ダイクロイックミラー（５）の次に設けられ、かつ該測定ゾーン（７）内の該粒子によって発せられた蛍光を検出するために設けられた第１の検出器（１３）と；該第３のビーム路（S_3）内に設けられ、かつ該測定ゾーン（７）内の該粒子によって生じた光散乱を検出するために設けられた第２の検出器（１６）とを有していることを特徴とする、該液体フローサイトメーター。

２．前記反射鏡（９）が球面凹面鏡であることを特徴とする、請求項１に記載のサイトメーター。

３．前記ビームスプリッタ（４）が偏光ビームスプリッタであることを特徴とする、請求項１に記載のサイトメーター。

４．前記第２のビーム路（S_2）内で、前記偏光ビームスプリッタ（４）と前記光源（１）との間に、該光源（１）からの光を該ビームスプリッタ（４）上に集束するための適切なレンズ装置（２，３）が設けられていることを特徴とする、請求項３に記載のサイトメーター。

５．前記光源（１）が、前記第２のビーム路（S_2）内に設けられたダイオードレーザであることを特徴とする、請求項４に記載のサイトメーター。

６．前記ダイクロイックミラー（５）を通る蛍光を前記第１の検出器（１３）上に集束するために、該第１の検出器（１３）と該ダイクロイックフィルター（５）との間に、光学フィルター（１０、１１）が適切なレンズ装置（１２）とともに設けられていることを特徴とする、請求項５に記載のサイトメーター。

７．前記第１の検出器（１３）がフォトダイオードを含むことを特徴とする、請求項６に記載のサイトメーター。

８．前記ビームスプリッタ（４）によって反射された散乱光を前記第２の検出器（１６）上に集束するために、該第２の検出器（１６）と該ビームスプリッタ（４）との間に、ビームストップ（１４）および適切なレンズ装置（１５）が設けられていることを特徴とする、請求項１に記載のサイトメーター。

９．前記第２の検出器（１６）がフォトダイオードを含むことを特徴とする、請求項７に記載のサイトメーター。

１０．前記フォトダイオードがなだれダイオードの形態であることを特徴とする、請求項７に記載のサイトメーター。

１１．前記第２の検出器（１６）に含まれる前記フォトダイオードがPINダイオードの形態であることを特徴とする、請求項９に記載のサイトメーター。

Electronic Patent Application Fee Transmittal

Application Number:	14555102
Filing Date:	26-Nov-2014
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Filer:	Gina N. Shishima/Hannah Wimberley
Attorney Docket Number:	BECC.P0002US.CIP(12US0040

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	29661946
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	129381
Filer:	Gina N. Shishima/Hannah Wimberley
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US.CIP(12US0040
Receipt Date:	30-JUN-2017
Filing Date:	26-NOV-2014
Time Stamp:	11:44:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$180
RAM confirmation Number	063017INTEFSW11524100
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		BECC02CIP_SIDS.pdf	162814 bc9d19cbc74af36423810d7eddc1d66a104c221b	yes	3
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	2	
	Information Disclosure Statement (IDS) Form (SB08)		3	3	
Warnings:					
Information:					
2	Foreign Reference	JPH04184241A.pdf	634794 be8c540f84cd34ae5e26babac918819c382686d0	no	10
Warnings:					
Information:					
3	Foreign Reference	JP2004341204A.pdf	678863 4d2fefcae7473b78cfb999a1c640ee65045e3dac	no	15
Warnings:					
Information:					
4	Foreign Reference	JP2012026837A.pdf	677046 558de3b147bc54d1933e09844ec05d446ae29354	no	19
Warnings:					
Information:					
5	Foreign Reference	JPH1073528A.pdf	369804 828c0ec384197d43b3143b63cadcf8e6b255a925	no	7
Warnings:					
Information:					

6	Foreign Reference	JPH09502794A.pdf	152302 50a82f868b6e3e929e214542e2c8dc0e9ab96b58	no	3
Warnings:					
Information:					
7	Non Patent Literature	OfficeAction.pdf	1046571 41e3b706fd4f62ed6a0723fc0c3268e2ae7fb542	no	12
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	30672 48b9bbbe0bef08b11e1dd5a6efc80679981fbfdc	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	3752866		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: IRIS INTERNATIONAL, INC.	Group Art Unit: 2884
Serial No.: 14/555,102	Examiner: Mindy D. Vu
Filed: November 26, 2014	Atty. Dkt. No.: BECC.P0002US.CIP/11412161
For: FLOW CYTOMETER	Confirmation No.: 4589

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

June 30, 2017	/Gina N. Shishima/
Date	Gina N. Shishima

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Commissioner:

In compliance with the duty of disclosure under 37 C.F.R. § 1.56, it is respectfully requested that this Supplemental Information Disclosure Statement be entered and the documents listed on attached Form PTO-1449 be considered by the Examiner and made of record. Copies of the listed documents required by 37 C.F.R. § 1.98(a)(2) are enclosed for the convenience of the Examiner.

In accordance with 37 C.F.R §§ 1.97(g), (h), this Supplemental Information Disclosure Statement is not to be construed as a representation that a search has been made, and is not to be construed to be an admission that the information cited is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

In accordance with 37 C.F.R § 1.97(e)(1), Applicant hereby certifies that each item of information contained in this Supplemental Information Disclosure Statement was first cited in

any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the present statement, as evidenced by the date of the enclosed office action.

In accordance with 37 C.F.R. § 1.97(d)(2), a fee as set forth in 37 C.F.R. § 1.17(p) in the amount of $180.00 (large entity) is enclosed. If an appropriate payment has not been enclosed, or if it is insufficient, the Commissioner is authorized to deduct the appropriate fee from Norton Rose Fulbright US LLP Deposit Account No.: 50-1212/BECC.P0002US.CIP/11412161.

Applicant respectfully requests that the listed documents be made of record in the present case.

Respectfully submitted,

/Gina N. Shishima/

Gina N. Shishima
Reg. No. 45,104
Attorney for Applicant

NORTON ROSE FULBRIGHT US LLP
98 San Jacinto Blvd., Suite 1100
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Date: June 30, 2017

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040	4589

7590 07/10/2017

NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

EXAMINER	
VU, MINDY D	

ART UNIT	PAPER NUMBER
2884	

NOTIFICATION DATE	DELIVERY MODE
07/10/2017	ELECTRONIC

NOTICE OF NON-COMPLIANT INFORMATION DISCLOSURE STATEMENT

An Information Disclosure Statement (IDS) filed 4/30/17 in the above-identified application fails to meet the requirements of 37 CFR 1.97(d) for the reason(s) specified below. Accordingly, the IDS will be placed in the file, but the information referred to therein has not been considered.

The IDS is not compliant with 37 CFR 1.97(d) because:

☐ The IDS lacks a statement as specified in 37 CFR 1.97(e).

☐ The IDS lacks the fee set forth in 37 CFR 1.17(p).

☒ The IDS was filed after the issue fee was paid. Applicant may wish to consider filing a petition to withdraw the application from issue under 37 CFR 1.313(c) to have the IDS considered. See MPEP 1308.

571-272-4200 or 1-888-786-0101
Application Assistance Unit
Office of Data Management

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP	Serial No.: 14/555,102
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: Yong Qin Chen	
	Filing Date: November 26, 2014	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 9*	*See Page 10*

U.S. Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
Change(s) applied to document, /B.H.H./ 4/24/2017	A139	7758324	7/20/10	Baumann et al.	417	477.2	5/12/05
	A140	7768120	8/3/10	Takashima	257	706	12/27/07
	A141	7781227	8/24/10	Mehrpouyan et al.	436	523	3/4/09
	A142	7787197	8/31/10	Chen	359	793	9/4/08
	A143	7835000	11/16/10	Graves et al.	356	338	11/3/06
	A144	7876436	1/25/11	Chu	356	338	10/31/07
	A145	7894047	2/22/11	Hamada et al.	356	39	7/24/09
	A146	7952981	5/31/11	Suzuki	369	121	10/23/08
	A147	7981661	7/19/11	Rich	435	286.5	4/17/07
	A148	8009189	8/30/11	Ortyn et al.	348	80	6/3/08
	A149	8049185	11/1/11	Hayashi et al.	250	459.1	2/4/09
	A150	8149525	4/3/12	Do	359	797	3/28/07
	A151	8157547	4/17/12	Vrielink	417	477.3	4/23/07
	A152	8187888	5/29/12	Rich	436	63	7/8/11
	A153	8213472	7/3/12	Hirai et al.	372	36	1/23/08
	A154	8229707	7/24/12	Olson et al.	702	189	7/4/11
	A155	8233146	7/31/12	Chen	356	246	1/5/10
	A156	8253938	8/28/12	Vacca et al.	356	337	5/26/11
	A157	8270098	9/18/12	Fukuta et al.	359	784	2/19/09
	A158	8284402	10/9/12	Frazier et al.	356	419	2/26/10
	A159	8337096	12/25/12	Shen et al.	385	89	11/30/09
	A160	8345237	1/1/13	Tsukii et al.	356	338	12/23/11
	A161	8405048	3/26/13	Hayashi	250	458.1	2/4/09

41600224.1

EXAMINER:	DATE CONSIDERED:

EXAMINER: INITIAL IF REFERENCE CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED. INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.V/

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP	Serial No.: 14/555,102
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: Yong Qin Chen	
	Filing Date: November 26, 2014	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 9*	*See Page 10*

U.S. Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A24	20130070243	3/21/13	Goehde	356	338	11/9/12
	A25	20130094102	4/18/13	Baba	359	784	7/13/10
	A26	20130204538	8/8/13	Rich	702	21	10/25/11
	A27	2385495	9/25/45	Brian	250	487.1	8/21/43
	A28	2669709	2/16/54	Glancy et al.	348	784	10/19/60
	A29	2764147	9/25/56	Brunner	126	247	2/23/51
Change(s) ~~applied~~ to document, /B.H.H./ 4/28/2017	A30	315667	4/14/~~85~~ 1885	Serdinko	417	475	10/20/84
	A31	3411293	11/19/68	Akins	60	454	10/20/66
	A32	3542491	11/24/70	Newman	417	295	5/27/69
	A33	3661460	5/9/72	Elking et al.	356	36	8/28/70
	A34	3726613	4/10/73	von Casimir	417	477.1	10/12/70
	A35	3826593	7/30/74	von Casimir	417	53	5/11/73
	A36	3873204	3/25/75	Friedman et al.	356	39	11/29/72
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	A42	4189236	2/19/80	Hogg et al.	356	317	3/20/78
	A43	4244045	1/6/81	Nosu et al.	398	86	1/31/79
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	A45	4515274	5/7/85	Hollinger et al.	209	3.1	12/2/81
	A46	4564342	1/14/86	Weber et al.	417	477.6	7/24/84

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REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	14/555,102	Filing Date	2014-11-26	Docket Number (if applicable)	BECC.P0002US.CIP	Art Unit	2884
First Named Inventor	Yong Qin Chen			Examiner Name	Mindy D. Vu		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☐ Amendment/Reply
- ☒ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☒ Other Quick Path IDS

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

☒ **The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.**
The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 501212

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

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Applicant Signature

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Signature of Registered U.S. Patent Practitioner			
Signature	/Gina N. Shishima/	Date (YYYY-MM-DD)	2017-07-11
Name	Gina N. Shishima	Registration Number	45104

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

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Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP/11412161	Serial No.: 14/555,102
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: IRIS INTERNATIONAL, INC.	
	Filing Date: November 26, 2014	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 1*	*See Page 1*

U.S. Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A169	5,257,917	11/02/93	Minarik et al.	417	475	10/02/92

Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	B36	JP H04-184241	07/01/92	Japan	Japanese (English Abstract)
	B37	JP 2004-341204	12/02/04	Japan	Japanese (English Abstract)
	B38	JP 2012-026837	02/09/12	Japan	Japanese (English Abstract)
	B39	JP H10-73528	03/17/98	Japan	Japanese (English Abstract)
	B40	JP H09-502794	03/18/97	Japan	Japanese (English Abstract)

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C6	Office Action issued in Japanese Patent Application No. 2015-515208, issued May 29, 2017 (English Translation Provided) (Attorney Docket No. BECC.P0002JP)

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Bibliographic data: JPH04184241 (A) — 1992-07-01

PARTICLE ANALYSER

Inventor(s): YAMAZAKI ISAO; OKI HIROSHI; MIYAKE AKIRA ± (YAMAZAKI ISAO, ; OKI HIROSHI, ; MIYAKE AKIRA)

Applicant(s): HITACHI LTD ± (HITACHI LTD)

Classification: **- international:** ***G01N15/14;*** (IPC1-7): G01N15/14
- cooperative:

Application number: JP19900315099 19901120 Global Dossier

Priority number (s): JP19900315099 19901120

Abstract of JPH04184241 (A)

PURPOSE:To obtain high analyzing accuracy by simply adjusting the width and uniformity of a laser beam irradiation region by providing a converting lens system on this side of the beam splitting element on laser beam and providing a converging lens system behind the element. CONSTITUTION:Laser beam 3 is split into two beams by a beam splitting element (Wollaston polarizing prism) 8 and these beams are respectively focused to an irradiation surface by converging lenses 2a, 2b but the spot positions thereof are separated at the interval equal to the diameter of each of the beams. When the focal distances of the lenses 2a, 2b are changed, the beam diameter and the spot interval are changed in the same ratio and, when the focal distance or magnifying power of a converting lens 4 is changed, only the beam diameter is largely changed. Therefore, by altering the converting lens 4, the overlap degree of the beams is changed and the uniformity of the intensity in an irradiation region can be altered.

⑲日本国特許庁（JP）　⑪特許出願公開

⑫公開特許公報（A）　平4－184241

⑤1Int. Cl.[5]	識別記号	庁内整理番号	⑬公開　平成4年(1992)7月1日
G 01 N 15/14	D	7005－2J	
	B	7005－2J	
	C	7005－2J	

審査請求　未請求　請求項の数 9　（全9頁）

⑤4発明の名称　粒子分析装置

⑳1特　願　平2－315099

⑳2出　願　平2(1990)11月20日

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明　細　書

1．発明の名称

粒子分析装置

2．特許請求の範囲

1．フローセルに被検粒子の懸濁液を供給する液供給手段と、該懸濁液中の被検粒子にレーザ光を照射する光学手段と、該被検粒子の像を結像させる集光手段と、該被検粒子から発散する光を検出する光検出手段と、該検出された光から前記被検粒子を分析する分析手段とを備えた粒子分析装置において、前記光学手段と前記フローセルの間のレーザ光の経路上に、該レーザ光をビーム径と同等あるいはそれ以下の間隔を隔てたビームに分岐するビーム分岐素子系を含み、該光学手段と該ビーム分岐素子系の間のレーザ光の経路上に焦点距離または拡大率可変の変換レンズ系を、該ビーム分岐素子系と該フローセルとの間のレーザ光の経路上に収束レンズ系を含むことを特徴とする粒子分析装置。

2．前記変換レンズ系は拡大率の異なる複数の変換レンズ・サブ系を並列して構成し、該変換レンズ・サブ系はその光軸を前記レーザ光の光軸に平行とし、該レーザ光の光軸を含む面内で平行移動可能に設置されたことを特徴とする請求項1記載の粒子分析装置。

3．前記ビーム分岐素子系は分岐角度の異なる複数のビーム分岐素子・サブ系を並列して構成し、該ビーム分岐素子・サブ系はその光軸を前記レーザー光の光軸に平行とし、該レーザ光の光軸を含む面内で平行移動可能に設置されたことを特徴とする請求項1または2記載の粒子分析装置。

4．前記収束レンズ系は収束率の異なる収束レンズ・サブ系を並列して構成し、該収束レンズ・サブ系はその光軸を前記レーザ光の光軸に平行とし、該レーザ光の光軸を含む面内で平行移動可能に設置されたことを特徴とする請求項1、2または3いずれか記載の粒子分析装置。

5．前記ビーム分岐素子・サブ系はそれぞれレーザ光進行方向に順次配列された1／2波長波と

ウォラストンプリズムとで構成されていることを特徴とする請求項4記載の粒子分析装置。

6．前記ビーム分岐素子系はレーザ光進行方向に順次配列された1／2波長板とウォラストンプリズムとで構成されていることを特徴とする請求項1記載の粒子分析装置。

7．前記ビーム分岐素子系は分割プリズムから構成されたことを特徴とする請求項1記載の粒子分析装置。

8．前記収束レンズ系は2枚のシリンドリカルレンズで構成し、該2枚のシリンドリカルレンズの一方は懸濁液の流れ方向と平行に、他方は該流れ方向に光を収束するように設置したことを特徴とする請求項1記載の粒子分析装置。

9．前記収束レンズ系は拡大率可変のレンズで構成したことを特徴とする請求項1記載の粒子分析装置。

3．発明の詳細な説明

〔産業上の利用分野〕

本発明は細管を流れる粒子にレーザ光を照射し、粒子から発散する散乱光や蛍光の強度を分析する粒子分析装置に係り、特にガウス分布を持つレーザビームのビーム断面における強度分布形状を平坦化するに好適なビーム強度平坦化装置を備えた粒子分析装置に関する。

〔従来の技術〕

従来、懸濁液サンプル中の粒子を速く効率的に識別できる方法の1つに流動細胞測定法がある。この方法では、粒子の懸濁液、典型的には血液サンプル中の細胞がフローセルの微小断面の流路を通して流され、そこで血液サンプル中の個々の粒子がレーザビームで照射される。そして個々の粒子とレーザビームの相互作用の結果が、一つもしくは複数の光検出装置により検出される。通常、光検出装置は特定の散乱角度における光の散乱または特定の波長の蛍光を測定するように設計されている。こうして、フローセルを流れる個々の粒子は、散乱光、蛍光もしくは他の光学的または電気的特性について関連した一つ以上の特性について特徴づけられる。それらの特性によって、個々の粒子は検出器で測定される光の強さまたは他の特性を軸とする特徴空間の中に写像される。理想的にはサンプル中の異なった種類の粒子が特徴空間の中の重複しない領域に写像され、特徴空間におけるその写像に基づいて個々の粒子の種類が推定できるとよい。粒子の分析精度を向上させるため、フローセル内の流れをつくる方法としてシースフロー方式が採用され、この方式によれば微小断面流路の中の中心部分のみを血液サンプルが流れる。

レーザビームのビーム断面における強度分布は、第9図に示すように中心部が高く周辺部が低いガウス分布になっている。粒子分析装置などの光源として使う場合には強度分布が一様である必要があるために、従来レーザビーム径を血液の流れに対して垂直な方向（幅方向）にのみ拡大した楕円ビームを用いてその中心部分のみを利用することが行われてきた。ガウス分布のレーザビームは一般に強度分布が中心強度の1／e^2 となる径dをもってビームの大きさとしている。照射領域幅Sの範囲内での強度の均一度Uをi／Iで定義すると、d／Sを大きくするほど均一度Uは高くなる。しかし、d／Sを大きくした場合、レーザビームの持つエネルギのうち、照射領域幅Sの中で利用できる割合は小さくなってしまう。従って、ガウス分布のレーザビームを用いる限り均一度Uとエネルギ効率Eを同時に高くすることはできない。均一度Uとエネルギ効率Eを同時に高くするためにはレーザビームの強度分布をガウス分布より効率のいい形に変換する必要がある。

レーザビームを平坦化する装置としては、特開平1－210923号公報に記載の装置などがある。これは、プリズムの稜線を用いて、ガウス分布のビームを分割し、強度分布を反転して重ね合わせることにより強度分布を平坦化するものであった。この方法でエネルギ効率Eを向上することができ、アライメントレーザ等に用いることができるが、粒子分析装置の光源として用いるには適していない。それは、粒子分析装置では、必要な照射領域幅が20μm程度と小さいため、ガウス

分布のビームを分割した場合干渉縞が発生して強度分布が滑らかにならないこと、及び、レーザビームの光軸とプリズムの中心軸がわずかでもずれると得られるビームの強度分布が非対称になり、均一度Uが低下してしまうことによる。

そこで本出願人は特願平1-279487にて簡単な構成でビーム強度を平坦化した粒子分析装置を提案した。これはガウス分布のレーザビームをウォラストンプリズムで微小角度を持たせて分離し、集光レンズで粒子の通過領域に照射するものである。この方法では、レーザビームを偏光成分によって分離し、偏光成分が直行したまま重ね合わせるために、照射領域では互いに干渉しないレーザビームがある間隔をおいて重なった状態になっている。個々のレーザビームはガウス分布を保っているために、干渉縞が生じない。またプリズムの稜線の部分を使わないので、光軸とプリズムの中心軸を高精度に一致させる必要がないなどの利点があった。

〔発明が解決しようとする課題〕

粒子分析装置では、分析対象によってレーザビームの照射領域の大きさやレーザビームの強度を変更する必要がある。例えば、血液中の赤血球の分析を行う場合には、赤血球は単位容積当りに含まれる数が非常に多いために、フローセルに流すサンプル液の量を小さくするので、照射領域を狭くしてよい。さらに、赤血球の中の網状赤血球の比率を検出するには、網状赤血球から放射される蛍光の強度を高めるために、照射するレーザビームのエネルギ密度を高める必要がある。この場合は、照射領域の均一度を多少落としてもエネルギ密度を高くした方が効果的である。また、血液中の白血球の分析を行うためには、白血球が赤血球に比べて単位容積当りの数が1／700と少ないために、フローセルに流すサンプル液の量を多くしなければならない。そのため、サンプル液の流れる領域の幅が大きくなるので、照射領域を広くしなければならない。この場合は、レーザビームのエネルギ密度が低くても白血球から放射される蛍光の強度は高いが、別の種類の白血球細胞と精度よく分類するには、レーザビームの照射均一度を高める必要がある。また、リンパ球のサブセットの分類を行う場合には、照射均一度を多少下げても、照射領域を広げ、かつエネルギ密度を高くする必要がある。このように、粒子分析装置では分析対象によってレーザビームの照射領域の大きさと領域内の照射均一度を独立に変更することが必要である。

従来のビーム強度平坦化装置を用いた粒子分析装置では、レーザビームが均一に照射している領域の大きさと領域内での照射の均一度を自由に変更することができなかった。2つのガウス分布のビームを重ね合わせると、照射面では第2図に示すように、強度分布形状は個々のビームの径dとビーム間隔Hで決まってしまう。ビーム径dは、収束レンズ2に入射するレーザビームの径Dと収束レンズ2の焦点距離f、レーザ光の波長λを用いて、

$$d = (4/\pi) \cdot f \cdot \lambda / D$$

であらわされる。また、照射面での2つのビームの間隔Hは、ウォラストンプリズム8の分岐角度θdを用いて、

$$H = f \cdot \theta d$$

で近似できる。分岐角度θdはウォラストンプリズム8の幾何学的形状で決定されてしまうので自由に変更できない。収束レンズ2に焦点距離fが異なるレンズを用いても強度分布は相似な形状のまま拡大、縮小するだけである。粒子分析装置では、照射領域の大きさを変更するだけでなく照射均一度も変更する必要があるので強度分布形状も変える必要がある。強度分布の形状を変更するためには、ビーム径Dの異なるレーザ光源を用いるか、分岐角度θdの異なるウォラストンプリズムを用いねばならず、高価になるという欠点があった。

また、粒子分析装置の照射領域を変更する場合以外にも、レーザ光源を交換したときなどにも強度分布形状を変更する必要がある。第2図は個々のビーム径dとビーム間隔Hの比H／dが0.530の場合の比較的平坦な強度分布であるが、H／dが0.607の場合の強度分布形状は第3図

のように二つの山形になる。レーザ光源によってビーム径は差があるが、ビーム径dが15%変化するだけで強度分布が第3図のように変化し照射均一度が下がってしまう。従来の粒子分析装置では、レーザ光源のビーム径の誤差によって照射均一度が低下して、分析精度が悪くなるという欠点があった。

本発明の目的は、レーザ光が均一に照射する領域の大きさと、その領域内の照射均一度を簡単に変更でき、分析精度の高い粒子分析装置を提供することにある。

〔課題を解決するための手段〕

上記目的を達成するために、本粒子分析装置は、フローセルに被検粒子の懸濁液を供給する液供給手段と該懸濁液中の被検粒子にレーザ光を照射する光学手段と、該被検粒子の像を結像させる集光手段と、該被検粒子から発散する光を検出する光検出手段と、該検出された光から前記被検粒子を分析する分析手段とを備えた装置において、前記光学手段と前記フローセルの間のレーザ光の経路上に、該レーザ光をビーム径と同等あるいはそれ以下の間隔を隔てたビームに分岐するビーム分岐素子系を含み、該光学手段と該ビーム分岐素子系の間のレーザ光の経路上焦点距離または拡大率可変の変換レンズ系を、該ビーム分岐素子系と該フローセルの間のレーザ光の経路上に収束レンズ系を含むことを特徴している。

そして、変換レンズ系は拡大率の異なる複数の変換レンズ・サブ系から構成しそれぞれの光軸がレーザ光の光軸に平行でかつ該レーザ光の光軸を含む面内で移動させる機構としてもよく、またビーム分岐素子系は分岐角度の異なる複数のビーム分岐素子・サブ系から構成しそれぞれの光軸がレーザ光の光軸に平行でかつ該レーザ光の光軸を含む面内で移動させる機構としてもよく、さらに収束レンズ系は収束率の異なる収束レンズ・サブ系から構成しかつそれぞれの光軸が前記レーザ光の光軸に平行でかつ該レーザ光の光軸を含む面内で移動させる機構としてもよい。

またビーム分岐素子系及びそのサブ系はそれぞれレーザ光進行方向に順次配列された1／2波長板とウォラストンプリズムで構成するのがよく、あるいはビーム分岐素子系は分割プリズムから構成することもできる。

また収束レンズ系は2枚のシリンドリカルレンズで構成し、該2枚のシリンドリカルレンズの一方は懸濁液の流れ方向と平行に他方は該流れ方向に光を収束するように設置するのがよい。また収束レンズ系は拡大率可変のレンズ組み合わせで構成することもできる。

〔作用〕

本発明の粒子分析装置において、レーザ光はビーム分岐素子により2つのビームに分離される。それぞれのビームは収束レンズで照射面に焦点を結ぶが、そのスポット位置はそれらのビームの径と同等またはそれ以下の間隔で隔たっている。収束レンズはレーザ光源から見てビーム分岐素子の背後にあるため収束レンズの焦点距離を変えるとビーム径及びスポット間隔が同じ割合で変化する。変換レンズはビーム分岐素子よりもレーザ光源側にあるために、変換レンズの焦点距離や拡大率を変化させるとスポットの間隔はあまり変化せずにビーム径のみが大きく変化する。したがって、変換レンズを変更することによりビームの重なり具合いを変化させることができ、照射領域内の強度の均一度を変更することができる。

〔実施例〕

本発明の実施例を図面を用いて説明する。

第1図は本発明の第1実施例の細胞分析装置の主要部分の構成を示す図である。レーザ光源15から出射するレーザビーム3の光軸の延長上に変換レンズ系4、1／2波長板7、ウォラストンプリズム8、収束レンズ2a、2b、フローセル1、ビームトラップ11が順次配置されている。フローセル1からレーザビーム3の進行方向には、集光レンズ6、ダイクロイックミラー21a、21bが順次設置され、そのダイクロイックミラー21a，21bの透過光路上及び2つの反射光路上に、それぞれフィルタ22（22a，22b，22c）、結像レンズ23（23a，23b，23

ｃ）、スリット２４（２４ａ，２４ｂ，２４ｃ）、光検出器１０（１０ａ，１０ｂ，１０ｃ）からなる検出系が３組配置されている。フローセル１にはシース液供給器１９とサンプル液供給器２０からそれぞれシース液と被検粒子を懸濁したサンプル液が送られる。光検出器１０ａ，１０ｂ，１０ｃには分析器１７が接続され分析器１７には表示器１８が接続されている。また、制御器１６が分析器１７およびシース液供給器１９、サンプル液供給器２０に接続されている。

フローセル１の中を、細胞を特異的に染色する蛍光染料で染色した細胞の懸濁液をシースフローを形成して流し、フローセル１中の観測点５においてレーザビーム３で照射すると細胞が光を発散する。細胞から発散する光には、蛍光、散乱光などが含まれるが、それらの強度は細胞の種類、性質によって変化する。それらの光は集光レンズ６で平行光線に変換された後、ダイックロイックミラー２１ａ，２１ｂで各検出系に導かれる。ダイックロイックミラー２１ａは、波長の長い蛍光を反射し、それ以外の光を透過する。ダイックロイックミラー２１ｂは、波長の短い蛍光を反射し、散乱光を透過する。フィルタ２２ａは、レーザビーム３と同じ波長の散乱光のみを透過する狭帯域フィルタである。結像レンズ２３ａは観測点５から発散される散乱光の像をスリット２４ａの面上に結ぶ。スリット２４ａは、観測点５から発する散乱光の結像領域よりも大きな矩形スリットであり、観測点５から出た散乱光は制限せずに光検出器１０ａの検出面上に透過し、他の迷光成分は除外される。こうして光検出器１０ａでは細胞から発散される散乱光のみが検出される。同様に、光検出器１０ｂ及び光検出器１０ｃでは、それぞれ細胞から発散される緑色蛍光、及び、赤色蛍光のみが検出される。ここで、フィルタ２２ｂ及び２２ｃは、それぞれ緑色蛍光及び赤色蛍光のみを透過する狭帯域フィルタである。個々の細胞からの散乱光、緑色蛍光、赤色蛍光の分析をすることにより血液サンプル中に含まれる種々の細胞の個数や比率を得ることができる。

第１実施例において変換レンズ系４はズームレンズ系を構成しており、レンズ間隔を変えることにより焦点距離を変更可能である。１／２波長板はレーザビーム３の偏光面を４５度回転する方向に結晶軸を向けて取り付けてあり、さらに結晶軸方向が回転可能になっている。ウォラストンプリズム８はフローセル１の流路方向に直交する面内で光線を分岐する向きに取り付けてある。収束レンズ系２ａ及び２ｂは光軸方向に移動可能に取り付けられた焦点距離の異なる２枚のシリンドリカルレンズであり２ａは懸濁液の流れに直交する方向、２ｂはその流れに平行な方向にレーザビームを収束させる。レーザ光源１５を出射したレーザビーム３は小さな角度で広がっている。変換レンズ系４により広がり角度を一旦変更された後、収束レンズ２によってビーム径が縮小され、フローセル１中の観測点５でレーザビーム３のビーム径は最も小さくなる。１／２波長板７、及びウォラストンプリズム８は両面が平行なためにビーム径に与える影響は小さい。また、レーザビーム３の偏光面はレーザ光源１５から出射した時点で水平方向であるが、１／２波長板を通過する際に垂直から４５度方向に偏光面が回転する。ウォラストンプリズム８は、レーザビーム３を水平な偏光成分と垂直な偏光成分とに分岐する。その分岐角度θdはプリズムの形状で決まっている。２つに分岐したレーザビームは収束レンズ系２で観測点５に収束されるが、収束レンズ系を構成している２枚のシリンドリカルレンズの焦点距離が違うために収束したビームは、懸濁流の流れに平行な方向より垂直な方向の方が径が小さい楕円ビームとなっている。レーザビームは２つに分岐しているために２つの楕円ビームとして収束する。

観測点５における２つのレーザビームの流れに垂直な面内のビーム径をｄ、間隔をＨとすると強度分布は第２図のようにほぼ平坦になる。図で個々のレーザビームの強度分布を破線、合成された強度分布を実線で示してある。第２図は、Ｈ／ｄが０．５３０の場合であるが、計算によると、Ｈ／ｄが０．５以上になると強度分布の中心に凹み

をもち、その両側に最大値がある形状になる。このような場合に最大値をとる点の外側まで含めた領域内の照射の均一度は中心上の強度と最大の強度の比以下になる。第４図はビーム間隔と光強度均一度の関係を示す図で、Ｈ／ｄを変化させた場合の中心上の強度と最大強度の比を計算した結果である。ビーム間隔とビーム径の比Ｈ／ｄを変えると強度比が大きく変化するので、必要な照射均一度に合わせてビーム間隔とビーム径の比を精度よく設定する必要がある。また、このような場合に最も効率よくレーザビームのエネルギを利用するには、強度が最大となる点の外側で、中心の凹みと同じ強度となる点までの領域を利用することである。その領域は第２図のＳで示した。レーザビームの持つエネルギの内、領域Ｓに含まれる割合がエネルギ効率Ｅである。第５図はこのように選んだ領域Ｓに対するエネルギ効率Ｅと均一度Ｕの関係を計算した結果である。均一度Ｕを１に近付けるとエネルギ効率Ｅが急激に小さくなるので均一度Ｕを必要以上に高くするとレーザビームのエネルギ密度を確保するためにはレーザ光源１５の出力を大きくする必要がある。したがって用途に合わせて必要な均一度Ｅ及び、照射領域の幅Ｓを定めて、それに合わせて、ビーム間隔Ｈとビーム径ｄを設定する必要がある。

第１図の実施例の場合はビーム間隔Ｈは、収束レンズ系２ａの焦点距離ｆａとウォラストンプリズム８の分岐角度 θd を用いて

$$H = fa \cdot \theta d$$

で近似できる。またビーム径ｄは収束レンズ２ａに入射するレーザビーム径Ｄとレーザの波長λを用いて、

$$d = (4/\pi) \cdot fa \cdot \lambda / D$$

で近似できる。変換レンズ系４はズームレンズを構成しているので、レンズ間の間隔を変えることによりレーザビーム径Ｄを無段階に変更できる。収束レンズ２ａは容易に交換可能なので、焦点距離ｆａを自由に選べる。したがって変換レンズ系４と収束レンズ２ａの焦点距離を個別に変化させることにより、観測点５におけるレーザビームの照射均一度Ｅと照射領域の幅Ｓを個別に変更することができる。そのため照射領域の幅Ｓをフローセル１の中をサンプル液が流れる太さに合わせることができ、無駄な部分をレーザ光が照射することを防げるので、出力の小さなレーザ光源１を用いることができ、小形で低価格の粒子分析装置を構成することができる。またレーザ光源を別のものと交換して元々のレーザビームの径が変わっても調整することができる。また、照射領域内の均一度を調整できるので分析の精度を向上できる。また、照射領域以外の部分を照射する割合が小さいので、細胞粒子以外からの散乱光などの迷光が光検出器１０に入射して分析精度を下げることを避けられる。また、レーザビームの強度が小さくてすむ上に狭い領域しか照射しないので、レンズやミラーなどの光学部品に与える損傷が小さい。

また、本実施例の場合は、収束レンズ系が２枚のシリンドリカルレンズで構成されているため観測点５におけるビーム径を流れに平行な方向と垂直な方向に対して別々に設定できる。そのため、流れに垂直な方向のビーム径を小さくしておけば、観測点を粒子が通過する時間が短くなり、分析時間を短縮できる。また観測点内を同時に複数の粒子が通過することによる分析誤差を防ぐことができる。

また、変換レンズの間隔を変えた場合には収束レンズ２ａ、２ｂによるビームの収束位置が変化するが収束レンズ２ａ、２ｂは光軸方向に移動可能なので、収束位置を容易に観測点５に一致させることができる。

また、ウォラストンプリズム８で分岐されるレーザビームは偏光成分によって分けられているため、それぞれの成分の強度が異なると観測点５における照射強度分布が対称でなくなり、照射の均一度が悪化する。しかし、本実施例の場合は、１／２波長板７を回転させることによりそれぞれの成分の強度を一致させることができる。この場合に、ウォラストンプリズム８の後方に光検出器をおき、偏光子を用いて垂直偏光成分と水平偏光成分の強度測定し、それらが一致するように調整

するのみで照射強度分布が対称になるので、調整に特殊な測定器が要らないという利点がある。

なお、この実施例において、被検粒子を特定の一つに限定するような場合には、変換レンズ系の拡大率を一定にするため、各レンズを固定する手段を設けておくのがよい。

第６図は本発明の第２実施例のビーム整形光学系の構成を説明する図である。この場合は３つのスライダ２６、２７、２８が配置されている。スライダ２６は３つブロックで構成されそれぞれのブロックには変換レンズ系４が固定されており、スライダ２６を移動することで１組の変換レンズ系がレーザビーム３の光路上にくる。それぞれの変換レンズ系は拡大率の異なるビーム拡大光学系をサブ系として構成しており、出射するビームは平行ビームとなるように調整されている。スライダ２７はビーム分岐素子系のサブ系としての３つのブロックで構成され、そのうち２つには１／２波長板７と分岐角度 θd の異なるウォラストンプリズム８が固定されている。スライダ２７を移動することで、レーザビーム３の光路上に３つのうち１つのブロックが選ばれる。スライダ２８も収束レンズ系のサブ系として３つのブロックで構成されており、それぞれには収束レンズ系が固定されている。そのうち２組は、シリンドリカルレンズ２ａ，２ｂで構成されており、もう１組は球面レンズ２ｃで構成されている。スライダ２８を移動することで、どれか１組の収束レンズ系が選ばれる。スライダ２６、２７、２８はそれぞれ独立に動かすことができ、光軸に対して十分な位置の再現性がある。

この実施例の場合は、スライダを動かすことで簡単に観測点５における照射領域の幅と均一度を変更できる。また、変換レンズ系４を出射したレーザビームが平行ビームになっているため、光路上のウォラストンプルズムなどが変わってもレーザビームの収束位置が変化しないので、３つのスライダをどの様な組合せで用いてもビーム収束位置の補正が不要であるという利点がある。また粒子分析の対象によって最適な照射強度分布を得るように、スライダを自動的に動かす手段を設けることも可能である。またスライダ式の代りにレボルバの如く回転式にすることも可能である。

第７図は本発明の第３実施例のビーム整形光学系の構成を示す図である。第１実施例と異なるのは、１／２波長板とウォラストンプリズムを用いる代わりに、分割プリズム９を用いている点である。分割プリズム９は山形をしているので、稜線の上下でレーザビームが分岐角度 θd をもって分割され、収束レンズ２ａによって観測点５において間隔Ｈだけ隔てて収束する。この場合の観測点５における照射強度分布は第８図のようになる。個々のビーム第８図の破線で示したように、ガウス分布を中心で切断した形状をしている。それを重ね合わせると実線のように中心部が平坦な分布になる。照射領域の幅と均一度を調整する必要があることは第１実施例と同じであり、それは変換レンズ系４と収束レンズ系２ａ、２ｂを変えることで可能である。この実施例の場合は、分割したレーザビームの偏光面が一致しているため、粒子分析に偏光面の方向が影響を与えることを防ぐことができる。また、偏光面を回転させる１／２波長板が不要なため小型化、低価格化が可能である。また、レーザビームを半分に切断して用いるため、照射領域の外を照射する割合が小さく、エネルギ効率が高くなるという利点がある。

〔発明の効果〕

本発明によれば、粒子分析装置のレーザビーム上のビーム分岐素子の手前に変換レンズ系、ビーム分岐素子の背後に収束レンズ系を備えているため、必要な幅と均一度に合わせてレーザビームの照射領域の幅と均一度を調整でき、分析精度の高い粒子分析装置を提供することができる。

４．図面の簡単な説明

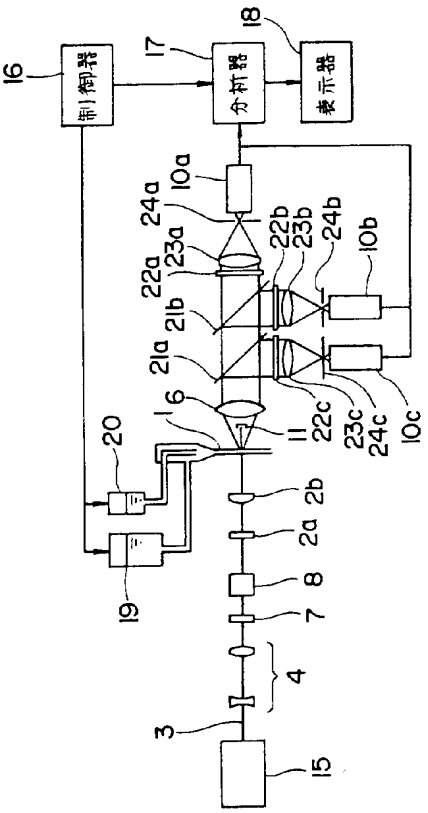
第１図は本発明の第１実施例を示す粒子分析装置の構成図、第２図～第５図は第１実施例の作用を説明する図、第６図は本発明の第２実施例の主要部分の構成図、第７図は第３実施例の主要部分の構成図、第８図は第３実施例の作用を説明する図、第９図は従来の光学系の作用を説明するため

の図。

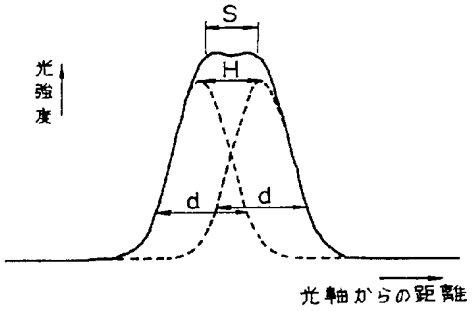
1…フローセル　　2…収束レンズ系
3…レーザビーム　　4…変換レンズ系
5…観測点　　6…集光レンズ
7…1／2波長板
8…ウォラストンプリズム
9…分割プリズム　　10…光検出器
15…レーザ光源　　19…シース液供給器
20…サンプル液供給器

代理人　鵜沼辰之

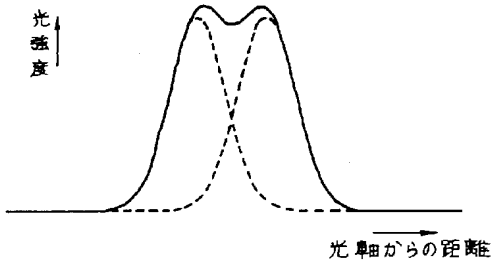
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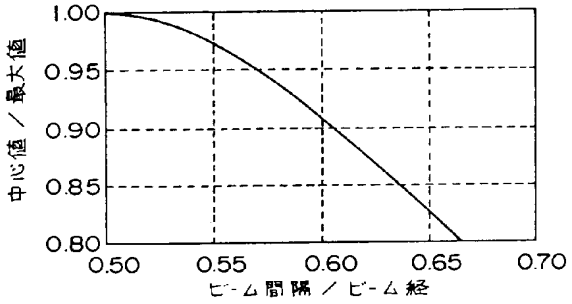
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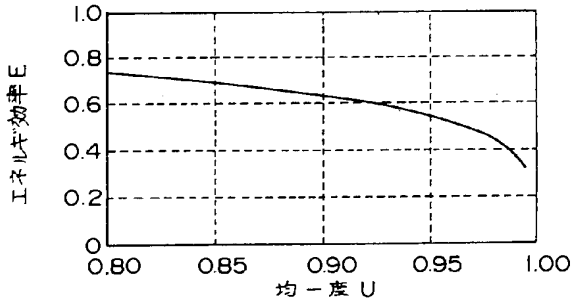
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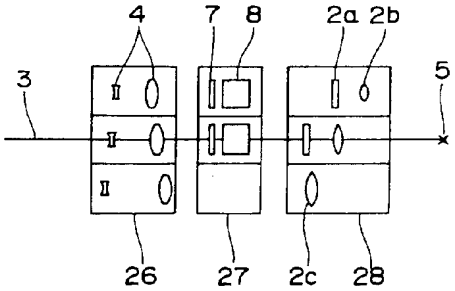
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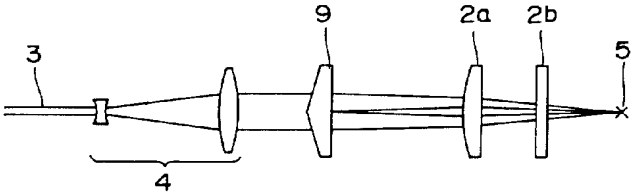
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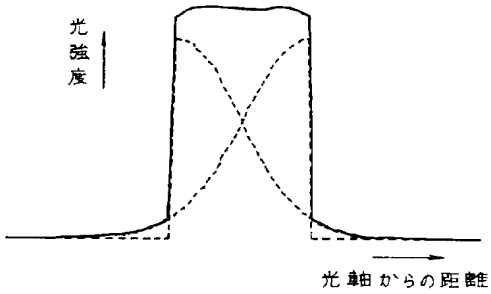
第 6 図



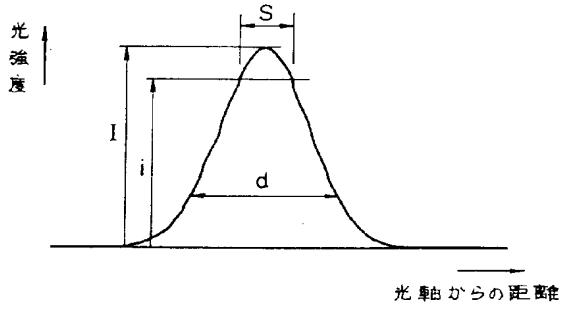
第 7 図



第 8 図



第 9 図



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Bibliographic data: JP2004341204 (A) — 2004-12-02

FLUORESCENT MICROSCOPE

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Applicant(s): OLYMPUS CORP ± (OLYMPUS CORP)

Classification: **- international:** ***G01N21/64; G02B21/06; G02B21/16;***
(IPC1-7): G01N21/64; G02B21/06; G02B21/16
- cooperative:

Application number: JP20030137483 20030515 Global Dossier

Priority number (s): JP20030137483 20030515

Abstract of JP2004341204 (A)

PROBLEM TO BE SOLVED: To provide a fluorescent microscope in which much fluorescent light reaches an optical detector which detects fluorescent light although the quantity of an undesired stimulating light reaching the optical detector is suppressed. ;SOLUTION: A scanning type fluorescent microscope has a light source part 1 which emits a light beam including stimulating light, a stimulating light irradiation optical system which irradiates a sample S with the light beam from the light source part 1, a separating optical element 5 which separates a fluorescent light generated by the sample S from the light beam traveling from the light source part 1 to the sample S, a light beam splitting part 15 which splits the light beam including the fluorescent light separated by the separating optical element 5 into two light beams, two fluorescent light selective transmission parts 18 and 23 which select fluorescent lights in specified wavelengths from the two split light beams respectively and transmit them, and two optical detectors 19 and 24 which detects the fluorescent lights transmitted through the fluorescent selective transmission parts 18 and 23 respectively. Further, the microscope has two stimulating light blocking optical elements 13 and 14 which are disposed between the separating optical element 5 and light beam splitting pat 15 and selectively block stimulating lights. ;COPYRIGHT: (C)2005,JPO&NCIPI

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G02B 21/06	G02B 21/06		

審査請求 未請求　請求項の数 7　ＯＬ　（全 14 頁）

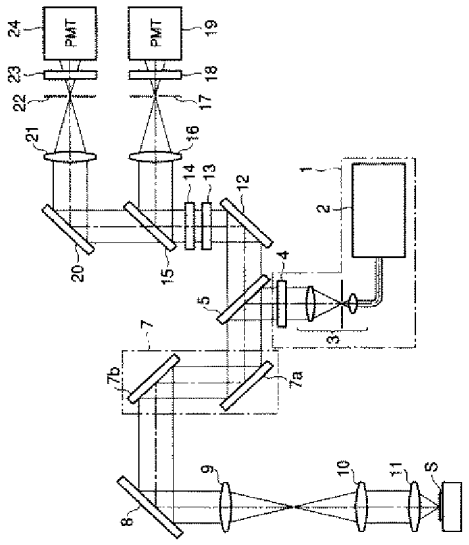
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最終頁に続く

(54)【発明の名称】蛍光顕微鏡

(57)【要約】
【課題】蛍光を検出する光検出器に到達する不所望な励起光の量を抑えつつも多く量の蛍光が光検出器に到達する蛍光顕微鏡を提供する。
【解決手段】走査型蛍光顕微鏡は、励起光を含む光ビームを射出する光源部１と、光源部１からの光ビームを試料Ｓに照射する励起光照射光学系と、試料Ｓから発生した蛍光を含む光ビームを光源部１から試料Ｓに向かう光ビームから分離する分離光学素子５と、分離光学素子５で分離された蛍光を含む光ビームを二本に分割する光ビーム分割部１５と、分割された二本の光ビームからそれぞれ特定の波長範囲内の蛍光を選択して透過する二つの蛍光選択透過部１８と２３と、蛍光選択透過部１８と２３を透過した蛍光をそれぞれ検出する二つの光検出器１９と２４と、分離光学素子５と光ビーム分割部１５の間に位置し、それぞれの励起光を選択的に遮断する二つの励起光遮断光学素子１３と１４とを有している。
【選択図】　図１



【特許請求の範囲】
【請求項1】
光ビームを試料に照射し試料から発する蛍光に基づいて試料を観察する蛍光顕微鏡であって、
少なくとも一種類の単一波長の励起光を含む光ビームを射出する光源部と、
光源部から射出された光ビームを試料に照射して試料から少なくとも一種類の蛍光を発生させる励起光照射光学系と、
試料から発生した少なくとも一種類の蛍光を含む光ビームを、光源部から試料に向かう光ビームから分離する分離光学素子と、
分離光学素子で分離された少なくとも一種類の蛍光を含む光ビームから特定の波長範囲内の蛍光を選択して透過する少なくとも一つの蛍光選択透過部を含む蛍光選択透過機構と、
蛍光選択透過機構を透過した蛍光を検出する少なくとも一つの光検出器を含む蛍光検出部と、
分離光学素子と蛍光選択透過部の間の光路上に位置し、光源部から射出された光ビームに含まれる少なくとも一種類の励起光を選択的に遮断する少なくとも一つの励起光遮断光学素子を含む励起光遮断部とを有している、蛍光顕微鏡。
【請求項2】
請求項１において、
光源部は、複数種類の単一波長の励起光を含む光ビームを射出し、
励起光照射光学系は、複数種類の単一波長の励起光を含む光ビームを試料に照射して試料から複数種類の蛍光を発生させ、
分離光学素子は、試料から発生した複数種類の蛍光を含む光ビームを光源部から試料に向かう光ビームから分離し、
蛍光顕微鏡は更に、分離光学素子で分離された複数種類の蛍光を含む光ビームを、複数種類の蛍光の波長帯域に基づいて複数に分割する光ビーム分割部を有しており、
蛍光選択透過機構は、光ビーム分割部で分割された複数の光ビームから、それぞれ、特定の波長範囲内の蛍光を選択して透過する複数の蛍光選択透過部を有しており、
蛍光検出部は、複数の蛍光選択透過部を透過した蛍光をそれぞれ検出する複数の光検出器を有しており、
励起光遮断部は、光源部から射出された光ビームに含まれる複数種類の励起光をそれぞれ選択的に遮断する複数の励起光遮断光学素子を有している、蛍光顕微鏡。
【請求項3】
請求項１または請求項２において、励起光遮断光学素子はホログラフィックノッチフィルターである、蛍光顕微鏡。
【請求項4】
請求項１ないし請求項３のいずれかひとつにおいて、励起光照射光学系は、励起光を含む光ビームを試料に収束させる対物レンズと、対物レンズにより試料に形成される励起光を含む光ビームのスポットを走査する走査光学系とを含んでいる、蛍光顕微鏡。
【請求項5】
請求項１ないし請求項４のいずれかひとつにおいて、蛍光選択透過部は、特定の波長範囲から外れた波長の光を吸収する吸収フィルターである、蛍光顕微鏡。
【請求項6】
請求項１ないし請求項４のいずれかひとつにおいて、蛍光選択透過部は、入射する光ビームを波長分散させる分散素子と、分散素子により分散された光ビームを収束させる収束レンズと、収束レンズにより収束された光ビームの一部の波長域を通す光学的開口とを有し、収束レンズは入射した光ビームを波長成分に応じて異なる位置に収束させ、光学的開口は特定の波長成分の光を選択的に透過する、蛍光顕微鏡。
【請求項7】
請求項６において、蛍光選択透過部は光学的開口に対する波長成分の収束位置を決め、分散素子の回転角度を変更可能とし、蛍光顕微鏡は、分散素子の回転角度を変えることによ

り、光学的開口を透過する光ビームの波長域を可変して、蛍光を細分化した波長帯域毎に検出し得る、蛍光顕微鏡。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】

本発明は、蛍光顕微鏡に関する。

【０００２】

【従来の技術】

蛍光顕微鏡は、一般に、染色された試料に、少なくとも一種類の波長の励起光を含む光ビームを照射して試料を励起し、試料から発生した少なくとも一種類の蛍光を、少なくとも一つの検出器をで検出し、検出された蛍光に基づいて試料の画像（蛍光画像）を取得する。

【０００３】

このような蛍光顕微鏡は、例えば特開平１１－２３１２２２号公報に開示されている。

【０００４】

【特許文献１】

特開平１１－２３１２２２号公報

【０００５】

【発明が解決しようとする課題】

蛍光顕微鏡においては、一般に、波長により分光し得るダイクロイックミラーを用いて、試料に照射される励起光から、試料から発生した蛍光が分離される。通常、試料からダイクロイックミラーに到達する光は、試料から発生した蛍光だけでなく、試料で反射した励起光も含まれている。

【０００６】

一般に、試料を励起する励起光と、試料から発生する蛍光は、比較的近い波長を有している。励起光と蛍光の分離のためには、ダイクロイックミラーは、特定の波長を境に二値的に変化する透過率特性を有していることが理想的であるが、実際の透過率特性は、比較的急峻に変化していても、その部分はある程度の幅を有している。また、その部分の両端における透過率も０と１ではない。このため、ダイクロイックミラーで分離された光には、蛍光だけでなく、試料で反射された励起光も含まれる。

【０００７】

また、蛍光を検出する光検出器の手前には、検出対象の蛍光だけを検出するために、検出対象の蛍光に合った波長帯域の光を選択的に透過するフィルター等の光学要素が配置される。前述したように、ダイクロイックミラーで分離された光には不所望な励起光も含まれるため、このようなフィルター等の光学要素は、単に検出対象の蛍光を透過するだけでなく、不所望な励起光を確実に遮断する機能も要求される。

【０００８】

通常、不所望な励起光を遮断することを優先すると、それに伴って光検出器に到達する検出対象の蛍光の量も減ってしまい、反対に、検出対象の蛍光を多く透過することを優先すると、光検出器に到達する不所望な励起光も増えてしまう。

【０００９】

このような事情は、複数種類の励起光を含む光ビームを試料に照射して複数種類の蛍光を発生させる場合、更に顕著である。

【００１０】

本発明は、このような実状を考慮して成されたものであり、その目的は、蛍光を検出する光検出器に到達する不所望な励起光の量を抑えつつ、多く量の蛍光が光検出器に到達する蛍光顕微鏡を提供することである。

【００１１】

【課題を解決するための手段】

本発明は、以下の各項に列記する蛍光顕微鏡を含んでいる。

【００１２】
１．本発明の蛍光顕微鏡は、少なくとも一種類の単一波長の励起光を含む光ビームを射出する光源部と、光源部から射出された光ビームを試料に照射して試料から少なくとも一種類の蛍光を発生させる励起光照射光学系と、試料から発生した少なくとも一種類の蛍光を含む光ビームを、光源部から試料に向かう光ビームから分離する分離光学素子と、分離光学素子で分離された少なくとも一種類の蛍光を含む光ビームから特定の波長範囲内の蛍光を選択して透過する少なくとも一つの蛍光選択透過部を含む蛍光選択透過機構と、蛍光選択透過機構を透過した蛍光を検出する少なくとも一つの光検出器を含む蛍光検出部と、分離光学素子と蛍光選択透過部の間の光路上に位置し、光源部から射出された光ビームに含まれる少なくとも一種類の励起光を選択的に遮断する少なくとも一つの励起光遮断光学素子を含む励起光遮断部とを有している。
【００１３】
この蛍光顕微鏡においては、不所望な励起光は、それ専用の励起光遮断光学素子によって、良好に遮断される。これにより、蛍光選択透過部に要求される光学的特性が緩和される。このため、蛍光選択透過部は、比較的広い波長範囲の光を透過してもよい。その結果、光検出器に、多くの量の蛍光が到達する。
【００１４】
２．本発明の別の蛍光顕微鏡は、第１項の蛍光顕微鏡において、光源部は、複数種類の単一波長の励起光を含む光ビームを射出し、励起光照射光学系は、複数種類の単一波長の励起光を含む光ビームを試料に照射して試料から複数種類の蛍光を発生させ、分離光学素子は、試料から発生した複数種類の蛍光を含む光ビームを光源部から試料に向かう光ビームから分離し、蛍光顕微鏡は更に、分離光学素子で分離された複数種類の蛍光を含む光ビームを、複数種類の蛍光の波長帯域に基づいて複数に分割する光ビーム分割部を有しており、蛍光選択透過機構は、光ビーム分割部で分割された複数の光ビームから、それぞれ、特定の波長範囲内の蛍光を選択して透過する複数の蛍光選択透過部を有しており、蛍光検出部は、複数の蛍光選択透過部を透過した蛍光をそれぞれ検出する複数の光検出器を有しており、励起光遮断部は、光源部から射出された光ビームに含まれる複数種類の励起光をそれぞれ選択的に遮断する複数の励起光遮断光学素子を有している。
【００１５】
この蛍光顕微鏡においては、複数種類の励起光を含む光ビームを試料に照射して複数種類の蛍光を発生させる場合においても、不所望な励起光が良好に遮断されつつも、光検出器に多くの量の蛍光が到達する。
【００１６】
３．本発明の別の蛍光顕微鏡は、第１項または第２項の蛍光顕微鏡において、励起光遮断光学素子はホログラフィックノッチフィルターである。
【００１７】
この蛍光顕微鏡は、不所望な励起光を良好に遮断する好適な励起光遮断光学素子の一態様を与える。
【００１８】
４．本発明の別の蛍光顕微鏡は、第１項ないし第３項のいずれかひとつの蛍光顕微鏡において、励起光照射光学系は、励起光を含む光ビームを試料に収束させる対物レンズと、対物レンズにより試料に形成される励起光を含む光ビームのスポットを走査する走査光学系とを含んでいる。
【００１９】
この蛍光顕微鏡は、走査型蛍光顕微鏡を提供する。
【００２０】
５．本発明の別の蛍光顕微鏡は、第１項ないし第４項のいずれかひとつの蛍光顕微鏡において、蛍光選択透過部は、特定の波長範囲から外れた波長の光を吸収する吸収フィルターである。
【００２１】

この蛍光顕微鏡は、蛍光選択透過部の一態様を与える。
【００２２】
６．本発明の別の蛍光顕微鏡は、第１項ないし第４項のいずれかひとつの蛍光顕微鏡において、蛍光選択透過部は、入射する光ビームを波長分散させる分散素子と、分散素子により分散された光ビームを収束させる収束レンズと、収束レンズにより収束された光ビームの一部の波長域を通す光学的開口とを有し、収束レンズは入射した光ビームを波長成分に応じて異なる位置に収束させ、光学的開口は特定の波長成分の光を選択的に透過する。
【００２３】
この蛍光顕微鏡は、蛍光選択透過部の別の一態様を与える。
【００２４】
７．本発明の別の蛍光顕微鏡は、第６項の蛍光顕微鏡において、蛍光選択透過部は光学的開口に対する波長成分の収束位置を決め、分散素子の回転角度を変更可能とし、蛍光顕微鏡は、分散素子の回転角度を変えることにより、光学的開口を透過する光ビームの波長域を可変して、蛍光を細分化した波長帯域毎に検出し得る。
【００２５】
この蛍光顕微鏡は、検出対象の蛍光のスペクトルデータを取得可能である。
【００２６】
【発明の実施の形態】
以下、図面を参照しながら本発明の実施の形態について説明する。
【００２７】
第一実施形態
本実施形態は、走査型蛍光顕微鏡に向けられている。以下、図１と図３～図８を参照しながら本実施形態について説明する。
【００２８】
図１に示されるように、本実施形態の走査型蛍光顕微鏡は、少なくとも一種類の単一波長の励起光を含む光ビームを射出する光源部１を有している。光源部１は、これに限定されないが、例えば、複数の単一波長の光を含む光ビームを発するレーザ光源２と、レーザ光源２から発せられた光ビームの径を拡大するビームエキスパンダ３と、試料Ｓの励起に必要な特定の波長の光を選択的に透過するレーザーラインフィルター４とを有している。
【００２９】
例えば、レーザ光源２は、４８８ｎｍと５６８ｎｍと６４７ｎｍの波長の光を同時発振するクリプトンアルゴンレーザであり、レーザーラインフィルター４は、レーザ光源２から発せられた光のうち、４８８ｎｍと５６８ｎｍの波長の光を選択的に透過する。
【００３０】
従って、光源部１は、二種類の単一波長の励起光を含む光ビームを射出する。しかし、光源部１は、これに限定されるものではなく、一種類の単一波長の励起光から成る光ビームを射出してもよく、あるいは、三種類以上の単一波長の励起光を含む光ビームを射出してもよい。
【００３１】
図１において、走査型蛍光顕微鏡は更に、光源部１から射出された二種類の励起光を含む光ビームを試料Ｓに照射する励起光照射光学系を有している。励起光照射光学系は、励起光を含む光ビームを試料Ｓに収束させる対物レンズ１１と、励起光を含む光ビームを対物レンズ１１に向けて偏向するミラー８と、ミラー８から対物レンズ１１に至る間の光路上に配置された瞳投影レンズ９と結像レンズ１０とを含んでいる。
【００３２】
励起光照射光学系は更に、対物レンズ１１により試料Ｓに形成される励起光を含む光ビームのスポットを二次元的に走査する走査光学系７を有している。走査光学系７は、光源部１とミラー８の間の光路上に配置された、ガルバノメータスキャナ７ａとガルバノメータスキャナ７ｂとで構成されている。
【００３３】

二つのガルバノメータスキャナ７ａと７ｂは、それぞれ、方向を変更可能な反射面を有しており、反射面の方向を変更することにより、反射面で反射された光ビームを一方向に沿って（つまり一次元的に）走査する。二つのガルバノメータスキャナ７ａと７ｂは、その反射面で反射された光ビームを互いに直交する方向（Ｘ方向とＹ方向）に走査する。このため、走査光学系７により、試料Ｓに形成される光ビームのスポットは、二次元的に走査される。

【００３４】

試料Ｓは光ビームの照射に反応して蛍光を発する。試料Ｓは、照射された光ビームに含まれる励起光の種類に対応した種類の蛍光を発する。本実施形態では、試料Ｓは二種類の試薬で染色されており、試料Ｓには二種類の励起光を含む光ビームが照射される。このため、試料Ｓからは二種類の蛍光が発生する。試料Ｓから発生する二種類の蛍光は、それぞれ、試料Ｓに照射される二種類の励起光の波長帯域に対応した波長帯域を有する。

【００３５】

走査型蛍光顕微鏡は更に、試料Ｓから発生した蛍光を含む光ビームを、光源部１から試料Ｓに向かう光ビームから分離する分離光学素子５を有している。簡単に言えば、分離光学素子５は励起光と蛍光とを分離する。分離光学素子５は、例えば、光源部１と試料Ｓの間の光路上に位置するダイクロイックミラーで構成される。

【００３６】

図１において、走査型蛍光顕微鏡は更に、分離光学素子５で分離された少なくとも一種類の蛍光を含む光ビームから特定の波長範囲内の蛍光を選択して透過する少なくとも一つの蛍光選択透過部を含む蛍光選択透過機構と、蛍光選択透過機構を透過した蛍光を検出する少なくとも一つの光検出器を含む蛍光検出部とを有している。

【００３７】

より詳しくは、試料Ｓからは二種類の蛍光が発生されることに対応して、走査型蛍光顕微鏡は更に、分離光学素子５で分離された蛍光を含む光ビームを、それに含まれる二種類の蛍光の波長帯域に基づいて二本に分割する光ビーム分割部１５を有している。蛍光選択透過機構は、光ビーム分割部１５で分割された二本の光ビームから、それぞれ、特定の波長範囲内の蛍光を選択して透過する二つの蛍光選択透過部１８と２３を有している。蛍光検出部は、二つの蛍光選択透過部１８と２３を透過した蛍光をそれぞれ検出する二つの光検出器１９と２４を有している。

【００３８】

光ビーム分割部１５は、これに限定されないが、例えば、ダイクロイックミラーで構成される。二つの蛍光選択透過部１８と２３は、それぞれ、これに限定されないが、例えば、特定の波長範囲から外れた波長の光を吸収する吸収フィルターで構成される。二つの光検出器１９と２４は、それぞれ、これに限定されないが、例えば、フォトマルチプライヤ（ＰＭＴ）で構成される。

【００３９】

走査型蛍光顕微鏡は更に、分離光学素子５で分離された蛍光を含む光ビームを光ビーム分割部１５に向けて偏向するミラー１２と、光ビーム分割部１５を透過した光ビームを光検出器２４に向けて偏向するミラー２０とを有している。

【００４０】

走査型蛍光顕微鏡は更に、光ビーム分割部１５と蛍光選択透過部１８の間の光路上に配置された集光レンズ１６およびピンホール１７と、ミラー２０と蛍光選択透過部２３の間の光路上に配置された集光レンズ２１およびピンホール２２とを有している。

【００４１】

ピンホール１７とピンホール２２は、それぞれ、対物レンズ１１により試料Ｓに形成される励起光を含む光ビームのスポットに対して共焦点の位置関係に配置されている。このため、ピンホール１７とピンホール２２は、共焦点効果により、試料Ｓに形成される光ビームのスポットから外れた位置、特に光軸に沿って外れた位置から発生された蛍光を効果的に遮断する。このため、光検出器１９と２４は、試料Ｓに形成される光ビームのスポット

の位置からの蛍光を選択的に検出し得る。

【００４２】

走査型蛍光顕微鏡は更に、光源部１から射出される光ビームに含まれる二種類の励起光をそれぞれ選択的に遮断する励起光遮断光学素子１３と励起光遮断光学素子１４とを有している。二つの励起光遮断光学素子１３と１４は共に分離光学素子５と光ビーム分割部１５の間の光路上に配置されている。二つの励起光遮断光学素子１３と１４は、好ましくは、それぞれ、ホログラフィックノッチフィルターで構成される。

【００４３】

走査型蛍光顕微鏡においては、光源部１から発せられた励起光を含む光ビームは対物レンズ１１により試料Ｓに収束される。対物レンズ１１により試料Ｓに形成された光ビームのスポットは、走査光学系７により試料Ｓの表面上あるいは内部を二次元的に走査される。試料Ｓに照射される光ビームは二種類の励起光を含んでおり、試料Ｓはそれに対応した二種類の試薬で染色されているため、試料Ｓからは二種類の蛍光が発生する。

【００４４】

試料Ｓから発生した二種類の蛍光は、それぞれ、光検出器１９と光検出器２４により検出される。特に、共焦点効果により、光ビームのスポットの位置から発生した蛍光が、光検出器１９と光検出器２４により選択的に検出される。光検出器１９と光検出器２４で得られる信号を、走査光学系７を制御する走査信号に同期させて処理することにより、それぞれの蛍光に基づく試料Ｓの蛍光画像が得られる。

【００４５】

前述したように、レーザ光源２は、４８８ｎｍと５６８ｎｍと６４７ｎｍの波長の光を発する。レーザーラインフィルター４は、図３に示される透過率特性を有しており、レーザ光源２で発せられた光のうち、４８８ｎｍと５６８ｎｍの光を選択的に透過する。従って、光源部１からは、４８８ｎｍと５６８ｎｍの励起光を含む光ビームが射出される。

【００４６】

光源部１から射出された４８８ｎｍと５６８ｎｍの励起光は試料Ｓに照射され、試料Ｓからは、４８８ｎｍの励起光に対応して、５００ｎｍ～５４０ｎｍの波長帯域の蛍光が発生され、５６８ｎｍの励起光に対応して、５８５ｎｍ～６５０ｎｍの波長帯域の蛍光が発生される。

【００４７】

励起光と蛍光とを分離する分離光学素子５を構成するダイクロイックミラーは、例えば図４に示される透過率特性を有しており、光源部１から射出される４８８ｎｍと５６８ｎｍの波長の励起光を反射する一方、試料Ｓから発生される５００～５４０の波長帯域の蛍光と５８５ｎｍ～６５０ｎｍの波長帯域の蛍光を良好に透過する。

【００４８】

図４から分かるように、分離光学素子５を構成するダイクロイックミラーは、４８８ｎｍと５６８ｎｍの波長の励起光をいくらかは透過する。このため、試料Ｓで反射された励起光のいくらかは、試料Ｓから発生された蛍光と一緒に分離光学素子５であるダイクロイックミラーを透過する。

【００４９】

励起光遮断光学素子１３を構成するホログラフィックノッチフィルターは、図５にＨＮＦ１３で示される透過率特性を有し、励起光遮断光学素子１４を構成するホログラフィックノッチフィルターは、図５にＨＮＦ１４で示される透過率特性を有している。

【００５０】

図５から分かるように、励起光遮断光学素子１３を構成するホログラフィックノッチフィルターは、４８８ｎｍの波長の近傍の帯域において、透過率がほとんど０である。従って、励起光遮断光学素子１３を構成するホログラフィックノッチフィルターは、４８８ｎｍの波長の励起光をほとんど完全に遮断する。

【００５１】

同様に、励起光遮断光学素子１４を構成するホログラフィックノッチフィルターは、５６

８ｎｍの波長の近傍の帯域において透過率がほとんど０である。従って、励起光遮断光学素子１４を構成するホログラフィックノッチフィルターは、５６８ｎｍの波長の励起光をほとんど完全に遮断する。

【００５２】

その結果、光ビーム分割部１５に到達した光ビームは、試料Ｓで反射された４８８ｎｍと５６８ｎｍの波長の励起光を殆ど含まない。つまり、光ビーム分割部１５に到達した光ビームは、実質的に、検出対象である試料Ｓから発生された５００ｎｍ～５４０ｎｍの波長帯域の蛍光と５８５ｎｍ～６５０ｎｍの波長帯域の蛍光で構成されている。

【００５３】

光ビーム分割部１５を構成するダイクロイックミラーは、例えば図６に示される透過率特性を有しており、５００ｎｍ～５４０ｎｍの波長帯域の蛍光を良好に反射する一方、５８５ｎｍ～６５０ｎｍの波長帯域の蛍光を透過する。

【００５４】

蛍光選択透過部１８を構成する吸収フィルターは、例えば図７にＢＡ１８で示される透過率特性を有し、蛍光選択透過部２３を構成する吸収フィルターは、図７にＢＡ２３で示される透過率特性を有している。

【００５５】

図７から分かるように、励起光遮断光学素子１３を構成するホログラフィックノッチフィルターの透過率特性ＨＮＦ１３と励起光遮断光学素子１４を構成するホログラフィックノッチフィルターの透過率特性ＨＮＦ１４に対応して、吸収フィルター１８と吸収フィルター２３は共に比較的広い透過帯域を有している。

【００５６】

比較例として、図１の構成から励起光遮断光学素子１３と励起光遮断光学素子１４とが省かれた構成（すなわち従来の走査型蛍光顕微鏡）において、蛍光選択透過部１８を構成する吸収フィルターに要求される透過率特性と、蛍光選択透過部２３を構成する吸収フィルターに要求される透過率特性を図８に示す。

【００５７】

図１の構成から励起光遮断光学素子１３と励起光遮断光学素子１４とが省かれた構成においては、蛍光選択透過部１８と蛍光選択透過部２３に到達した光は、検出対象の蛍光の他に、試料Ｓで反射された不所望な励起光も含んでいる。このため、蛍光選択透過部１８と蛍光選択透過部２３は、単にそれぞれの検出対象の蛍光を透過するだけでなく、不所望な励起光を良好に遮断することも要求される。

【００５８】

このため、蛍光選択透過部１８を構成する吸収フィルターの透過特性は、４８８ｎｍと５６８ｎｍの波長の励起光を良好に遮断するように、図８にＢＡ１８で示されるように、透過帯域が狭いものにならざるを得ない。同様に、蛍光選択透過部２３を構成する吸収フィルターの透過特性は、５６８ｎｍの波長の励起光を良好に遮断するように、図８にＢＡ２３で示されるように、透過帯域が狭いものものにならざるを得ない。

【００５９】

その結果、例えば、蛍光選択透過部１８は、５００ｎｍ～５４０ｎｍの透過帯域を有する吸収フィルターで構成され、蛍光選択透過部２３は、５８５ｎｍ～６５０ｎｍの透過帯域を有する吸収フィルターで構成される。図８において、「５８５ｎｍ（蛍光）」は蛍光のピーク波長を示しており、実際の蛍光はこの両側にある帯域を持って広がっている。従って、ＢＡ２３で示される特性の吸収フィルターであっても、蛍光を検出することは可能である。

【００６０】

このような透過帯域の狭い吸収フィルターは、好ましくないことに、検出対象である蛍光をも吸収してしまい、その後方に位置する光検出器１９や光検出器２４に到達する蛍光の量を減少させてしまう。

【００６１】

これに対して、本実施形態の走査型蛍光顕微鏡においては、不所望な４８８ｎｍと５６８ｎｍの波長の励起光は、分離光学素子５と光ビーム分割部１５の間の光路上に配置された二つの励起光遮断光学素子１３と１４によって遮断される。このため、蛍光選択透過部１８と蛍光選択透過部２３に到達した光は、不所望な励起光を殆ど含まない。

【００６２】

従って、蛍光選択透過部１８と蛍光選択透過部２３には、不所望な励起光を良好に遮断することは要求されない。このため、蛍光選択透過部１８と蛍光選択透過部２３に、透過帯域の広い吸収フィルターを使用することが可能になる。具体的には、本実施形態における吸収フィルター（図７）の励起光遮断レベルは、従来例における吸収フィルター（図８）の励起光遮断レベルに比べて１桁から３桁程度低くてよく、その分、蛍光の透過帯域を広げることができる。これにより、蛍光選択透過部１８と蛍光選択透過部２３の後方にそれぞれ位置する光検出器１９と光検出器２４に、より多くの量の蛍光を到達させることができる。

【００６３】

その結果、本実施形態の走査型蛍光顕微鏡は、励起光の波長に近い波長の蛍光も検出できる。これにより、例えば、より明るい蛍光画像を得ることができる。

【００６４】

第二実施形態

本実施形態は、走査型蛍光顕微鏡に向けられている。以下、図２を参照しながら本実施形態について説明する。

【００６５】

図２は、本発明の第二実施形態の走査型蛍光顕微鏡を示している。図２において、図１中の部材と同一の参照符号で指示された部材は同様の部材であり、その詳しい説明は省略する。

【００６６】

本実施形態の走査型蛍光顕微鏡は、主に蛍光選択透過機構の構成の点で、第一実施形態の走査型蛍光顕微鏡と相違している。

【００６７】

図２に示されるように、本実施形態の走査型蛍光顕微鏡は、分離光学素子５とミラー１２の間の光路上に、分離光学素子５で分離された蛍光を含む光ビームを収束させる結像レンズ２５と、結像レンズ２５の結像位置に配置された共焦点絞り２６と、共焦点絞り２６を通過し発散光ビームを平行光ビームに変えるレンズ２７とを有している。

【００６８】

共焦点絞り２６は、対物レンズ１１により試料Ｓに形成される励起光を含む光ビームのスポットに対して共焦点の位置関係に配置されている。このため、共焦点絞り２６は、共焦点効果により、試料Ｓに形成される光ビームのスポットから外れた位置、特に光軸に沿って外れた位置から発生された蛍光を効果的に遮断する。これにより、最終的に得られる蛍光画像の分解能の向上に貢献する。

【００６９】

本実施形態の走査型蛍光顕微鏡は、光ビーム分割部１５から光検出器１９に至る光路上に蛍光選択透過部２８を有し、光ビーム分割部１５からミラー２０を経て光検出器２４に至る光路上に蛍光選択透過部３２を有している。

【００７０】

別の観点から、図１に示される第一実施形態の走査型蛍光顕微鏡との相違に注目して述べれば、本実施形態の走査型蛍光顕微鏡は、分離光学素子５とミラー１２の間の光路上に結像レンズ２５と共焦点絞り２６とレンズ２７とが追加されており、第一実施形態の走査型蛍光顕微鏡の集光レンズ１６とピンホール１７と蛍光選択透過部１８が蛍光選択透過部２８に置き換えられ、集光レンズ２１とピンホール２２と蛍光選択透過部２３が蛍光選択透過部３２に置き換えられた構成をしている。

【００７１】

蛍光選択透過部２８は、入射する光ビームを波長分散させる分散素子（反射回折格子）２９と、分散素子２９により分散された光ビームを収束させる収束レンズ３０と、収束レンズ３０により収束された光ビームの一部の波長域を通す光学的開口すなわちスリット３１とを有している。

【００７２】

同様に、蛍光選択透過部３２は、入射する光ビームを波長分散させる分散素子（反射回折格子）３３と、分散素子３３により分散された光ビームを収束させる収束レンズ３４と、収束レンズ３４により収束された光ビームの一部の波長域を通す光学的開口すなわちスリット３５とを有している。

【００７３】

蛍光選択透過部２８と蛍光選択透過部３２は、例えば、グレーティングガルバノで構成される。蛍光選択透過部２８と蛍光選択透過部３２は全く同様の機能を有している。このため、以下では、代表的に、蛍光選択透過部２８の動作について述べる。

【００７４】

蛍光選択透過部２８において、分散素子２９に入射した光ビームは、波長に従って異なる方向に分散される。つまり、入射光ビームに含まれる光は、波長成分毎に異なる方向に変更される。このため、収束レンズ３０に入射した光は、光軸を横切る方向に関して、波長成分に応じて異なる位置に収束される。つまり、収束レンズ３０は入射した光ビームを波長成分に応じて異なる位置に収束させる。このため、蛍光選択透過部２８に入射した光のうち、特定の波長範囲の光だけがスリット３１を通過し得る。つまり、スリット３１は特定の波長成分の光だけを選択的に透過する。その結果、スリット３１を通過した特定の波長範囲の光だけが光検出器１９で検出される。

【００７５】

全く同様にして、蛍光選択透過部３２に入射した光ビームのうち、特定の波長範囲の光だけが、スリット３５を通過して光検出器２４で検出される。

【００７６】

本実施形態の走査型蛍光顕微鏡においては、二つの励起光遮断光学素子（好ましくはホログラフィックノッチフィルター）１３と１４によって、蛍光選択透過部２８と蛍光選択透過部３２に到達する前に、不所望な励起光が除去される。このため、蛍光選択透過部２８と蛍光選択透過部３２に入射した光は、不所望な励起光を殆ど含まない。このため、スリット３１とスリット３５は、開口の大きさを比較的大きくすることが可能である。これにより、スリット３１とスリット３５の後方にそれぞれ位置する光検出器１９と光検出器２４に、より多くの量の蛍光を到達させることができる。

【００７７】

その結果、本実施形態の走査型蛍光顕微鏡は、励起光の波長に近い波長の蛍光も検出できる。これにより、例えば、より明るい蛍光画像を得ることができる。

【００７８】

より好ましくは、蛍光選択透過部２８は分散素子２９の方向を調整可能であり、蛍光選択透過部３２は分散素子３３の方向を調整可能であるとよい。例えば、蛍光選択透過部２８において、分散素子２９の方向を変えることにより、蛍光を細分化した波長帯域毎に検出することも可能である。同様に、蛍光選択透過部３２において、分散素子３３の方向を変えることにより、蛍光を細分化した波長帯域毎に検出することも可能である。

【００７９】

つまり、本実施形態の走査型蛍光顕微鏡は、蛍光選択透過部２８と蛍光選択透過部３２をこの様に構成することにより、検出対象の蛍光のスペクトルデータを取得することも可能である。

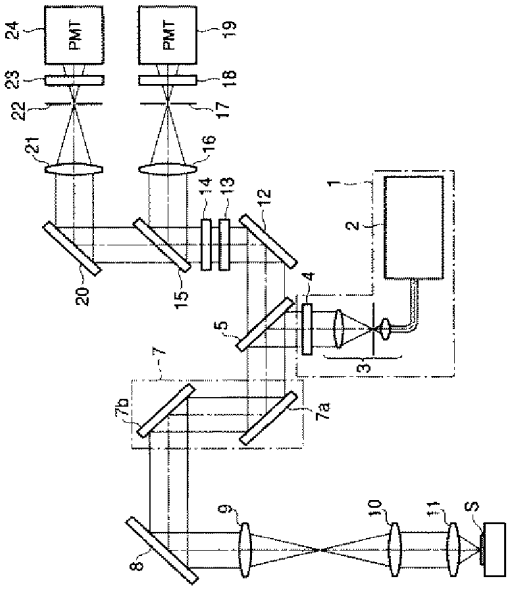
【００８０】

これまで、図面を参照しながら本発明の実施の形態を述べたが、本発明は、これらの実施の形態に限定されるものではなく、その要旨を逸脱しない範囲において様々な変形や変更が施されてもよい。

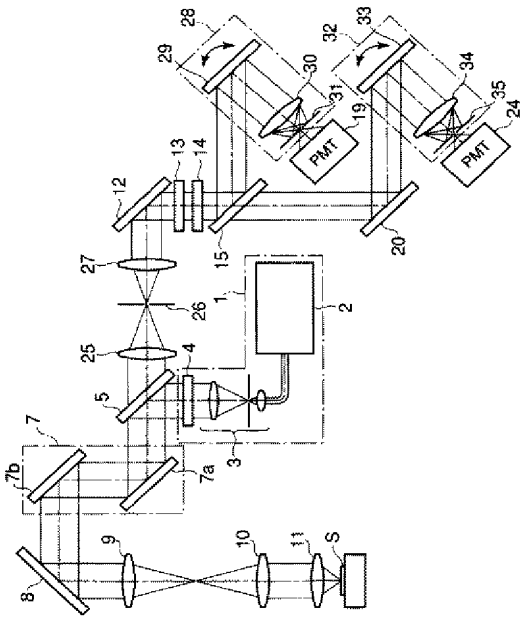
【００８１】
上述した実施形態では、光源部１は二種類の単一波長の励起光を含む光ビームを射出し、試料Ｓからは二種類の蛍光が発生されることに対応して、蛍光選択透過機構は二つの蛍光選択透過部を有し、蛍光検出部は二つの光検出器１９と２４を有し、励起光遮断部は二つの励起光遮断光学素子１３と１４を有している。
【００８２】
しかし、蛍光選択透過部と光検出器と励起光遮断光学素子の個数は、これに限定されるものではなく、光源部１から射出される光ビームに含まれる単一波長の励起光の種類、あるいは、試料Ｓから発生される蛍光の種類に対応して、適宜変更されてよい。
【００８３】
前述したように、光源部１は一種類の単一波長の励起光を含む光ビームを射出してもよい。その場合、蛍光選択透過機構は一つの蛍光選択透過部を有し、蛍光検出部は一つの光検出器を有し、励起光遮断部は一つの励起光遮断光学素子を有していればよい。
【００８４】
また、光源部１は三種類以上の単一波長の励起光を含む光ビームを射出してもよい。その場合、蛍光選択透過機構は対応する個数の蛍光選択透過部を有し、蛍光検出部は対応する個数の光検出器を有し、励起光遮断部は対応する個数の励起光遮断光学素子を有しているとよい。
【００８５】
結局、光源部１は少なくとも一種類以上の単一波長の励起光を含む光ビームを射出し、それに対応して、蛍光選択透過機構は少なくとも一つの蛍光選択透過部を有し、蛍光検出部は少なくとも一つの光検出器を有し、励起光遮断部は少なくとも一つの励起光遮断光学素子を有していればよい。
【００８６】
また、光源部１から射出される光は、レーザー光のようなほぼ単一波長の光が最も好ましいが、レーザー光でなくとも帯域の狭い光であってもよい。帯域の狭い光は、それ自体が光源から射出されるものに限らず、光源から射出された光からフィルター等の波長選択手段によって作られたものであってもよい。
【００８７】
【発明の効果】
本発明によれば、蛍光を検出する光検出器に到達する不所望な励起光の量を抑えつつ、多く量の蛍光が光検出器に到達する蛍光顕微鏡が提供される。
【図面の簡単な説明】
【図１】本発明の第一実施形態の走査型蛍光顕微鏡の構成を概略的に示している。
【図２】本発明の第二実施形態の走査型蛍光顕微鏡の構成を概略的に示している。
【図３】図１と図２に示された光源部内のレーザーラインフィルターの透過率特性を示している。
【図４】図１と図２に示された分離光学素子であるダイクロイックミラーの透過率特性を示している。
【図５】図１と図２に示された励起光遮断素子であるホログラフィックノッチフィルターの透過率特性を示している。
【図６】図１と図２に示された光ビーム分割部であるダイクロイックミラーの透過率特性を示している。
【図７】図１と図２に示された蛍光選択透過部である吸収フィルターの透過率特性を示している。
【図８】図１の構成からホログラフィックノッチフィルターが省かれた構成である従来の走査型蛍光顕微鏡において要求される吸収フィルターの透過率特性を示している。
【符号の説明】
１…光源部、２…レーザ光源、３…ビームエキスパンダ、４…レーザーラインフィルター、５…分離光学素子、７…走査光学系、７ａ…ガルバノメータスキャナ、７ｂ…ガルバノ

メータスキャナ、８…ミラー、９…瞳投影レンズ、１０…結像レンズ、１１…対物レンズ、１２…ミラー、１３…励起光遮断光学素子、１４…励起光遮断光学素子、１５…光ビーム分割部、１８…蛍光選択透過部、１９…光検出器、２３…蛍光選択透過部、２４…光検出器、２８…蛍光選択透過部、３２…蛍光選択透過部。

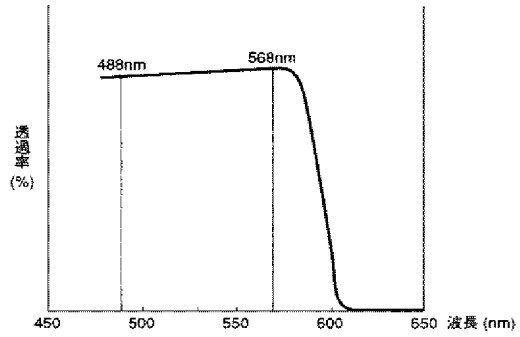
【図1】



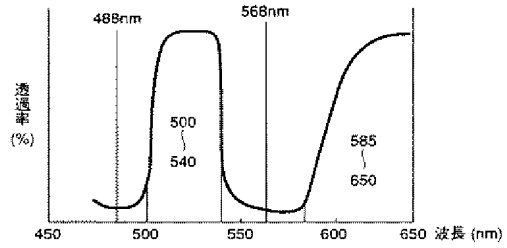
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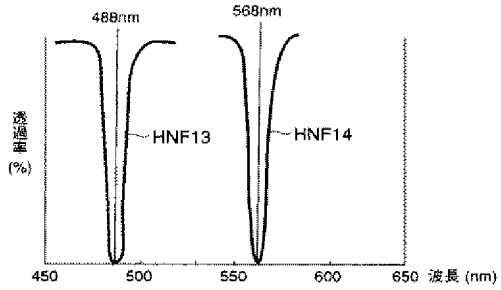
【図3】



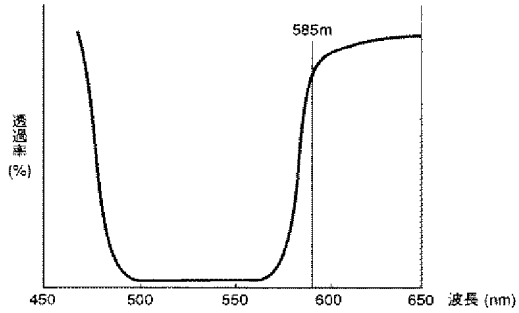
【図4】



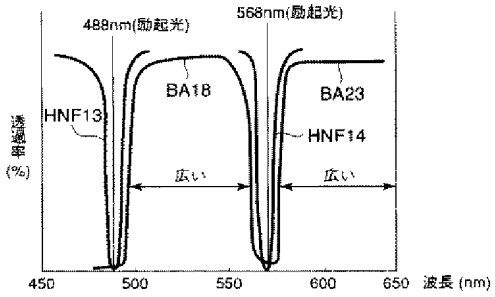
【図5】



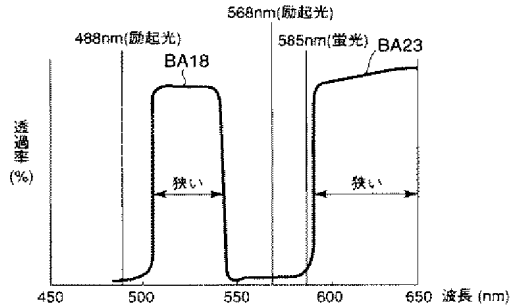
【図6】



【図7】



【図8】



Ｆターム(参考) 2G043 AA03 DA02 EA01 FA01 FA06 GA04 GB01 HA01 HA02 HA09
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FINE PARTICLE MEASUREMENT INSTRUMENT

Inventor(s): TOISHI MITSURU; SEO KATSUHIRO; TAKASAKI KOJI; YAMADA SHINJI; FUKUMOTO ATSUSHI ± (TOISHI MITSURU, ; SEO KATSUHIRO, ; TAKASAKI KOJI, ; YAMADA SHINJI, ; FUKUMOTO ATSUSHI)

Applicant(s): SONY CORP ± (SONY CORP)

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Abstract of JP2012026837 (A)

PROBLEM TO BE SOLVED: To provide a fine particle analyzer which efficiently acquires necessary light components, in particular, back-scattered light and fluorescent light. ;SOLUTION: In a fine particle measurement instrument, an optical filter divided into a plurality of areas is provided on an optical path through which light generated from a fine particle irradiated with light is guided to an optical detector, and the optical filter has a first area having a wavelength selectivity to block reflected light from the fine particle and unnecessary scattered light components but transmit fluorescent light, and a second area disposed at least around the first area and having no wavelength selectivities to transmit the necessary scattered light components. ;COPYRIGHT: (C) 2012,JPO&INPIT;PROBLEM TO BE SOLVED: To provide a fine particle analyzer which efficiently acquires necessary light components, in particular, back-scattered light and fluorescent light.SOLUTION: In a fine particle measurement instrument, an optical filter divided into a plurality of areas is provided on an optical path through which light generated from a fine particle irradiated with light is guided to an optical detector, and the optical filter has a first area having a wavelength selectivity to block reflected light from the fine particle and unnecessary scattered light components but transmit

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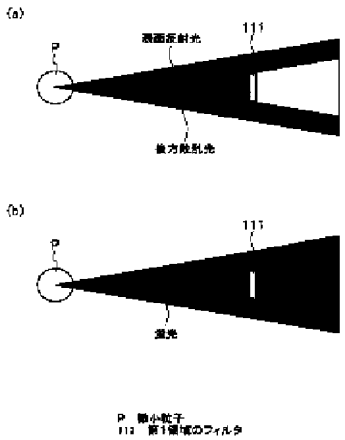
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最終頁に続く

(54)【発明の名称】微小粒子測定装置

(57)【要約】
【課題】　必要とする光成分、特に後方散乱光と蛍光を高効率に取得する微小粒子分析装置を提供すること。
【解決手段】　光を照射された微小粒子から発生する光を光検出器に導光する光学経路上に、複数の領域に分割された光学フィルタを備え、該光学フィルタは、微小粒子からの反射光と不要な散乱光成分を遮断し、蛍光を透過する波長選択性を有する第１領域と、少なくとも該領域の周囲に配され、必要な散乱光成分を透過する波長選択性のない第２領域と、を有する、微小粒子測定装置。
【選択図】図２



【特許請求の範囲】
【請求項１】
光を照射された微小粒子から発生する光を光検出器に導光する光学経路上に、複数の領域に分割された光学フィルタを備え、
該光学フィルタは、微小粒子からの反射光と不要な散乱光成分を遮断し、蛍光を透過する波長選択性を有する第１領域と、
少なくとも該領域の周囲に配され、必要な散乱光成分を透過する波長選択性のない第２領域と、を有する、微小粒子測定装置。
【請求項２】
前記波長選択性のない第２領域の貫通部分に前記波長選択性を有する第１領域を配する、請求項１記載の微小粒子測定装置。
【請求項３】
前記波長選択性を有する第１領域の外周に該第１領域を支持するフレームを備えるとともに、前記波長選択性のない第２領域が中空である、請求項１又は２記載の微小粒子測定装置。
【請求項４】
前記光学フィルタは、前記波長選択性を有する第１領域の周囲に配された波長選択性のない第２領域の周囲に、さらに波長選択性を有する第３領域を有する、請求項１～３のいずれか１項記載の微小粒子測定装置。
【請求項５】
前記光学経路上に、蛍光・後方散乱光検出器に蛍光および後方散乱光を導光する光ファイバーを備え、
前記複数の領域に分割された光学フィルタを、該光ファイバーによって前記光学フィルタを透過した光が受光されるように配置する、請求項１～４のいずれか１項記載の微小粒子測定装置。
【発明の詳細な説明】
【技術分野】
【０００１】
本発明は、微小粒子測定装置に関する。より詳しくは、微小粒子の特性を光学的に分析する微小粒子分析装置に関する。
【背景技術】
【０００２】
従来、フローセル内やマイクロチップ上に形成された流路内を通流する微小粒子に光を照射し、微小粒子からの散乱光や、微小粒子そのもの或いは微小粒子に標識された蛍光物質から発生する蛍光を検出して、微小粒子の光学特性を測定する微小粒子測定装置が用いられている。
先行技術文献１には、より高感度に計測するため、検出光受光用光ファイバを有する検体識別装置が開示されている。この装置では、検体からの後方散乱光の受光量変動を測定することにより、検体の状態（透過率、吸収等）を検出することができる。
【先行技術文献】
【特許文献】
【０００３】
【特許文献１】国際公開第２００５／１０３６４２号パンフレット
【発明の概要】
【発明が解決しようとする課題】
【０００４】
従来の微小粒子検出装置において、最適な散乱取得角度を得るためには、蛍光と散乱光を検出する光学検出系内に散乱光用電子倍増管や蛍光用電子倍増管に反射光成分が入射しないように、反射光を遮光するマスクを入れる必要がある。しかしながら、そのマスクによって蛍光などの必要な光成分も必要以上に遮断され、必要とする光成分の感度が劣化す

るという問題があった。尚、このような従来使用されていたマスクを、以下、「通常（従来）のマスク」という。

【０００５】

そこで、本発明は、必要な光成分、特に後方散乱光と蛍光をより効率的に取得する微小粒子分析装置を提供することを主な目的とする。

【課題を解決するための手段】

【０００６】

本発明は、光を照射された微小粒子から発生する光を光検出器に導光する光学経路上に、複数の領域に分割された光学フィルタを備え、該光学フィルタは、微小粒子からの反射光と不要な散乱光成分を遮断し、蛍光を透過する波長選択性を有する第１領域と、少なくとも該領域の周囲に配され、必要な散乱光成分を透過する波長選択性のない第２領域と、を有する、微小粒子測定装置を提供するものである。

これにより、前記第１領域で、微小粒子からの反射光や不要な散乱光成分をカットしつつ、必要な光成分の一つである蛍光を透過させることが可能となる。かつ、前記第２領域で、蛍光および必要な散乱光成分（例えば、後方散乱光）を通過させることも可能となる。

【０００７】

また、本発明は、前記波長選択性のない第２領域の貫通部分に前記波長選択性を有する第１領域を配するのが好適である。

また、本発明は、前記波長選択性を有する第１領域の外周に該第１領域を支持するフレームを備えるとともに、前記波長選択性のない第２領域が中空であるのが好適である。

これにより、第１領域と第２領域との界面での屈折率が異なるのを低減できる。

【０００８】

また、本発明は、前記光学フィルタは、前記波長選択性を有する第１領域の周囲に配された波長選択性のない第２領域の周囲に、さらに波長選択性を有する第３領域を有するのが好適である。前記第１領域でビーム系の中心をカットするのに加えて、この第３領域により、ビーム系の外側も必要に応じてカットすることが可能となる。よって、所望の角度からの後方散乱光を得ることが可能となる。

【０００９】

また、本発明は、前記光学経路上に、蛍光・後方散乱光検出器に蛍光および後方散乱光を導光する光ファイバーを備え、前記複数の領域に分割された光学フィルタを、該光ファイバーによって前記光学フィルタを透過した光が受光されるように配置するのが好適である。これにより、後方散乱光及び蛍光を高感度に検出できる。

【発明の効果】

【００１０】

本発明によれば、後方散乱光と蛍光とを効率的に取得する微小粒子分析装置が提供される。

【図面の簡単な説明】

【００１１】

【図１】本発明に係わる微小粒子分析装置の光学経路を説明するための模式図である。

【図２】本発明に係わる光学フィルタを備えた微小粒子分析装置において、レーザーを照射した微小粒子から発せられる反射光、後方散乱光及び蛍光を示した模式図である。

【図３】本発明に係わる微小粒子分析装置に備える光学フィルタの第１領域の透過スペクトラムを示す図である。

【図４】本発明に係わる微小粒子分析装置に備える光学フィルタの実施形態（上面方向）の例示を示す。（ａ）第１領域が円形状である場合、（ｂ）第１領域が四角形状である場合、（ｃ）第１～第３の各領域が円形状である場合、（ｄ）第１～第３の各領域が四角形状である場合。

【図５】本発明に係わる微小粒子分析装置に備える光学フィルタの左右方向の断面図の例示を示す。（ａ）第２領域の（平面）フィルタの上部に第１領域の（平面）フィルタの下

部が接合する場合、（ｂ）第２領域の（平面）フィルタの貫通部分に第１領域の（平面）フィルタが配され、第２領域の（平面）フィルタの上面よりも第１領域の（平面）フィルタの上面が突出する場合、（ｃ）第２領域の（平面）フィルタの貫通部分に第１領域が配され、第１領域と第２領域の上下面が面一である場合。
【図６】本発明に係わる微小粒子分析装置に備える光学フィルタの例示を示す。第１領域の（平面）フィルタが円筒状のフレームによって支持され、第２領域が中空である場合。
【図７】本発明の光学フィルタの変形実施形態の一例（反射型光学フィルタ）を示す図である。（ａ）反射型光学フィルタによる反射光、後方散乱光及び蛍光の流路を示す図である。（ｂ）本発明の反射型光学フィルタの第１領域の透過スペクトラムを示す図である。
【図８】（ａ）通常（従来）のマスクを備える微小粒子分析装置；（ｂ）本発明に係わる異なる透過波長の領域を有するマスクを備える微小粒子分析装置；（ｃ）マスクを備えない微小粒子分析装置による蛍光感度の測定結果を示す図である。
【図９】（ａ）通常（従来）のマスクを備える微小粒子分析装置；（ｂ）本発明に係わる異なる透過波長の領域を有するマスクを備える微小粒子分析装置による後方散乱の測定結果を示す図である。
【図１０】通常（従来）のマスクを備えた微小粒子分析装置において、レーザーを照射した微小粒子から発せられる反射光、後方散乱光及び蛍光を示した模式図である。
【発明を実施するための形態】
【００１２】
以下、本発明を実施するための好適な形態について図面を参照しながら説明する。なお、以下に説明する実施形態は、本発明の代表的な実施形態の一例を示したものであり、これにより本発明の範囲が狭く解釈されることはない。尚、説明は以下の順に行う。
１．微小粒子測定装置の装置構成
（１）光学検出手段
（１－１）ＦＬ・ＢＳ集光系－光学フィルタ
２．微小粒子測定装置の動作
３．変形例
（１）反射型光学フィルタ
（２）反射型光学フィルタを備えた場合の動作
【００１３】
＜１．微小粒子測定装置の構成＞
図１は、本発明に係わる微小粒子測定装置の概略構成、特に光学検出系（光学流路）を模式的に示す図である。
当該微小粒子装置は、フローセルやマイクロチップなどの流路系、このフローセル内やマイクロチップ上に形成された流路内を通過する微小粒子Ｐからの複数の蛍光、後方散乱光、前方散乱光などの光成分を検出する光学検出手段などを備えている。尚、当該微小粒子測定装置は、必要に応じて測定後の微小粒子Ｐを回収や分離するソート手段を備えていてもよい。
【００１４】
（１）光学検出手段
図１に示すように、前記光学検出手段は、光学照射系１０１、蛍光・後方散乱光集光系１０２、前方散乱光検出系１０３、および蛍光・後方散乱光検出系１０４から構成されている。これによって、光が照射された微小粒子から発生する光成分を各光検出器に導入し、所望の光成分を検出、測定することが可能となる。
【００１５】
前記光学照射系１０１は、光（励起光）を照射する光源（λ１～４）１、この光源１からの光を平行光とする集光レンズ２、更に光を同軸上に配置するダイクロイックミラー３などを備えている。
前記光学照射系１０１より照射された光（励起光）は、蛍光・後方散乱光（以下、「ＦＬ・ＢＳ」ともいう）集光系内にあるハーフミラー４及び対物レンズ５を透過し、流路６

内を通流する微小粒子Ｐに照射される。このとき、微小粒子Ｐから蛍光および必要な散乱光成分と共に反射光が発せられ、また不要な散乱光成分も発せられる。

ここで、蛍光および必要な散乱光成分は、微小粒子Ｐの光学的情報（特性）を得る上で重要な光成分である。当該必要な散乱光成分としては、例えば、後方散乱光、側方散乱光などが挙げられる。また、不要な散乱光成分は、照射された光（励起光）が流路系や基板又は細胞などに当たることにより生じるものである。

【００１６】

尚、本発明において、「微小粒子」には、細胞や微生物、リポソーム等の生体関連微小粒子、およびラテックス粒子やゲル粒子、工業用粒子等の合成粒子などが広く含まれているものとする。

生体関連微小粒子には、各種細胞を構成する染色体、リポソーム、ミトコンドリア、オルガネラ（細胞小器官）などが含まれる。対象とする細胞には、動物細胞（血球系細胞など）および植物細胞が含まれる。対象とする微生物には、大腸菌等の細菌類、タバコモザイクウイルス等のウイルス類、イースト菌等の菌類などが含まれる。さらに、生体関連微小粒子には、核酸やタンパク質、これらの複合体などの生体関連高分子も包含され得るものとする。

また、工業用粒子は、例えば有機もしくは無機高分子材料や金属等であってもよい。有機高分子材料には、ポリスチレン、スチレン・ジビニルベンゼン、ポリメチルメタクリルレートなどが含まれる。無機高分子材料には、ガラス、シリカ、磁性体材料などが含まれる。金属には、金コロイド、アルミ等が含まれる。

上記微小粒子の形状は、一般には球形であるのが普通であるが、非球形であってもよく、また大きさや質量なども特に限定されない。

【００１７】

前記前方散乱（以下、「ＦＳ」ともいう）検出系１０３は、ＦＳ集光レンズ７、前方散乱光を所望の角度成分でカットするＦＳマスク８、所望の波長成分のみを透過するバンドパスフィルタ９、透過した光を検出するＦＳ光学検出器１０を備えている。

【００１８】

前記蛍光・後方散乱光集光系１０２は、微小粒子Ｐからの光成分を集光する対物レンズ５、これら光成分を表面で反射させるハーフミラー４、この光成分が導光される複数の領域に分割された光学フィルタ１１、およびＦＬ・ＢＳ集光レンズ１２を備えている。

ここで、当該複数の領域に分割された光学フィルタ（以下、「光学フィルタ」や「ノッチフィルタマスク」ともいう）１１は、ハーフミラー４で反射された光成分から反射光および不要な散乱光成分を遮断（低減）し、必要な光成分（例えば、蛍光および後方散乱光）を透過させるためのものである。また、ＦＬ・ＢＳ集光レンズ１２は、必要な光成分を光ファイバー１３に導光するためのものである。

【００１９】

前記蛍光・後方散乱検出系１０４は、光ファイバー１３から出た光成分の蛍光および後方散乱光を検出するように構成されている。

具体的には、光ファイバー１３からの光成分を平行光とするＦＬ・ＢＳ集光レンズ１４、この平行光のうちの後方散乱光を反射し、蛍光を透過するノッチフィルタ１５と、後方散乱光を検出するＢＳ光学検出器１６が備えられている。さらに、ノッチフィルタ１５で透過した蛍光成分のうち所望の波長成分を透過し、その他の波長成分を反射させるＦＬダイクロックミラー１７、この所望の波長成分をさらに選択するＦＬバンドパスフィルタ１８，２０、および所望の波長成分を検知する光学電子倍増管１９，２１が備えられている。

なお、必要に応じて、ノッチフィルタマスク、ダイクロイックミラー、バンドパスフィルタ、光学電子倍増管および光学検出器を増減してもよい。

【００２０】

尚、上述のした微小粒子測定装置の光学照射系１０１、前方散乱光検出系１０３、および蛍光・後方散乱検出系１０４は、従来公知の微小粒子測定装置と同様の構成とすること

ができる。

【００２１】

（１－１）ＦＬ・ＢＳ集光系－光学フィルタ

図２～６を参照して、以下に、本発明に係わる微小粒子測定装置におけるＦＬ・ＢＳ集光系１０２およびこれに備えられている光学フィルタ１１について説明する。

【００２２】

まず、前記光学フィルタ１１について説明する。

前記光学フィルタ１１は、微小粒子Ｐよりの光成分からの反射光および不要な散乱光成分を低減すると共に、蛍光成分および必要な散乱光成分（特に後方散乱光成分）を高効率に得るためのものである。このため、当該光学フィルタ１１の略中心部分には、微小粒子Ｐよりの（表面）反射光および不要な散乱光成分が入射する第１領域１１１のフィルタが配されており（例えば、図２参照）、当該第１領域１１１のフィルタは特定の波長選択性を有する。この特定の波長選択性によって、反射光および不要な散乱光成分を遮断（低減）しつつ、かつ蛍光成分を透過させることが可能となる。

前記第１領域１１１のフィルタは、不必要な所望の光成分のみを高精度に反射させ、必要な光成分を透過させることが可能となるような透過スペクトラムを有していればよい。より具体的には、一般的に入射光や反射光の波長は蛍光波長よりも低いことから、低い波長を透過させないフィルタが好ましい。

例えば、図３に示すように、前記第１領域１１１の特定の波長選択性を有するフィルタは、４８８ｎｍ付近、具体的には４８０～５００ｎｍという特定の波長成分のみを透過させないものが好適である。当該波長選択性を有するフィルタは、当該特定の波長成分のみを他の透過成分に対して５桁透過率が低い特性を有するように調製するのが好適である。これにより、反射光および不要な散乱光成分を遮断（低減）しつつ、かつ蛍光成分を高精度に透過させるので、蛍光の光量を高効率に取得することが可能となる。

【００２３】

ここで、従来のマスクを用いた場合の微小粒子Ｐから発せられる反射光、後方散乱光および蛍光を示した模式図を図１０に示す。このように、通常のマスクによって、表面反射光と共に蛍光も遮断されることとなる。これにより光検出器に入る蛍光の光量が少なくなり、この蛍光感度が劣化してしまう。

これに対し、図２（ａ）に示すように、前記第１領域１１１のフィルタは、上述のように特定の波長選択性を有するので、微小粒子Ｐからの伝播角の小さい反射光と不要な散乱光成分とを遮断しつつ、蛍光を透過させる。しかも、図２（ｂ）に示すように、後方散乱光や蛍光は原理的に反射光成分よりも伝播角が大きくなるので、前記第１領域１１１のフィルタの周囲を、必要な光成分（後方散乱光成分や蛍光成分）が通過することとなる。これにより、光学検出系に入る蛍光の光量のロスが低減できる。さらに、後方散乱光も取得することができるので、後方散乱光と蛍光とを同時に高効率に取得することも可能となる。加えて、微小粒子測定装置に前記光学フィルタをセットするだけでよく、複雑な構成も必要としない。これにより、後方散乱光および蛍光を高効率に取得できる光学検出系および微小粒子測定装置をシンプルかつコンパクトに実現することも可能となる。

【００２４】

図４および図５に、前記特定の波長選択性を有する第１領域と、少なくとも該領域の周囲に配された前記波長選択性のない第２領域を有する前記光学フィルタ１１を例示するが、前記光学フィルタ１１の形状や構造はこれに限定されるものではない。

まず、図４（ａ）および（ｂ）に示すような前記特定の波長選択性を有する第１領域１１１のフィルタと、該領域の周囲に配された前記波長選択性のない第２領域１１２のフィルタを有する前記光学フィルタ１１について説明する。

前記光学フィルタ１１は、第１領域１１１のフィルタと第２領域１１２のフィルタを有し、前記光学フィルタの略中央付近に、第１領域１１１のフィルタが配置されている。当該略中央付近とは、反射光や不要な散乱光成分などの光成分が入射される部分である。

前記第１領域１１１のフィルタは、微小粒子Ｐからの反射光と不要な散乱光成分とを遮

断し、蛍光を透過するという特定の波長選択性を有する。
また、第２領域１１２のフィルタは、特定の波長選択性のないものであり、蛍光および必要な散乱光成分を透過する。ここで、特定の波長選択性のないフィルタとは、上記第１領域１１１に使用されるフィルタ（光学材料）ではなく、後方散乱光や蛍光が透過するもの（例えばガラスなど）であればよい。

【００２５】

さらに、図５に、上記図４（ａ）の前記光学フィルタの左右方向の断面図の例示を示すが、これに限定されるものではない。
図５（ａ）に示すような前記光学フィルタ１１は、第２領域１１２のフィルタの上面かつ略中央付近で、第１領域１１１のフィルタが接合しているものである。当該接合の際には、第１領域１１１や第２領域１１２のフィルタの光学的な特性を阻害しないような接着剤や光硬化樹脂を用いるのが好適である。さらに、可視域においての反射を防ぐため、図５（ａ）に示すような接合するタイプの前記光学フィルタにおいて、第２領域１１２のフィルタ表面にＡＲコート（Anti Refledion Coating）を施すのが好適である。

【００２６】

また、図５（ｂ）および（ｃ）に示すような前記光学フィルタ１１は、第２領域１１２のフィルタの略中央付近に貫通穴又は未貫通穴を形成し、当該貫通穴又は未貫通穴に第１領域１１１フィルタを嵌合や挿入などによって配する。さらに、図５（ｂ）に示すように第１領域１１１のフィルタが、第２領域１１２のフィルタの上面及び／又は下面より突出してもよい。また、図５（ｃ）に示すように第１領域１１１のフィルタが、第２領域１１２のフィルタの上面及び／又は下面と、面一になるようにしてもよい。なお、第１領域１１１のフィルタと第２領域フィルタとを左右方向や高さ方向で積層構造としてもよい。
図５（ｂ）や（ｃ）に示すような一方の領域の貫通穴に他方の領域を配する光学フィルタを形成することによって、図５（ａ）に示すような光学フィルタの場合に界面の屈折率が異なることによって生じ易いフィルタの性能劣化を低減することが可能となる。

【００２７】

さらに、図４（ｃ）や（ｄ）に示すような前記光学フィルタ１１は、前記特定の波長選択性を有する第１領域の周囲に配された特定の波長選択性のない第２領域の周囲に、さらに波長選択性を有する第３領域を有するものである。

【００２８】

図４（ｃ）および（ｄ）に示すような前記光学フィルタ１１は、上記第１領域１１１のフィルタの周囲に配された上記第２領域１１２のフィルタの周囲に、更に特定の波長特性を有する第３領域１１３のフィルタを配するものである。一般的に後方散乱光は粒子サイズに対する角度依存性があり、所望の角度成分のみの散乱光を取得する必要がある場合もある。このような場合に、図４（ｃ）および（ｄ）に示すような光学フィルタ１１の構造を採用することにより、第１領域１１１のフィルタでビーム系の中心をカットするだけでなく、その外側も第３領域１１３のフィルタでカットすることが可能となる。これにより、所望の角度からの後方散乱光を得ることが可能となる。
このとき、第１領域１１１の幅Ｌと、第２領域１１２の幅Ｍと、第３領域１１３の幅Ｎとの各幅は、それぞれ、中心から０～４ｍｍ：４～６ｍｍ：６～８ｍｍとするのが、所望の後方散乱光を得るため好適である。
ここで、幅Ｌは、第１領域１１１の半径の距離である。幅Ｍは、第１領域１１１の中心から第２領域１１２の外周までの距離－幅Ｌである。幅Ｎは、第１領域１１１の中心から第３領域１１３の外周までの距離－（幅Ｌ＋幅Ｍ）である。尚、多角形の場合には、中心から外辺までの距離（垂線の長さ）を基準とする。
尚、図４（ｃ）および（ｄ）に示すような光学フィルタ１１は、上述のように各領域のフィルタを接合や嵌合などを行うによって形成することが可能である。

【００２９】

上述のような前記光学フィルタは、特定の波長特性を有する光学材料や波長特性のない光学材料などといった異なる波長特性を有する光学材料（フィルタ）を少なくとも２種以

上組み合わせて調製すればよい。
異なる光学特性の光学材料を２種以上用いる光学フィルタの形成方法としては、特に限定されないので、上述の形成方法以外に、フォトニック結晶を形成する方法、例えばエッチング法（例、半導体リソグラフィ法）を用いればよい。フォトニック結晶を形成する方法であれば、各領域が積層状となった光学フィルタ１１も形成しやすいので、好適である。
【００３０】
また、図６に示すように、第１領域１１１のフィルタと、これを単数又は複数のワイヤー１２１を介して支持する円筒状のフレーム１２０とを備える光学フィルタ１１であってもよい。さらに、当該フレーム１２０の略中央付近に第１領域１１１のフィルタが配置され、当該第１領域の周囲で、かつ中空である空間１１５を第２領域とするのが好適である。このような簡単な構造を採用することにより、フィルタの材質の相違によって生じ易いフィルタの性能劣化を低減することが可能である。
【００３１】
前記光学フィルタ１１は、上述のとおり、ＦＬ・ＢＳ集光系に備えられている。前記光学フィルタ１１の配置は、前記ＦＬ・ＢＳ光学検出系（前記蛍光・後方散乱光検出器）に蛍光および後方散乱光を導光する光ファイバー１３の前に配置するのが好適である。具体的には、前記光学フィルタを、該光ファイバーによって前記光学フィルタを透過した光が受光されるように配置するのが好適である。
このような構成を採用することにより、前記光学フィルタ１１をＦＬ・ＢＳ光学検出系内に配置した場合よりも、蛍光の光量のロスが少なくなるので好適である。
【００３２】
＜２．微小粒子測定装置の動作＞
以下に、上述した前記光学フィルタを備えた微小粒子測定装置の動作について、図１を参照して説明する。
前記光学照射系１０１内の複数の波長の異なる各レーザー光源１からの各光（励起光）が、それぞれの集光レンズ２によって平行光となり、ダイクロイックミラー３によって同軸上に配置される。この光（励起光）は、ＦＬ・ＢＳ集光系１０２のハーフミラー４および対物レンズ５を透過し、流路６内の微小粒子Ｐに照射される。そして、当該微小粒子Ｐから、蛍光および必要な散乱光成分（前方散乱光、後方散乱光など）や反射光が発せられると共に不要な散乱光成分や反射光も生じる。
このときの微小粒子Ｐの前方散乱光は、ＦＳ検出系１０３内のＦＳ集光レンズ７、ＦＳマスク８、ＦＳバンドパスフィルタ９を経てＦＳ光学検出器１０に導光される。
また、微小粒子Ｐからの反射光、蛍光や必要な散乱光成分（後方散乱光など）並びに不要な散乱光成分は、ＦＬ・ＢＳ集光系１０２の集光レンズ５を通過してハーフミラー４で反射し、ノッチフィルタマスク（前記光学マスク）１１を通過する。このときノッチフィルタマスク１１の略中央部分にある第１領域１１１のフィルタにより、光成分の中心にある反射光や不要な散乱光成分および後方散乱光を遮断し、かつ蛍光成分が透過する。また、後方散乱光および蛍光は反射光成分よりも広い伝搬角であるため、後方散乱光および蛍光が第１領域１１１のフィルタの周囲に配された第２領域１１２のフィルタを透過する。このようにして、ノッチフィルタマスク１１を透過した後方散乱光および蛍光は、ＦＬ・ＢＳ集光レンズ１２を介して光ファイバー１３にカップリングされる。
この光ファイバー１３を出た後方散乱光および蛍光は、ＦＬ・ＢＳ集光レンズ１４によって平行光とされ、後方散乱光のみを反射するノッチフィルタ１５によって後方散乱光はＢＳ検出器１６に導光され、このＢＳ検出器１６で後方散乱光が検出される。また、蛍光は、ノッチフィルタ１５、ＦＬダイクロイックミラー１７、次いでＦＬバンドパスフィルタ１７を経ることによって、蛍光成分の中でも所望の波長成分が選択され、その選択された成分が光電子倍増管１９によって検出される。また、ノッチフィルタ１５、ＦＬダイクロイックミラー１７、次いでＦＬバンドパスフィルタ２０を経ることによって、蛍光成分の所望の波長成分がさらに選択され、この成分も同様にして光学電子倍増管２１によって

検出される。

【００３３】

＜３．変形例＞

（１）反射型光学フィルタ

ＦＬ・ＢＳ集光系１０２に備えられている前記光学フィルタ１１を、図７に示す反射型光学フィルタ４０に変更してもよい。

当該反射型光学フィルタ４０は、特定の波長選択性を有する第１領域３０と、該領域の周囲に配され、波長選択性のない第２領域３１とを有する。第１領域３０は、微小粒子Ｐからの反射光と不要な散乱光成分を透過し、蛍光を反射させるような特定の波長選択性を有する。また、第２領域３１は、必要な光成分（例えば、微小粒子Ｐからの蛍光や後方散乱光などの必要な散乱光成分）を反射させる波長選択性のない第２領域を有する。

より詳細に説明すると、前記反射型光学フィルタ４０は、その略中央部分にバンドパスフィルタ３０、かつそのバンドパスフィルタ３０の両端にミラー３１を有する。当該バンドパスフィルタ３０は、微小粒子Ｐからの反射光、また不要な散乱光成分を透過させ、かつ蛍光を反射させるものである。

当該バンドパスフィルタ３０の透過スペクトラムの一例を、図７（ｂ）に示す。

前記反射型光学フィルタの第１領域のフィルタは、上述した前記光学フィルタの第１領域１１１のフィルタの特性とは逆に、所望の不必要な光成分のみを高精度に透過させ、必要な光成分を反射させることが可能となるようなスペクトラムを有する。具体的には、バンドパスフィルタ３０（第１領域のフィルタ）は、前記光学フィルタ１１の第１領域１１１のフィルタの透過スペクトラムとは逆の波長選択性を有しており、例えば、４８８付近、具体的には４８０～５００ｎｍが透過するのみでこれ以外の波長は反射する。

一方、当該ミラー３１は、全波長域の光成分が反射するものであり、いわゆる波長選択性のないものである。

前記反射型光学フィルタ４０を採用することにより、蛍光成分などは反射成分を使うため、前記光学フィルタ１１よりも光の利用率がよくなる。

【００３４】

そして、前記反射型光学フィルタ４０を備えたＦＬ・ＢＳ集光系１０２は、前記反射型光学フィルタ４０にて反射された光成分（例えば、後方散乱光および蛍光成分など）が光ファイバー１３に受光されるように、構成されていればよい。例えば、ＦＬ・ＢＳ集光系１０２内に、後方散乱光および蛍光を反射するミラーを集光レンズ１２の前に設けてもよいし、或いは、光ファイバー１３の受光部の配置を変更してもよい。

【００３５】

（２）反射型光学フィルタを備えた場合の動作

図７に示すように、微小粒子Ｐからの光成分（反射光、後方散乱光、蛍光）や不要な散乱光成分は、反射型光学フィルタ４０に導光される。このうちの反射光は、伝搬角が小さいので、前記反射型光学フィルタ４０の略中央部分にあるバンドパスフィルタ３０（第１領域）を透過する。後方散乱光は、伝播角が広いので、前記反射型光学フィルタ４０のミラー全面に当たり、このとき略中央部分のバンドパスフィルタ３０（第１領域）を透過し、この周囲にあるミラー３１（第２領域）によって反射する。蛍光は、伝播角が広いので、前記反射型光学フィルタ４０のミラー全面に当たり、このとき略中央部分のバンドパスフィルタ３０（第１領域）およびこの周囲にあるミラー３１（第２領域）によって反射する。

反射した蛍光および後方散乱光は、反射ミラーなど（図示せず）を経て、光ファイバー１３に導光される。

【００３６】

なお、前記反射型光学フィルタ４０の形状や構造は、必要な光成分（例えば、蛍光および後方散乱光などの必要な散乱光成分）を反射させることができれば特に限定されないが、前記光学フィルタ１１と同じ形状や構造とするのが好適である。例えば、上述した図４および５に示すようなものが挙げられる。このとき、前記反射型光学フィルタ４０の微小

粒子Ｐからの光成分や不要な散乱光成分が当たる面は、蛍光および後方散乱光を効率よく反射させるため、面一とするのが好適である。

【実施例】

【００３７】

前記光学フィルタ１１を用いたときの効果を下記に示す。

製造例１：本発明の微小粒子測定装置に備える光学フィルタ

波長特性を有する第１領域のフィルタは、図３に示すようなスペクトラムを有していた。この第１領域のフィルタは、所望の４８８ｎｍの波長成分のみを他の透過成分に対して５桁透過率の低い特性を有していた。

また、波長特性のない第２領域のフィルタは、市販のカバーガラスを用い、可視域において反射を防ぐＡＲコートをした。

上記第１領域のフィルタを半径４～５ｍｍにカットし、上記第２領域のフィルタの上に貼り付けて、図４（ａ）および図５（ａ）のような形状および構造を有するノッチフィルタマスクを作製した。

【００３８】

試験例１：各微小粒子測定装置による蛍光感度の測定

下記の実験は、８種類の異なる明るさを持ったビーズを用いて、４８８ｎｍの励起光で蛍光を取得し、そのときのピークを、図８（ａ）通常のマスク、（ｂ）ノッチフィルタマスク、（ｃ）マスクを用いない時を比較した。

ここで、本実験では、８つのビーズでのヒストグラムのピークが全て独立して存在する状態であれば感度が高い状態といえる。強度の一番弱いピーク（一番左側）は蛍光体が付着していないビーズのため、この成分はノイズ成分となる。よってこのノイズ成分と他のピークとが完全に離れている状態が感度の高い状態で望ましい。

上記（ａ）と（ｂ）の実験では、同じ一辺４．５ｍｍのサイズのマスクを用いた。

（ａ）の通常のマスクを用いたときには、右から６，７，８番目のピークが繋がっていたのに対して、（ｂ）のノッチフィルタマスクを用いたときには、７－８個目のピークが分離していた。（ｃ）のマスクなしの時とほぼ変わらない特性であることから、ノッチフィルタマスクを用いたときは、蛍光の光量のロスがないことが確認できた。

【００３９】

試験例２：各微小粒子測定装置による散乱の測定

血液のサンプルを３種類の細胞に分けたもので、そのサイズと、細胞の複雑さによって前方散乱光と後方散乱光の信号が変化する。当然後方散乱光のマスクのサイズが最適化されていなかったり、マスクの消光比が悪いと、特性は劣化した。

上述のような（ａ）と（ｂ）の各マスクを用いた実験では、同じ一辺４．５ｍｍのサイズのマスクを用いた。

（ａ）の通常のマスクを用いたときのような、完全に中央の光をブロックしたときとほぼ同じ結果が、（ｂ）のノッチフィルタマスクを用いたときにも得られている。

【００４０】

以上のこれら２つの実験から、本発明の装置に用いる前記光学フィルタ（領域分割フィルタ）が、蛍光などの光量のロスがないにも拘らず、所定の波長に関してはフィルタとして機能して遮断していることが確認できた。

【産業上の利用可能性】

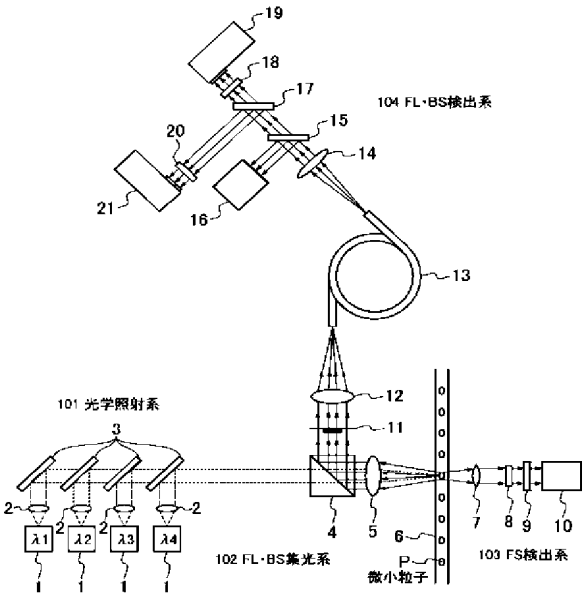
【００４１】

本発明に係わる微小粒子測定装置は、特定の波長選択性を有する領域と波長選択性のない領域とを組み合わせた領域分割の光学フィルタを用いることにより、微小粒子から発生する反射光や不要な散乱光成分を遮断し、後方散乱光の光量を低減させることなく、かつ蛍光の光量のロスを低減する。よって、本発明に係わる微小粒子測定装置は、散乱光と蛍光の何れをも高効率で取得することができる。さらに後方散乱光および蛍光を高効率に取得できる光学検出系をシンプルかつコンパクトに形成することも可能となる。

【符号の説明】

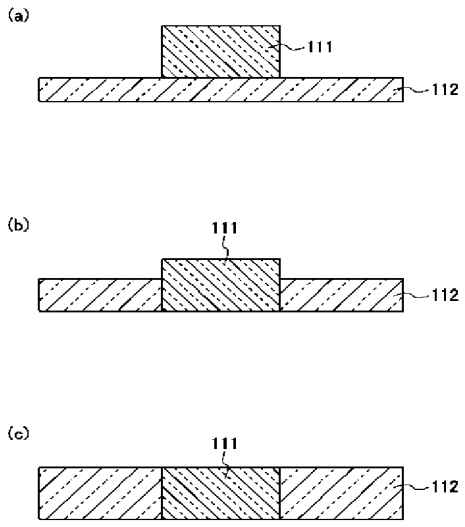
【００４２】
１１　光学フィルタ
１１１　第１領域
１１２　第２領域
１１３　第３領域
１１４　第４領域
３０　バンドパスフィルタ
３１　ミラー
４０　反射型光学フィルタ
１０１　光学照射系
１０２　蛍光・後方散乱光集光系
１０３　前方散乱検出系
１０４　蛍光・後方散乱光検出系

【図１】



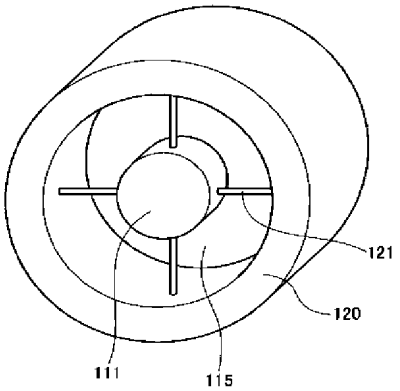
1　光源
2　集光レンズ
3　ダイクロイックミラー
4　ハーフミラー
5　対物レンズ
6　流路
7　FS集光レンズ
8　FSマスク
9　FSバンドパスフィルタ
10　FS光学検出器
11　ノッチフィルタマスク
12　FL･BS集光レンズa
13　光ファイバー
14　FL･BS集光レンズb
15　ノッチフィルタ
16　BS光学検出器
17　FLダイクロイックミラー
18　FLバンドパスフィルタa
19　光学電子倍増管a
20　FLバンドパスフィルタb
21　光学電子倍増管b

【図５】



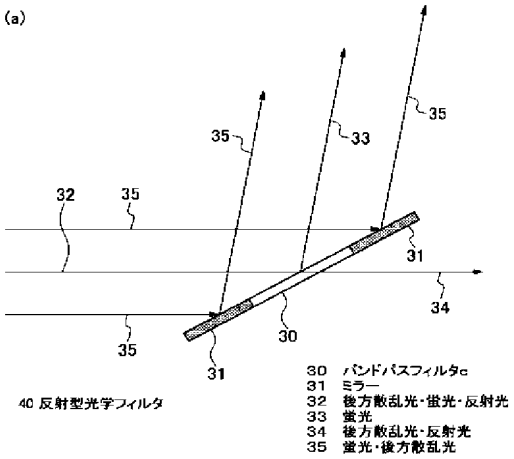
111　第1領域
112　第2領域

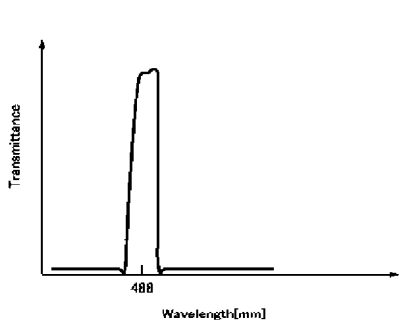
【図６】



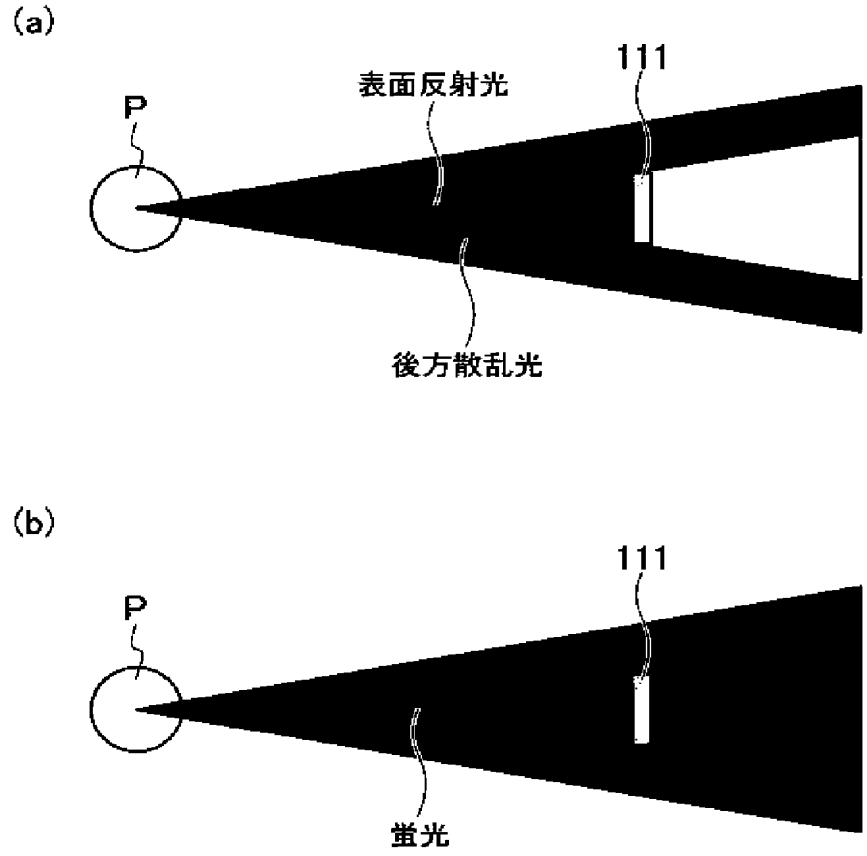
111 第1領域
115 空間
120 フレーム
121 ワイヤー

【図７】



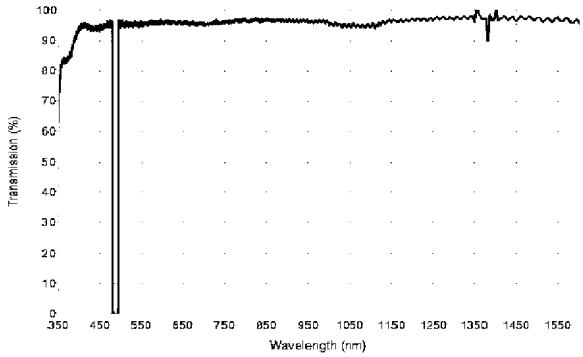


【図２】

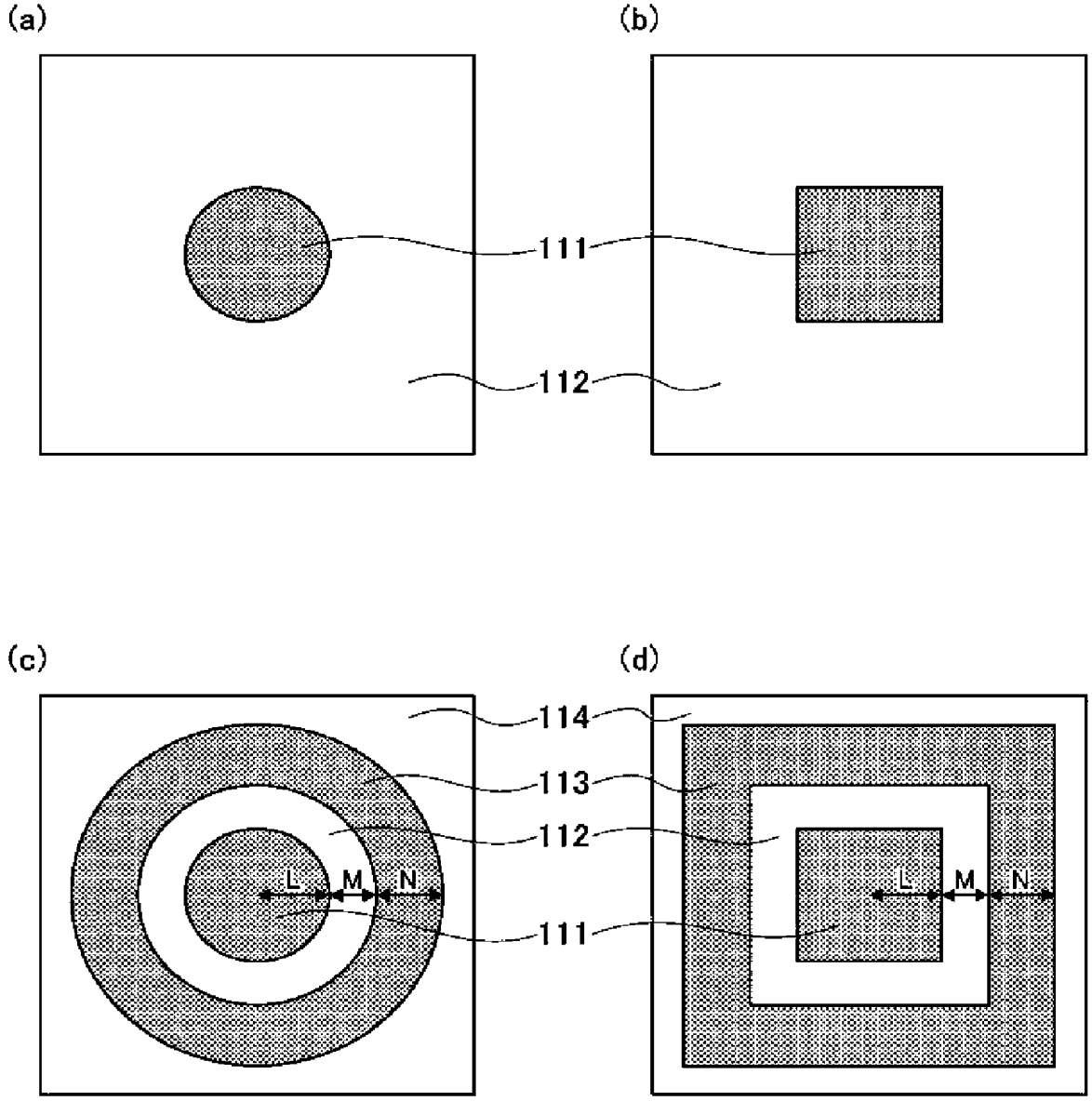


P　微小粒子
111　第１領域のフィルタ

【図３】


100
90
80
70
60
50
40
30
20
10
0
Transmission (%)
350
450
550
650
750
850
950
1050
1150
1250
1350
1450
1550
Wavelength (nm)

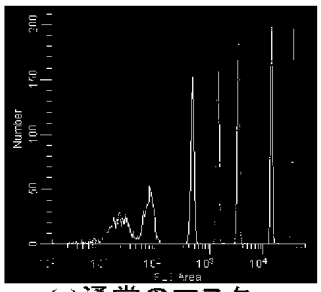
【図４】



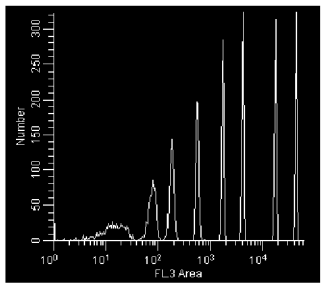
111　第1領域
112　第2領域
113　第3領域
114　第4領域

L,M,N　各領域の幅

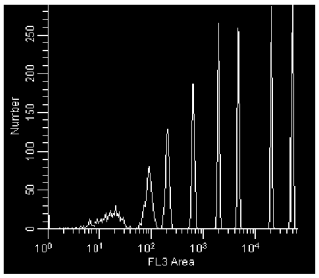
【図８】



(a)通常のマスク

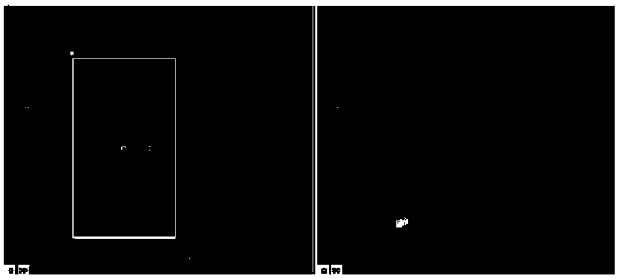


(b)ノッチフィルタマスク

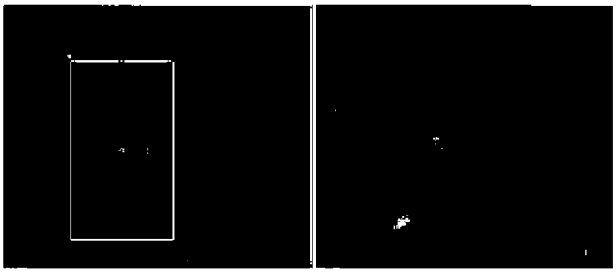


(c)マスクなし

【図９】

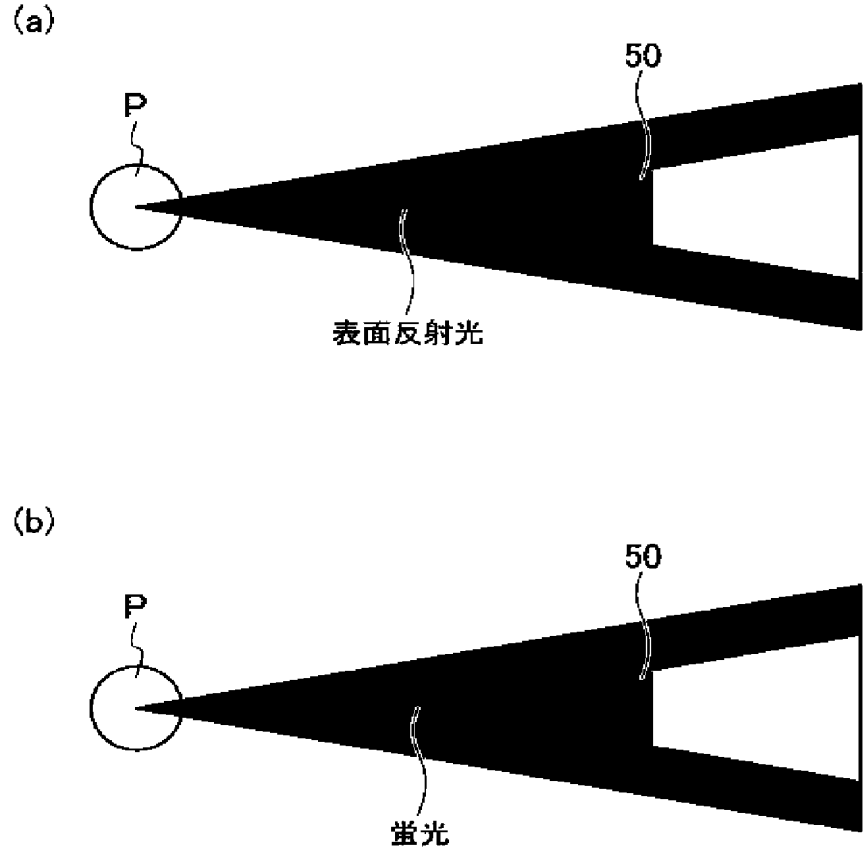


(a)通常のマスク



(b)ノッチフィルタマスク

【図１０】



P　微小粒子
50　マスク

フロントページの続き

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Espacenet

Bibliographic data: JPH1073528 (A) — 1998-03-17

FLOW CYTOMETER EQUIPPED WITH IMAGING FUNCTION

Inventor(s): KUSUSAWA HIDEO; KUBOTA FUMIO + (KUSUSAWA HIDEO, ; KUBOTA FUMIO)

Applicant(s): TOA MEDICAL ELECTRONICS + (TOA MEDICAL ELECTRONICS CO LTD)

Classification: **- international:** ***G01N15/14; G01N33/483;*** (IPC1-7): G01N15/14; G01N33/483

- cooperative:

Application number: JP19960248875 19960830 Global Dossier

Priority number (s): JP19960248875 19960830

Abstract of JPH1073528 (A)

PROBLEM TO BE SOLVED: To obtain a flow cytometer by which a cell in an intrinsically three- dimensional shape is analyzed with high accuracy by a method wherein a sample which contains particles is changed into a sample flow by using a sheath flow cell and the sample is irradiated with near-infrared rays so as to be imaged. SOLUTION: A pulse light source 2 is a light source of a type which emits light only for an instant by a light-emitting tigger signal, and flowing particles can be imaged without any blur even when a sample flow is fast at a high speed by several m/sec. Pulsed light L2 is guided to a collimating lens 12 by a kaleidoscope 2a, and it is narrowed down to be fine by a condensing lens 14 via a cold mirror 13 so as to irradiate the sample flow in a sheath flow cell 3. The pulsed light which is transmitted through the sample flow is image- formed by a video camera 17 via a collimating lens 8, dischroic mirror 9, a condensing lens 15 and a band-pass filter 16, and the transmitted-light image of a cell is imaged. In addition, the pulsed light L2 is irradiated via an optical fiber 2a, its coherency is dropped, and the image of the nucleus part of the cell can be imaged with good contrast. An image signal from the video camera 17 is transferred to an image processing part so as to be analyzed and processed.

(19)日本国特許庁（ＪＰ）　(12) **公開特許公報（Ａ）**　(11)特許出願公開番号

特開平10－73528

(43)公開日　平成10年(1998)３月17日

(51)Int.Cl.[6]	識別記号	庁内整理番号	ＦＩ		技術表示箇所
Ｇ０１Ｎ　15/14			Ｇ０１Ｎ　15/14	Ｄ	
33/483			33/483	Ｃ	

審査請求　未請求　請求項の数６　ＦＤ　（全　６　頁）

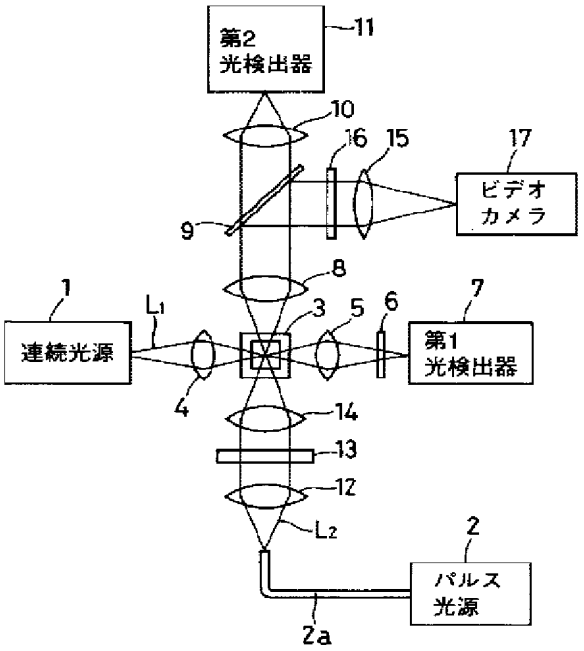
(21)出願番号　特願平8－248875

(22)出願日　平成８年(1996)８月30日

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(54)【発明の名称】　撮像機能付きフローサイトメータ

(57)【要約】
【課題】　細胞を変形させることなく、その核部分をコントラストよく撮像する。
【解決手段】　粒子含有試料を試料流に変換するシースフローセルと、近赤外光で前記試料流を照明する近赤外光源と、近赤外光で照明された試料流中の粒子を撮像する撮像器と、撮像された粒子像を画像処理する画像処理部を備える。



【特許請求の範囲】
【請求項１】　粒子含有試料を試料流に変換するシースフローセルと、近赤外光で前記試料流を照明する近赤外光源と、近赤外光で照明された試料流中の粒子を撮像する撮像器と、撮像された粒子像を画像処理する画像処理部を備えた撮像機能付きフローサイトメータ。
【請求項２】　近赤外光源は、７２０～１５００ｎｍの波長の光を試料流に照明するものである請求項１記載のフローサイトメータ。
【請求項３】　試料流を照明する近赤外線は、コヒーレンス長が粒子径と同程度、又はそれ以下である請求項１記載のフローサイトメータ。
【請求項４】　近赤外光源が、パルスレーザーからなる請求項１記載のフローサイトメータ。
【請求項５】　試料流を可視光で照明する可視光源と、可視光で照明された試料流中の粒子からの光を検出する光検出素子と、試料流から光検出素子までの光路中に設けられ可視光を光検出素子に入射せしめて近赤外光を排除する第１光選択器と、試料流から撮像器までの光路中に設けられ近赤外光を撮像器に入射せしめ可視光を排除する第２光選択器と、光検出素子から得られた粒子信号に基づいて粒子を分析する粒子分析部とをさらに備えた請求項１記載のフローサイトメータ。
【請求項６】　可視光源は、４００～７００ｎｍの波長の光を試料流に照明するものである請求項５記載のフローサイトメータ。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】この発明は、撮像機能付きフローサイトメータに関し、特に細胞などの粒子撮像するための撮像系を備えるフローサイトメータに関する。
【０００２】
【従来の技術】従来、細胞の核部分を細胞質部分から弁別して分析する方法として次のような方法が知られている（例えば、特開平６－５８９２６号公報参照）。つまり、細胞をスライド上に定着して洗浄した後、特別な染料で染色し、更に洗浄して余分な染料を除去し、これにカバーグラスをかけて細胞標本を作成し、そして、作成した標本に赤外線を照射して、細胞を撮像し、細胞像をデジタル化してコンピュータで分析を行うようにしている。
【０００３】
【発明が解決しようとする課題】しかしながら、従来のこのような分析方法においては、細胞標本の作成に手間を要するばかりでなく、細胞がスライドとカバーグラスとに挟まれて偏平化し、立体的な形状を有する細胞本来の像を撮像することができないため、例えば、核部分の直径やプロフィールのような情報を正しく得ることができないという問題点がある。
【０００４】この発明は、このような事情を考慮してなされたもので、粒子を含有する試料流に近赤外光を照射して撮像することにより、本来の立体的な形をした細胞の核部分をコントラストよく撮像することが可能で、またそれによって細胞の分析を高精度に行うことができる撮像機能付きフローサイトメータを提供するものである。
【０００５】
【課題を解決するための手段】この発明は、粒子含有試料を試料流に変換するシースフローセルと、近赤外光で前記試料流を照明する近赤外光源と、近赤外光で照明された試料流中の粒子を撮像する撮像器と、撮像された粒子像を画像処理する画像処理部を備えた撮像機能付きフローサイトメータを提供するものである。
【０００６】
【発明の実施の形態】この発明のシースフローセルは、粒子（細胞）を含む試料をシース液で包んで流すことにより流体力学的効果によって細い試料液の流れを形成させることのできるフローセルであり、これには従来公知のものを用いることができる。
【０００７】この発明の対象とする粒子は、血液や尿に含まれる血球や細胞を主としているが、特に限定されない。
【０００８】粒子を撮像するについて、粒子を照明する光の波長が短いと、光が粒子表面及び内部において散乱や吸収による減光が大きくなり、粒子内部を画像化することができない。
【０００９】逆に光の波長が長いと、粒子による減光が少なくなる（粒子透過性が大きくなる）。水の吸光度が増大する。画像分解能が低下する。これらのことにより良好な画像が得られない。
【００１０】よって、粒子内部をコントラストよく撮像するために近赤外光源は、波長が７２０～１５００ｎｍの範囲にある近赤外光を試料流に照明するものであることが好ましい。具体的には、近赤外パルス半導体レーザ（７２０～９８０ｎｍ）、カラーセンターレーザ（６００～９００ｎｍ）、色素レーザ（６００～１２００ｎｍ）、ＹＡＧレーザ（１．０６４μｍ）、ＹＬＦレーザ（１．０４８μ）、又はＴｉサファイアレーザー（モードロックタイプでキャビテイダンパー付）などにより構成される。
【００１１】粒子を撮像する撮像器には、一般的な２次元画像を撮像するビデオカメラを使用してもよいが、その場合には微弱な蛍光増を増幅するイメージインテンシファイアを備えたものを用いることが好ましい。さらに、そのイメージインテンシファイアにはシャッター手段を備えてもよい。
【００１２】画像処理部は、ＣＰＵ、ＲＯＭおよびＲＡＭからなるマイクロコンピュータによって構成することができる。さらに、近赤外光源から試料流への光路中に設けられ試料流を照明する近赤外光のコヒーレンシー

（可干渉性）を低下させる光学素子をさらに備え、コヒーレンス長を粒子径と同程度又はそれ以下にすることが好ましい。

【００１３】コヒーレンシーを低下させる光学素子としては、特開平７－１３４０９３号公報に記載されたものを使用することができる。

【００１４】この発明は、試料流を可視光で照明する可視光源と、可視光で照明された試料流中の粒子からの光を検出する光検出素子と、試料流から光検出素子までの光路中に設けられ可視光を光検出素子に入射せしめ近赤外光を排除する第１光選択器と、試料流から撮像器までの光路中に設けられ近赤外光を撮像器に入射せしめ可視光を排除する第２光選択器と、光検出素子から得られた粒子信号に基づいて粒子を分析する粒子分析部をさらに備えてもよい。第１光選択器と第２光選択器は１つの光学素子で実現されていてもよく、別々の光学素子で実現されていてもよい。

【００１５】可視光源は、試料流に照明する光が４００～７００ｎｍの波長を有することが好ましい。

【００１６】また、近赤外光の波長が７２０～１５００ｎｍ、可視光の波長が４００～７００ｎｍの場合には、第１光選択器として７００ｎｍより短い波長を光検出素子に入射せしめ、第２光選択器として７００ｎｍあるいは７２０ｎｍよりも長い波長を撮像器に入射せしめる光学素子をそれぞれ使用すればよい。

【００１７】

【実施例】この発明の実施例における光学系を図１に示す。この実施例では、散乱光や蛍光を検出するための連続発光光源１と、細胞像を撮像するためのパルス光源２との２つの光源を設けている。光源１は可視光源、光源２は近赤外光源である。

【００１８】なお、光源１には、中心波長が４８８ｎｍの可視光を発するＡｒレーザーを使用し、光源２には、中心波長が７８０ｎｍで、スペクトル幅が７ｎｍの図３に示すスペクトル特性を備えた近赤外光を発するパルスレーザダイオードを使用している。

【００１９】この２つの光源１、２からの連続可視光Ｌ１とパルス近赤外光Ｌ２は、角型のシースフローセル３（図１では紙面に垂直方向に試料流が流れる）に対して互いに直交するように照射している。

【００２０】この照射領域に細胞が流れてくると、その細胞による前方散乱光が集光レンズ５によって集められ、バンドパスフィルター６を介してフォトダイオードからなる第１光検出器７で受光される（ビームストッパは図示しない）。細胞の側方散乱光は、コリメートレンズ８、ダイクロイックミラー９およびコンデンサレンズ１０を介して光電子増倍管からなる第２光検出器１１で受光、増倍される。

【００２１】なお、第１光検出器７は波長選択された複数の光をそれぞれ検出する複数の光検出素子を含んでもよい。この場合、各光検出素子により、前方散乱光や前方蛍光などを検出することができる。

【００２２】同様に、第２光検出器１０は波長選択された複数の光をそれぞれ検出する複数の光検出素子を含んでもよい。この場合、各光検出素子により、側方散乱光や側方蛍光を検出することができる。

【００２３】図２はこの実施形態の信号処理系の構成を示し、第１および第２光検出器７、１１によってそれぞれ検出された粒子信号、即ち、前方散乱光強度信号Ｓ１および側方蛍光強度信号Ｓ２は、データ処理部１００の粒子分析部１００ａに入力され、各検出信号パルスの高さ情報がＡ／Ｄ変換され、散乱光強度と蛍光強度の２つの特徴パラメータが生成される。

【００２４】撮像制御部１００ｃは信号Ｓ１、Ｓ２がデータ処理部１００に入力されると同時又は所定時間後に細胞を撮像するための発光トリガ信号Ｔｓをパルス光源２に対して供給する。

【００２５】パルス光源２は、発光トリガ信号Ｔｓによって一瞬だけ（２５ナノ秒程度）発光するタイプの光源であり、試料流の流速が数ｍ／秒と高速であっても、流れる粒子をブレ無く撮像することができる。

【００２６】パルス光Ｌ２は図１に示すように、コヒーレンス低下手段の一例であるカライドスコープ（マルチモード光ファイバーでも可能である）２ａによってコリメートレンズ１２へ導かれ、コールドミラー１３を通過してコンデンサレンズ１４によって細く絞られて試料流に照射される。

【００２７】光ファイバー２ａを介して照射することにより、パルス光Ｌ２のコヒーレンシーが落ち、後述するように細胞の核部分の像をコントラスト良く撮像することができる。なお、光ファイバー２ａには、コア－径８００μｍ、長さ２ｍ（住友電工株式会社製ＭＫＨ－８００）のものを用いている。

【００２８】試料流を透過したパルス光は、コリメートレンズ８によって平行光に変換されダイクロイックミラー９に反射されて、コンデンサレンズ１５とバンドパスフィルター１６を介してビデオカメラ１７の受光面に結像され、細胞の透過光像が撮像される。ビデオカメラ１７からの画像信号Ｖｓは図２に示す画像処理部１００ｂに渡され、ディジタル画像として記憶、保存される。

【００２９】粒子分析部１００ａは、前方散乱光および側方蛍光強度の特徴パラメータに基づいてスキャッタダラム（２次元散布図）を生成し、その解析を行う。

【００３０】なお、入力部２００はキーボードやマウスからなり、データ処理部１００に対して各種の指令や条件の設定などを行う。また、出力部３００はＣＲＴやプリンタからなり粒子分析部１００ａと画像分析部１００ｂで得られたスキャッタダラム、粒子像および解析結果などを出力する。

【００３１】ここで、波長４８８ｎｍの光で励起され波

長５００～７００ｎｍの光として発せられる蛍光を検出するために、５００～７００ｎｍの波長の光を透過させ７００ｎｍ以上の波長の光を反射するダイクロイークミラー９を使用している。そして、波長７８０ｎｍ以上の近赤外光画像を得るために、さらに、７８０ｎｍの波長の光を透過させるバンドパスフィルター１６を使用している。

【００３２】従って、ビデオカメラ１７には、可視光Ｌ１（４８８ｎｍ）によって細胞から生じた側方の光は入射しないので、ビデオカメラ１７は光源１からの光の影響を受けない良好な細胞画像を得ることができる。

【００３３】同様に、第２検出器１１にはダイクロイックミラー９の作用により、可視光Ｌ１によって生じた側方の光のみが入射するので、第２検出器１１は、光源２からの光の影響を受けない良好な粒子信号を得ることができる。

【００３４】また、バンドパフルター６には、４８８ｎｍの波長の光を透過させるものを使用しているので、第１光検出器７には、可視光Ｌ１によって生じた前方散乱光のみが入射し、近赤外光Ｌ２によって生じた側方の光は全く入射しないので、第１光検出器は光源２からの光の影響を受けない良好な粒子信号を得ることができる。

【００３５】試料流に含まれる細胞は球形に近い形状を有するので、可視光を用いると、細胞表面及び細胞質部分において散乱・吸収による減光により細胞内部を鮮明に画像化することは難しい。しかし、近赤外領域の波長の光については、細胞表面及び細胞質部分での減光が小さいために内部を鮮明に画像化しやすい。

【００３６】図４は、この実施例によって得られた細胞画像の７つの例を示し、図５はその比較例（撮像用のパルス光源２として波長が５２３ｎｍの可視光源を使用したもの）として２つの細胞画像例を示している。

【００３７】なお、この実施形態では、近赤外線を照射する光源として赤外光パルスレーザーのような単色性の高い光源を使用し、光学素子により、その光のコヒーレンシーを低下させている。そのことにより、核部分の細胞質部分に対する減光比を最適に調整し、それによって核部分が鮮明に写った画像を得るようにしている（この実施形態では、コヒーレンス長さを５～６μｍに設定している）。

【００３８】また、近赤外線光源を、波長帯域の広い赤外線ランプと、波長幅を制限する狭帯域フィルターとで代用することも可能である。

【００３９】この実施形態では、図１に示すように、レンズ１２とレンズ１４との間の光のコリメート領域には、赤外光を透過し可視光を反射するコールドミラー１３が設けられている。従って、可視光Ｌ１によって照射された細胞から生じる側方散乱光や側方蛍光の内、レンズ１４側に入射したものは、コールドミラー１３で反射されて第２光検出器１へ導かれる。これによって、検出される光の強度が増大し、とくに第２光検出器１１の収集効率が向上する。

【００４０】なお、このようなコールドミラーをシースフローセル３とレンズ１４の間に設置することも可能であるが、この場合には、コールドミラーを凹面ミラーとする必要がある上、その位置の設定が容易でない。

【００４１】これに対して、この実施形態におけるコールドミラー１３は光路コリメート領域に設けられているので、平面ミラーを用いることができると共に、その位置設定が容易である。

【００４２】

【発明の効果】この発明によれば、細胞を変形させることなくその核部分をコントラストよく撮像することができる。

【図面の簡単な説明】

【図１】この発明の実施例の光学系を示す構成図である。

【図２】この発明の実施例の信号処理系を示す構成図である。

【図３】この発明の実施例の光源のスペクトル特性図である。

【図４】この発明の実施例によって得られた細胞画像例（顕微鏡写真）である。

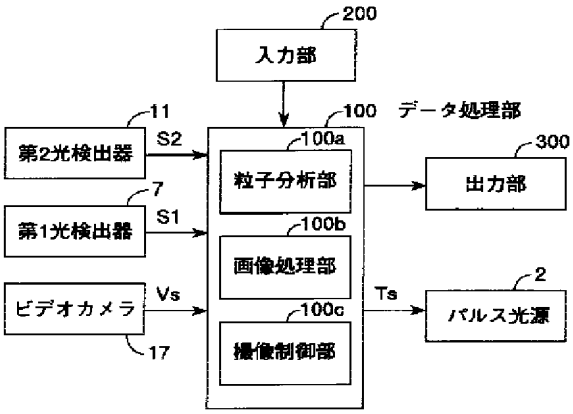
【図５】図４の画像に対する比較例を示す画像例（顕微鏡写真）である。

【符号の説明】

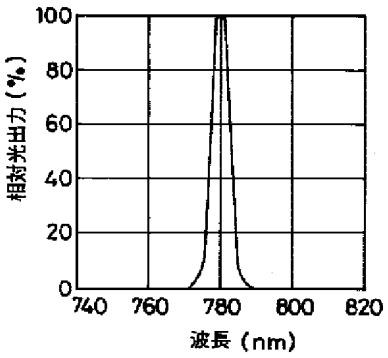
１　連続発光光源
２　パルス光源
２ａ　光ファイバー
３　シースフローセル
４　コンデンサレンズ
５　集光レンズ
６　バンドパスフィルター
７　第１光検出器
８　コリメートレンズ
９　ダイクロイックミラー
１０　コンデンサレンズ１０
１１　第２光検出器
１２　コリメートレンズ
１３　コールドミラー
１４　コンデンサレンズ
１５　コンデンサレンズ
１６　バンドパスフィルター
１７　ビデオカメラ

【図１】

【図２】

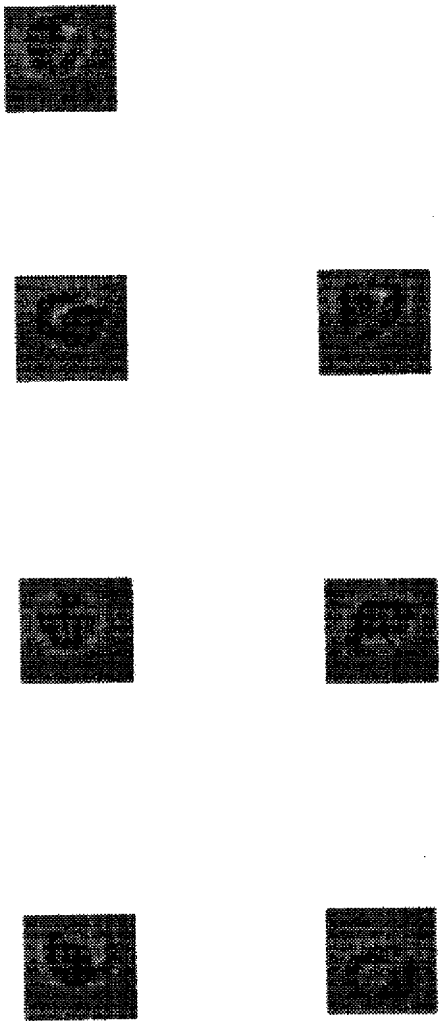


【図３】



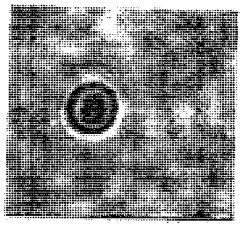
【図４】

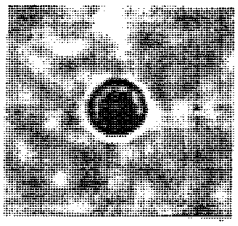
図面代用写真



【図５】

図面代用写真





Espacenet

Bibliographic data: JPH09502794 (A) — 1997-03-18

LIQUID FLOW CYTOMETER

Inventor(s):

Applicant(s):

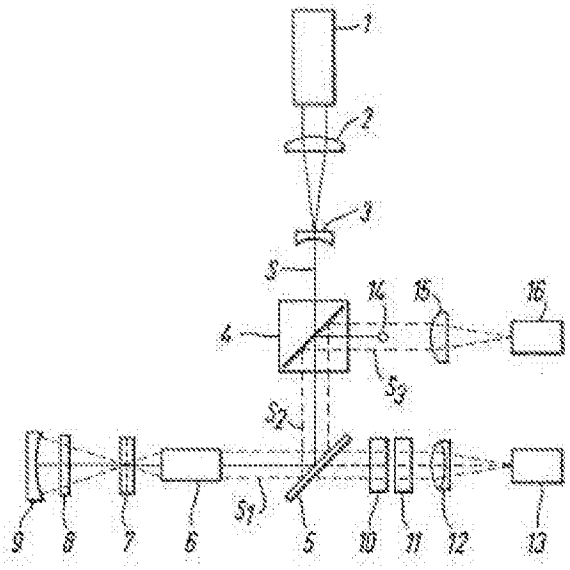
Classification: - international: ***G01N15/14; G01N21/53; G01N21/64; G01N33/49;*** (IPC1-7): G01N15/14; G01N21/53; G01N21/64; G01N33/49
- cooperative: **G01N15/14**

Application number: JP19950501624 19940608 Global Dossier

Priority number (s): WO1994NO00106 19940608 ; NO19930002088 19930608

Also published as: AU6986094 (A) DE69412646 (T2) DK0737307 (T3) EP0737307 (A1) EP0737307 (B1) more

Abstract not available for JPH09502794 (A)
Abstract of corresponding document: WO9429695 (A1)



A liquid flow cytometer for counting and classification of particles such as biological cells and other microscopic particles in liquids, where the cytometer comprises an optical system for irradiation and detection in a metering zone (7), thus enabling optical dispersion and fluorescence caused by the particles to be simultaneously detected when the particles pass the metering zone. The optical system comprises in a first beam path (S1) a mirror reflector (9) with its focal point in the metering zone (7), a quarter-wave plate (8) between the metering zone (7) and the mirror reflector (9), a lens (6) with its focal point in the metering zone (7) and located on the opposite side of it together with a dichroic mirror (5) behind the lens and provided in

【公報種別】特許法第１７条第１項及び特許法第１７条の２の規定による補正の掲載
【部門区分】第６部門第１区分
【発行日】平成１３年１１月１３日（２００１．１１．１３）

【公表番号】特表平９－５０２７９４
【公表日】平成９年３月１８日（１９９７．３．１８）
【年通号数】
【出願番号】特願平７－５０１６２４
【国際特許分類第７版】
G01N 15/14
21/53
21/64
33/49
【ＦＩ】
G01N 15/14 D
21/53 Z
21/64 Z
33/49 A

手続補正書

平成１３年６月６日

特許庁長官　殿

１．事件の表示
平成７年特許願第５０１６２４号

２．補正をする者
住所　ノルウェー国　オスロ　エヌ　１１８７，スペインバイエン　２９エイ
名称　ジェルスネス，　オッドジェーン
国籍　ノルウェー国

住所　ノルウェー国　オスロ　エヌ－１１５８，ヘラゲルドバイエン
５７ビィ
名称　タンゲン，　ライダー
国籍　ノルウェー国

３．代理人
住所　〒540-6015　大阪府大阪市中央区城見一丁目２番２７号
クリスタルタワー１５階
氏名　（７８２８）　弁理士　山本　秀策
電話（大阪）０６－６９４１－３９１０

４．補正対象書類名
請求の範囲および明細書

５．補正対象項目名
請求の範囲および明細書

６．補正の内容
6.1　請求の範囲を別紙のとおり補正します。
6.2　明細書を以下のとおり補正します。
6.2.1　明細書第２頁第２行の次に、以下の２段落を挿入します：
「
米国特許第4,662,742号は、蛍光を透過し、そして散乱光を反射するためのビームスプリッタを有する光路を含む、フローサイトメトリー装置を開示する。
EP-A-0 539 351は、蛍光のみを放出するためのフローサイトメーターを開示する。
」
6.2.2　明細書第４頁下から第４行～最下行の「その反対側に設けられたレンズと、レンズの背後に位置し、かつレンズ軸に対して実質的に横線方向に部分反射を行うように設けられたダイクロイックミラーとを有し、」を「この測定ゾーンとダイクロイックミラーとの間に設けられたレンズとを有し、このダイクロイックミラーが、レンズの軸に対して法線方向の部分反射を行うように設けられ、」に補正します。
6.2.3　明細書第５頁第１行～第２行の「それ自体公知の光源」を「光源」に補正します。
6.2.4　明細書第５頁第３行～第４行の「実質的に法線方向に」を「伝播方向の」に補正します。
6.2.5　明細書第５頁第５行の「背後に」を「次に」に補正します。
6.2.6　明細書第９頁第18行の２つの「pc.ml.」を「粒子/ml.」に補正します。

請求の範囲

１．液体中の生物細胞または他の微粒子のような粒子を計測し、そして分類するための液体フローサイトメーターであって、ここで該サイトメーターは、測定ゾーン（７）における屈折および検出のための光学系を有することによって、該粒子が該測定ゾーンを通過するときに該粒子によって発生する光散乱および蛍光を同時に検出することを可能にし、

該光学系が、第１のビーム路（S_1）内に、該測定ゾーン（７）内に焦点を有する反射鏡（９）と；該測定ゾーン（７）と該反射鏡（９）との間に位置する1/4波板（８）と；該測定ゾーン（７）内に焦点を有し、かつ該測定ゾーン（７）とダイクロイックミラー（５）との間に設けられたレンズ（６）とを有し；

該ダイクロイックミラー（５）が、該レンズ（６）の軸に対して法線方向の部分反射を行うように設けられ、

該反射の方向が該光学系の第２のビーム路（S_2）内にあり、そして該第２のビーム路（S_2）内で、該ダイクロイックミラー（５）と光源（１）との間に位置するビームスプリッタ（４）を有しており、

該ビームスプリッタ（４）が該第２のビーム路（S_2）に対して法線方向の第３のビーム路（S_3）を創出し、そして該サイトメーターがさらに、該第１のビーム路（S_1）内で、該ダイクロイックミラー（５）の次に設けられ、かつ該測定ゾーン（７）内の該粒子によって発せられた蛍光を検出するために設けられた第１の検出器（１３）と；該第３のビーム路（S_3）内に設けられ、かつ該測定ゾーン（７）内の該粒子によって生じた光散乱を検出するために設けられた第２の検出器（１６）とを有していることを特徴とする、液体フローサイトメーター。

２．前記反射鏡（９）が球面凹面鏡であることを特徴とする、請求項１に記載のサイトメーター。

３．前記ビームスプリッタ（４）が偏光ビームスプリッタであることを特徴とする、請求項１に記載のサイトメーター。

４．前記第２のビーム路（S_2）内で、前記偏光ビームスプリッタ（４）と前記光源（１）との間に、該光源（１）からの光を該ビームスプリッタ（４）上に集束するための適切なレンズ装置（２，３）が設けられていることを特徴とする、請求項３に記載のサイトメーター。

５．前記光源（１）が、前記第２のビーム路（S_2）内に設けられたダイオードレーザであることを特徴とする、請求項４に記載のサイトメーター。

６．前記ダイクロイックミラー（５）を通る蛍光を前記第１の検出器（１３）上に集束するために、該第１の検出器（１３）と該ダイクロイックフィルター（５）との間に、光学フィルター（１０、１１）が適切なレンズ装置（１２）とともに設けられていることを特徴とする、請求項５に記載のサイトメーター。

７．前記第１の検出器（１３）がフォトダイオードを含むことを特徴とする、請求項６に記載のサイトメーター。

８．前記ビームスプリッタ（４）によって反射された散乱光を前記第２の検出器（１６）上に集束するために、該第２の検出器（１６）と該ビームスプリッタ（４）との間に、ビームストップ（１４）および適切なレンズ装置（１５）が設けられていることを特徴とする、請求項１に記載のサイトメーター。

９．前記第２の検出器（１６）がフォトダイオードを含むことを特徴とする、請求項７に記載のサイトメーター。

１０．前記フォトダイオードがなだれダイオードの形態であることを特徴とする、請求項７に記載のサイトメーター。

１１．前記第２の検出器（１６）に含まれる前記フォトダイオードがPINダイオードの形態であることを特徴とする、請求項９に記載のサイトメーター。

Electronic Patent Application Fee Transmittal

Application Number:	14555102
Filing Date:	26-Nov-2014
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Filer:	Gina N. Shishima/Sean Minorini
Attorney Docket Number:	BECC.P0002US.CIP(12US0040

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
PETITION FEE- 37 CFR 1.17(H) (GROUP III)	1464	1	140	140
RCE- 1st Request	1801	1	1200	1200
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1340**

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Decision Date : July 11, 2017

In re Application of :

Yong Chen

DECISION ON PETITION

UNDER CFR 1.313(c)(2)

Application No : 14555102

Filed : 26-Nov-2014

Attorney Docket No : BECC.P0002US.CIP(12US0040

This is an electronic decision on the petition under 37 CFR 1.313(c)(2), filed July 11, 2017 , to withdraw the above-identified application from issue after payment of the issue fee.

The petition is **GRANTED.**

The above-identified application is withdrawn from issue for consideration of a submission under 37 CFR 1.114 (request for continued examination). See 37 CFR 1.313(c)(2).

Petitioner is advised that the issue fee paid in this application cannot be refunded. If, however, this application is again allowed, petitioner may request that it be applied towards the issue fee required by the new Notice of Allowance.

Telephone inquiries concerning this decision should be directed to the Patent Electronic Business Center (EBC) at 866-217-9197.

This application file is being referred to Technology Center AU 2884 for processing of the request for continuing examination under 37 CFR 1.114 .

Office of Petitions

Electronic Acknowledgement Receipt

EFS ID:	29741700
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	129381
Filer:	Gina N. Shishima/Sean Minorini
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US.CIP(12US0040
Receipt Date:	11-JUL-2017
Filing Date:	26-NOV-2014
Time Stamp:	09:03:28
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1340
RAM confirmation Number	071117INTEFSW09031901
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Petition automatically granted by EFS	petition-request.pdf	31376 084b383997a9bc21296a6edbf51695a0f2f997eb	no	2
Warnings:					
Information:					
2	Request for Continued Examination (RCE)	RCE.pdf	697818 07f9b3a75bfcd360a6a62c3a41e4d173b9f9d61a	no	3
Warnings:					
Information:					
3	Quick Path Information Disclosure Statement	QPIDS.pdf	342872 8e885376b3eb6986aef495f97f2ee394e0d41d98	no	2
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	1449.pdf	127279 20ae246c6ed175e4a21a2e9393f04ee4e5335448	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
5	Non Patent Literature	NPL.pdf	1045992 cb7f9ec561af0656db67430671f94c0ed93f5a0c	no	12
Warnings:					
Information:					
6	Foreign Reference	B36.pdf	634796 d2471baa21206fbee52ba177848a47cf44670f28	no	10
Warnings:					

Information:					
7	Foreign Reference	B37.pdf	678878 d52329dde8c778fafdfd032c221297c728a6a009	no	15
Warnings:					
Information:					
8	Foreign Reference	B38.pdf	677055 f2c39211d1b513fea03d38ecab998703e65fd229	no	19
Warnings:					
Information:					
9	Foreign Reference	B39.pdf	369806 0497c2a27f721d8ba206e308b0c82d9081126b67	no	7
Warnings:					
Information:					
10	Foreign Reference	B40.pdf	152304 27547795cb98fe00c713f51c6ced40d13048bd55	no	3
Warnings:					
Information:					
11	Fee Worksheet (SB06)	fee-info.pdf	32044 ed5cf2208000e41b5714c78ff11f1d5a54e94761	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	4790220	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: PET.AUTO Document Description: Petition automatically granted by EFS-Web	PTO/SB/140 U.S. Patent and Trademark Office Department of Commerce

Electronic Petition Request	**PETITION TO WITHDRAW AN APPLICATION FROM ISSUE AFTER PAYMENT OF THE ISSUE FEE UNDER 37 CFR 1.313(c)**
Application Number	14555102
Filing Date	26-Nov-2014
First Named Inventor	Yong Chen
Art Unit	2884
Examiner Name	MINDY VU
Attorney Docket Number	BECC.P0002US.CIP(12US0040
Title	FLOW CYTOMETER

An application may be withdrawn from issue for further action upon petition by the applicant. To request that the Office withdraw an application from issue, applicant must file a petition under this section including the fee set forth in § 1.17(h) and a showing of good and sufficient reasons why withdrawal of the application from issue is necessary.

APPLICANT HEREBY PETITIONS TO WITHDRAW THIS APPLICATION FROM ISSUE UNDER 37 CFR 1.313(c).

A grantable petition requires the following items:
(1) Petition fee; and
(2) One of the following reasons:
(a) Unpatentability of one or more claims, which must be accompanied by an unequivocal statement that one or more claims are unpatentable, an amendment to such claim or claims, and an explanation as to how the amendment causes such claim or claims to be patentable;
(b) Consideration of a request for continued examination in compliance with § 1.114 (for a utility or plant application only); or
(c) Express abandonment of the application. Such express abandonment may be in favor of a continuing application, but not a CPA under 37 CFR 1.53(d).

Petition Fee

○	Small Entity
○	Micro Entity
◉	Regular Undiscounted

Reason for withdrawal from issue

◯ One or more claims are unpatentable ◉ Consideration of a request for continued examination (RCE) (List of Required Documents and Fees) ◯ Applicant hereby expressly abandons the instant application (any attorney/agent signing for this reason must have power of attorney pursuant to 37 CFR 1.32(b)).
RCE request,submission, and fee. ☐ I certify, in accordance with 37 CFR 1.4(d)(4) that : The RCE request ,submission, and fee have already been filed in the above-identified application on ☒ Are attached.
THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES I certify, in accordance with 37 CFR 1.4(d)(4) that I am: ◉ An attorney or agent registered to practice before the Patent and Trademark Office who has been given power of attorney in this application. ◯ An attorney or agent registered to practice before the Patent and Trademark Office, acting in a representative capacity. ◯ A sole inventor ◯ A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application ◯ A joint inventor; all of whom are signing this e-petition

Signature	/Gina N. Shishima/
Name	Gina N. Shishima
Registration Number	45104

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 07/12/2017

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01 FC : 1806 180.00 DA

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/555,102	11/26/2014	Yong Qin Chen	BECC.P0002US.CIP(12US0040	4589

129381 7590 07/27/2017

NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

EXAMINER
VU, MINDY D

ART UNIT	PAPER NUMBER
2884	

NOTIFICATION DATE	DELIVERY MODE
07/27/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

aoipdocket@nortonrosefulbright.com
PatentDocket@Beckman.com

Corrected Notice of Allowability	Application No. 14/555,102	Applicant(s) CHEN, YONG QIN	
	Examiner MINDY VU	Art Unit 2884	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *submissions of July 11, 2017*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *364-382 and 387*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 07/11/17
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☐ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/DAVID PORTA/
Supervisory Patent Examiner, Art Unit 2884

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP/11412161	Serial No.: 14/555,102
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: IRIS INTERNATIONAL, INC.	
	Filing Date: November 26, 2014	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 1*	*See Page 1*

U.S. Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A169	5,257,917	11/02/93	Minarik et al.	417	475	10/02/92

Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	B36	JP H04-184241	07/01/92	Japan	Japanese (English Abstract)
	B37	JP 2004-341204	12/02/04	Japan	Japanese (English Abstract)
	B38	JP 2012-026837	02/09/12	Japan	Japanese (English Abstract)
	B39	JP H10-73528	03/17/98	Japan	Japanese (English Abstract)
	B40	JP H09-502794	03/18/97	Japan	Japanese (English Abstract)

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C6	Office Action issued in Japanese Patent Application No. 2015-515208, issued May 29, 2017 (English Translation Provided) (Attorney Docket No. BECC.P0002JP)

42540182.1

EXAMINER: /MINDY D VU/	DATE CONSIDERED: 07/21/2017

EXAMINER: INITIAL IF REFERENCE CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED. INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.V/

Electronic Acknowledgement Receipt

EFS ID:	29741700
Application Number:	14555102
International Application Number:	
Confirmation Number:	4589
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	129381
Filer:	Gina N. Shishima/Sean Minorini
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US.CIP(12US0040
Receipt Date:	11-JUL-2017
Filing Date:	26-NOV-2014
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The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

Electronic Patent Application Fee Transmittal

Application Number:	14555102
Filing Date:	26-Nov-2014
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Filer:	Gina N. Shishima/Sean Minorini
Attorney Docket Number:	BECC.P0002US.CIP(12US0040

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
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RCE- 1st Request	1801	1	1200	1200
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

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ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 64 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

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IRIS INTERNATIONAL, INC., Chatsworth, CA;

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