PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: IRIS INTERNATIONAL, INC.	Group Art Unit: Not yet assigned
Serial No.: Filed Concurrently	Examiner: Not yet assigned
Filed: June 30, 2017	Atty. Dkt. No.: BECC.P0002US.CIP.C1/11706355
For: FLOW CYTOMETER	Confirmation No.: Not yet assigned

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

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June 30, 2017	/Gina N. Shishima/
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.

Amendment to the Specification beginning on page 2.

Amendments to the Claims beginning on page 3.

Remarks begin on page 8.

CYTEK V. BECKMAN
IPR2025-01319
BECKMAN 2016

AMENDMENT TO THE SPECIFICATION

Please replace the first full paragraph on page 1 of the Specification with the following amended paragraph:

This application is a continuation of U.S. Patent Application No. 14/555,102 filed November 26, 2014, which 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~~ U.S. Patent Application No. 14/555,102 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.

AMENDMENT TO THE CLAIMS

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

1-363. (cancelled)

364. (New) An optical module for a flow cytometer, the optical module comprising:

(a) a light source;

(b) a collimating optical element arranged to receive light from the light source, the collimating optical element configured to project a collimated beam; and

(c) an optical relay arranged to receive at least a portion of the collimated beam from the collimating optical element, the optical relay configured to project at least a portion of the collimated beam to a first focusing optical element,

wherein the optical relay is one of a curved mirror and a refractive lens.

365. (New) The optical module of claim 364, wherein the light source is one of light passing through a pinhole and light emitted from a facet of a multimode optical fiber.

366. (New) The optical module of claim 365, wherein the first focusing optical element is configured to focus the collimated beam to a size smaller than 1 millimeter in diameter to a first semiconductor detector.

367. (New) The optical module of claim 364, wherein the light source forms an object, wherein the collimating optical element is configured to project a magnified image of the object, wherein the optical relay 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.

368. (New) The optical module of claim 364, wherein the optical relay is a concave-shaped dichroic filter.

369. (New) The optical module of claim 364, wherein the collimating optical element is an achromatic doublet lens.

370. (New) The optical module of claim 364, further comprising a first optical filter arranged between the collimating optical element and the optical relay, the first optical filter configured to separate the collimated beam into a first branch and a second branch.

371. (New) The optical module of claim 370, wherein the collimated beam comprises light of a plurality of wavelengths, wherein the first optical filter is configured to reflect light of a first range of wavelengths to form the first branch of the collimated beam, wherein the first optical filter is configured to pass light of a second range of wavelengths to form the second branch of the collimated beam, wherein the first branch is directed to the optical relay.

372. (New) The optical module of claim 371, further comprising a second focusing optical element arranged to receive the second branch of the collimated beam, the second focusing optical element configured to focus the second branch of the collimated beam to a second semiconductor detector.

373. (New) The optical module of claim 372, wherein the diameter of the collimated beam between the optical relay and the first focusing optical element is substantially the same as the diameter of the collimated beam between the collimating optical element and the second focusing optical element.

374. (New) The optical module of claim 372, further comprising a second optical filter, the second optical filter arranged between the optical relay and the first focusing optical element, the second optical filter configured to pass light of a third range of wavelengths in the first branch of the collimated beam to the first focusing optical element, wherein the third range of wavelengths is a subset of the first range of wavelengths.

375. (New) The optical module of claim 374, wherein successive combinations of optical relay, optical filter, and focusing optical element are cascaded to produce focused spots having a diameter of less than 1 millimeter for multiple ranges of wavelengths of light of the collimated beam.

376. (New) An optical module for a flow cytometer, the optical module comprising:

(a) a light source;

(b) a collimating optical element arranged to receive light from the light source, the collimating optical element configured to project a collimated beam and a first image, wherein the collimated beam has a collimated distance; and

(c) an optical relay arranged to receive the collimated beam, the optical relay configured to extend the collimated distance of the collimated beam.

377. (New) The optical module of claim 376, wherein the optical relay is one of a curved mirror and a refractive lens.

378. (New) The optical module of claim 376, wherein the optical relay projects a second image, wherein the second image and the first image are substantially the same size.

379. (New) The optical module of claim 376, wherein the optical relay projects a second image, wherein the collimated beam from the collimating optical element to the first image has substantially the same diameter as the collimated beam from the optical relay to the second image.

380. (New) The optical module of claim 376, further comprising a first focusing optical element arranged to receive the collimated beam from the optical relay at the extended collimated distance, the first focusing optical element configured to focus the extended collimated beam to a first semiconductor detector.

381. (New) The optical module of claim 380, further comprising:

an optical filter arranged between the collimating optical element and the optical relay, the optical filter configured to separate the collimated beam into a first branch and a second branch, wherein the first branch and the second branch are different colors, wherein the optical relay receives the first branch; and

a second focusing optical element arranged to receive the second branch, the second focusing optical element configured to focus the second branch to a second semiconductor detector.

382. (New) The optical module of claim 380, wherein the optical distance from the collimating optical element to the optical relay is substantially the same as the optical distance from the optical relay to the first focusing optical element.

383. (New) An optical module for a flow cytometer, the optical module comprising:

(a) a light source providing light that forms and object;

(b) a collimating optical element arranged to receive light from the light source, the collimating optical element configured to project a first image of the object; and

(c) an optical relay arranged near the first image, the optical relay configured to receive light from the collimating optical element and to project a second image of the object; wherein the size of the first image is substantially the same as the size of the second image.

384. (New) The optical module of claim 383, wherein the optical distance from the collimating optical element to the first image is substantially the same as the optical distance from the optical relay to the second image.

385. (New) The optical module of claim 383, wherein the light source is an extended light source that forms the object, wherein the first image is a magnified image of the object.

386. (New) The optical module of claim 385, wherein the light source is one of light passing through a pinhole and light emitted from a facet of a multimode optical fiber.

387. (New) The optical module of claim 385, wherein the collimating optical element has an effective size defined by its cross section, wherein the size of the first image is substantially the same as the effective size of the collimating optical element.

388. (New) The optical module of claim 385, wherein the first image is less than approximately 10 times the size of the object.

389. (New) The optical module of claim 385 further comprising a first focusing optical element arranged near the second image, the first focusing optical element configured to focus the light from the optical relay to a size smaller than the object of the light source to a first semiconductor detector.

REMARKS

The specification has been amended to update the priority claim paragraph.

The claims have been amended without prejudice or disclaimer to manage the claims fees. Claims 1-363 have been canceled. Claims 364-389 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 Norton Rose Fulbright US LLP Deposit Account No. 50-1212/BECC.P0002US.CIP.C1/11706355.

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/

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

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Date: June 30, 2017

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

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☐ 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.)

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Address					
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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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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:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

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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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/Sean Minorini
Attorney Docket Number:	BECC.P0002US.CIP.C1

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

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	6	80	480
Miscellaneous Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**2480**

Electronic Acknowledgement Receipt

EFS ID:	29659253
Application Number:	15638461
International Application Number:	
Confirmation Number:	9847
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	32425
Filer:	Gina N. Shishima/Sean Minorini
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US.CIP.C1
Receipt Date:	30-JUN-2017
Filing Date:	
Time Stamp:	09:29:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

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Payment was successfully received in RAM	$2480
RAM confirmation Number	063017INTEFSW09311000
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	ADS.pdf	1823403 9c7f2cce1337f7bbba78b386071f88c4dea25fb2	no	9
Warnings:					
Information:					
2		Specification.pdf	505440 305d2b2731b7eaee7f3b934cff9c1679e1ad6b54	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-only black and white line drawings	Figures.pdf	529991 c8151d7b037cda22e2059cb1b55625ba51d5413e	no	35
Warnings:					
Information:					
4	Preliminary Amendment	prelimamend.pdf	119969 7205cfa623669ca2edde936f285702a7fa06641f	no	8
Warnings:					
Information:					
5	Power of Attorney	POA.pdf	121286 21bf1e7c3997c5b3c737a450d4138b3a32f0e4cb	no	1

Warnings:					
Information:					
6	Oath or Declaration filed	Oath.pdf	63613 182c557bb1bdf14df0127166a46e0c30673fef2e	no	1
Warnings:					
Information:					
7	Fee Worksheet (SB06)	fee-info.pdf	38305 c3d7122b17f4193c4c9d383a2e9c7ca3d20b42dc	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	3202007		

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<u>National Stage of an International Application under 35 U.S.C. 371</u>
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US.CIP.C1
	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	State/Province	Country of Residence
San Jose	CA	US

Mailing Address of Inventor:

Address 1	4097 Cortona Court
Address 2	

City	State/Province	Postal Code	Country
San Jose	CA	95135	US

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

Correspondence Information:

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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.CIP.C1	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	35	**Suggested Figure for Publication (if any)**	

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US.CIP.C1
	Application Number	
Title of Invention	FLOW CYTOMETER	

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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).
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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, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim 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" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	14/555102	2014-11-26

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US.CIP.C1
	Application Number	
Title of Invention	FLOW CYTOMETER	

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14/555102	Claims benefit of provisional	61/911859	2013-12-04
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14/555102	Continuation in part of	PCT/US2013/043453	2013-05-30
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/653245	2012-05-30
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/653328	2012-05-30
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/715819	2012-10-18
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/715836	2012-10-19
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2013/043453	Claims benefit of provisional	61/816819	2013-04-29
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Foreign Priority Information:

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US.CIP.C1
	Application Number	
Title of Invention	FLOW CYTOMETER	

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. 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(i)(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).

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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.
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US.CIP.C1
	Application Number	
Title of Invention	FLOW CYTOMETER	

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When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US.CIP.C1
	Application Number	
Title of Invention	FLOW CYTOMETER	

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Applicant 1 Remove

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● 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	

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Name of the Deceased or Legally Incapacitated Inventor:

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Organization Name	IRIS INTERNATIONAL, INC.		
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US.CIP.C1
	Application Number	
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Signature	/Gina N. Shishima/			Date (YYYY-MM-DD)	2017-06-30
First Name	Gina N.	Last Name	Shishima	Registration Number	45104

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	BECC.P0002US.CIP.C1
	Application Number	
Title of Invention	FLOW CYTOMETER	

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet 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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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.

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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 μm, 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.

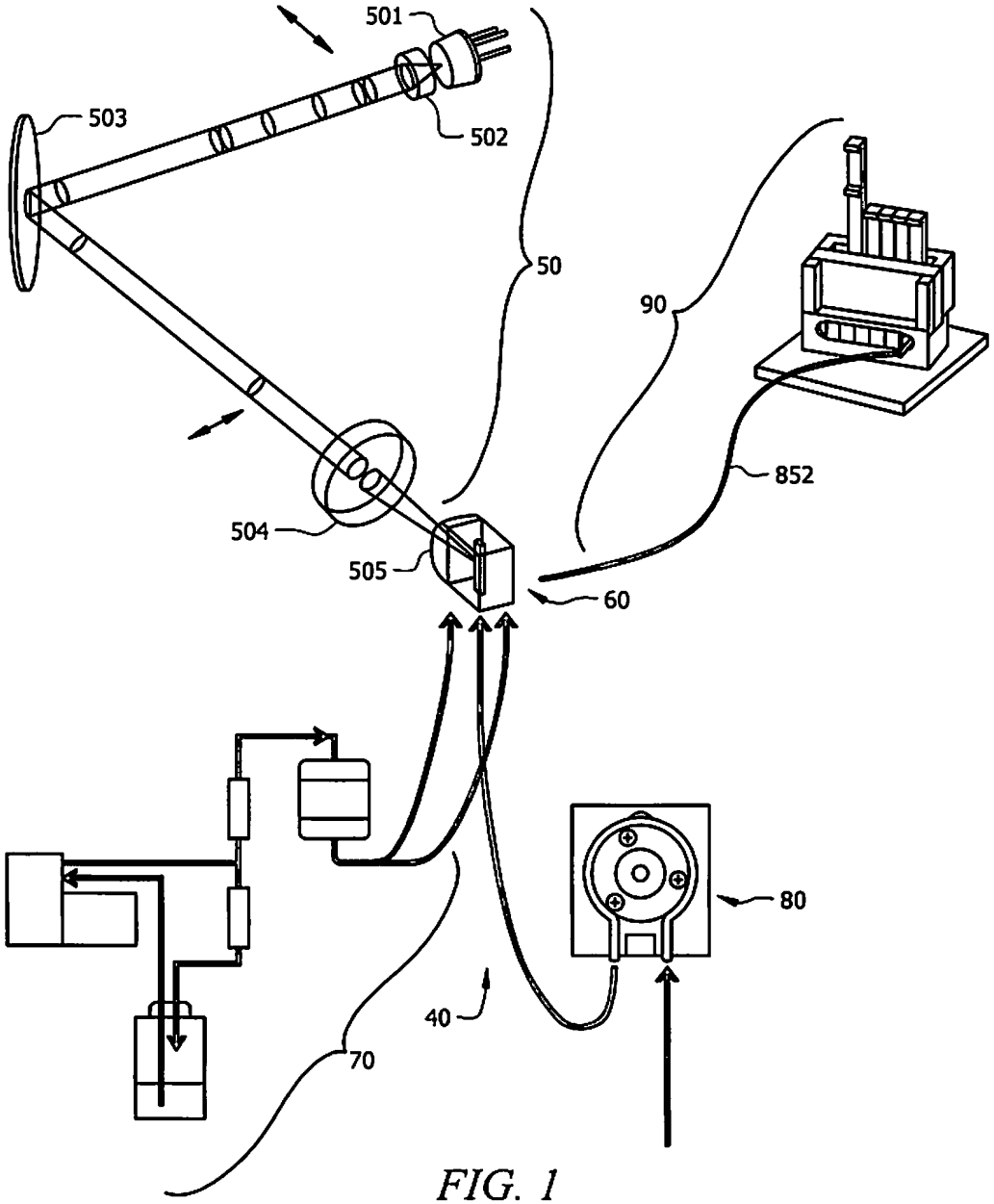


FIG. 1

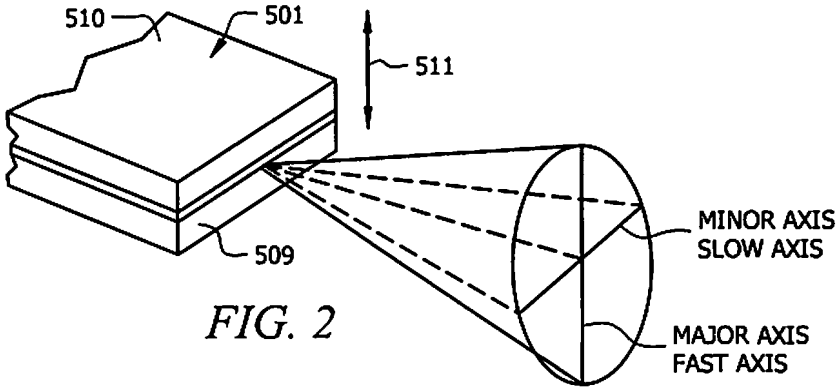


FIG. 2

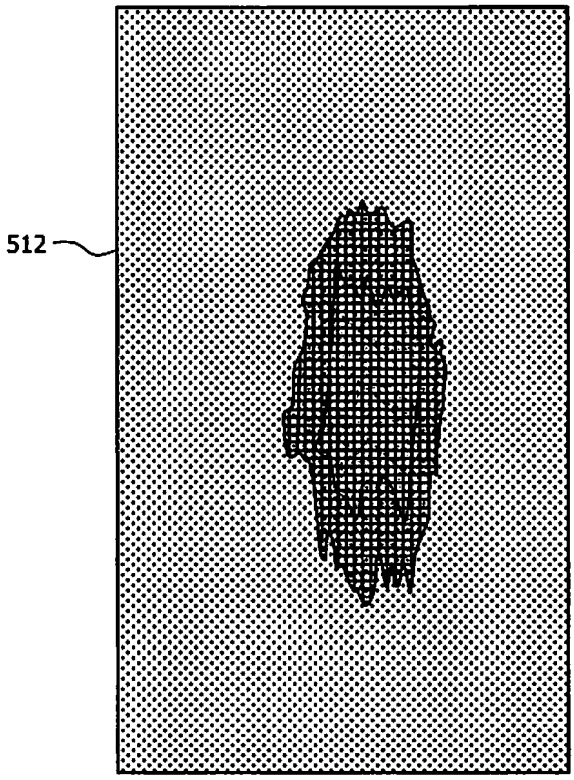


FIG. 2A

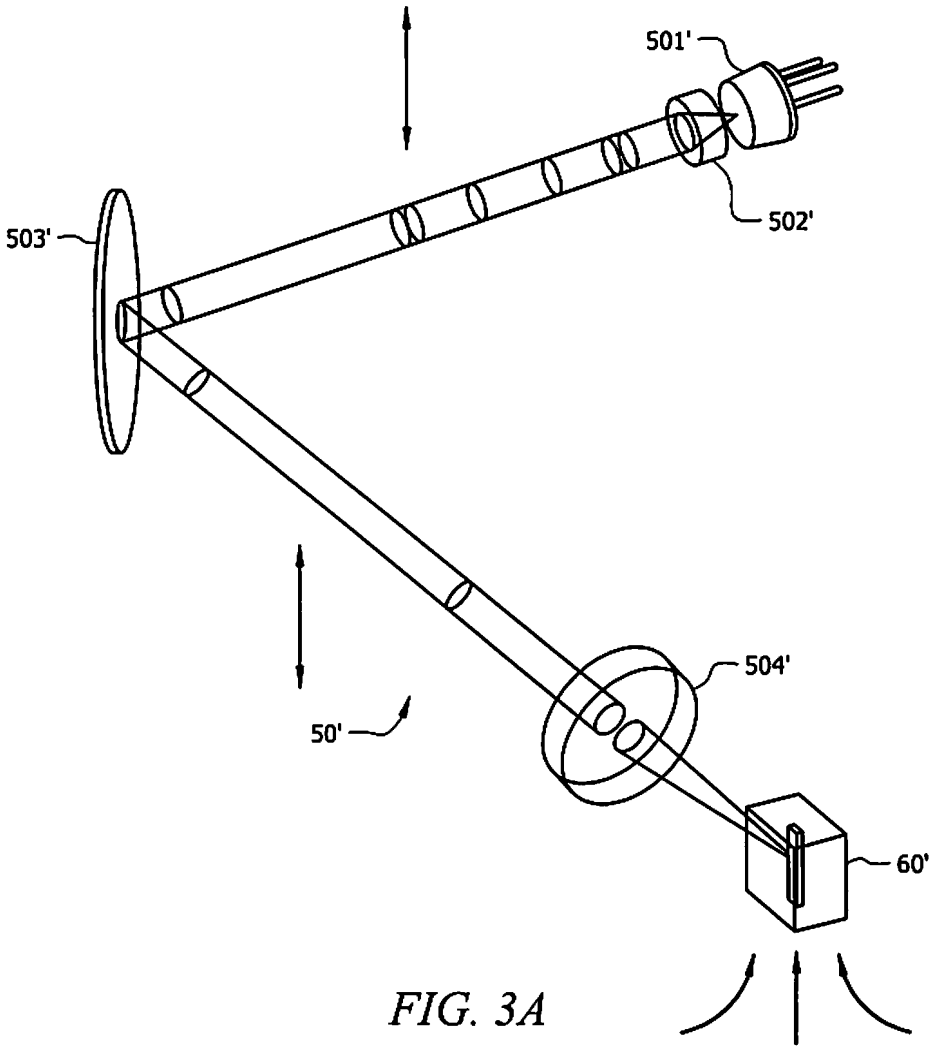


FIG. 3A

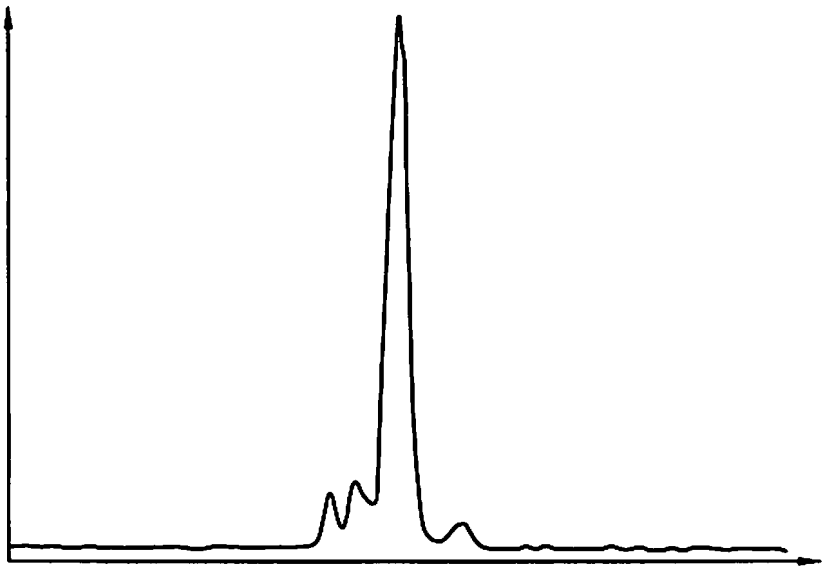


FIG. 3B

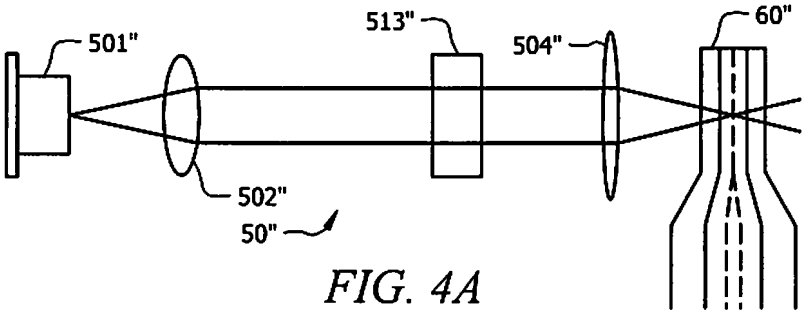


FIG. 4A

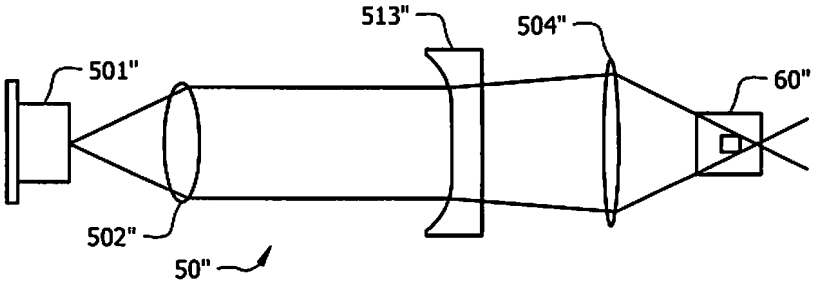


FIG. 4B

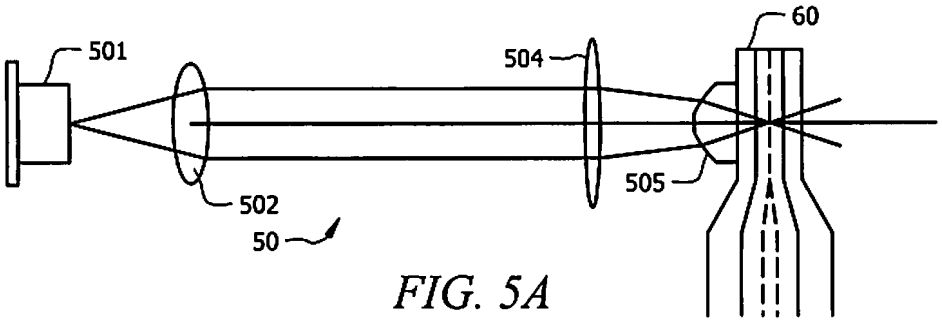


FIG. 5A

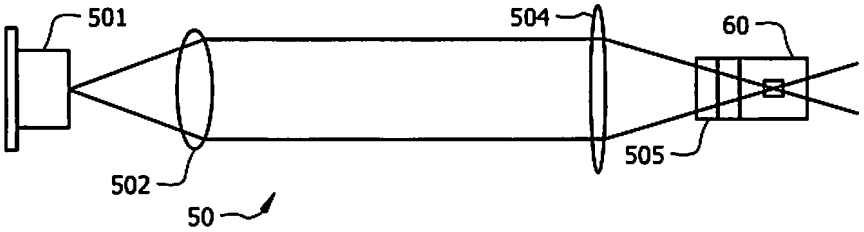


FIG. 5B

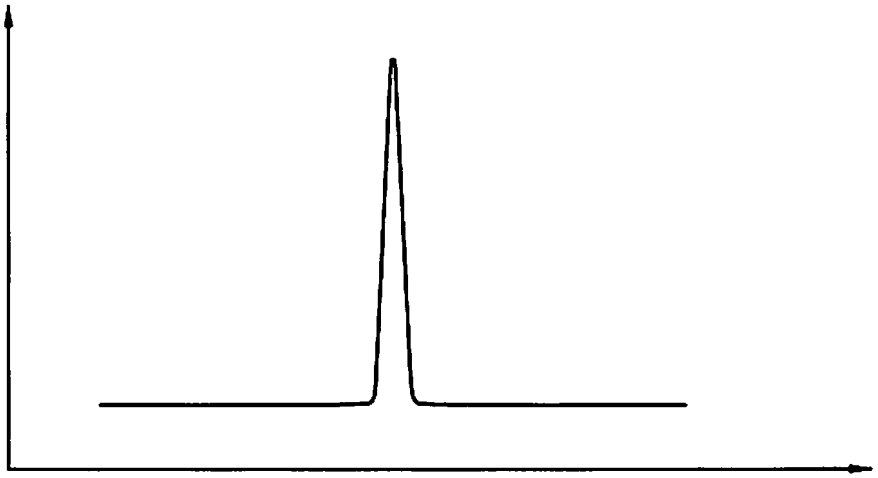


FIG. 5C

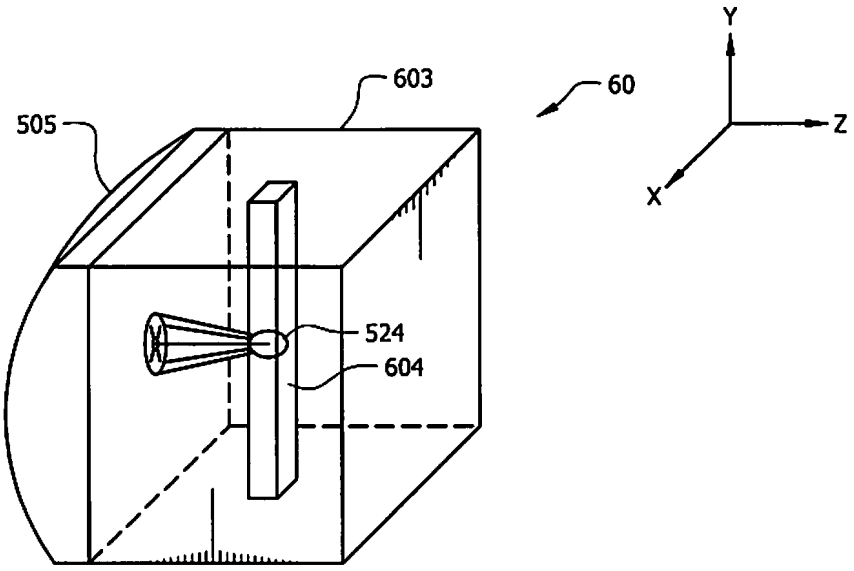


FIG. 5D

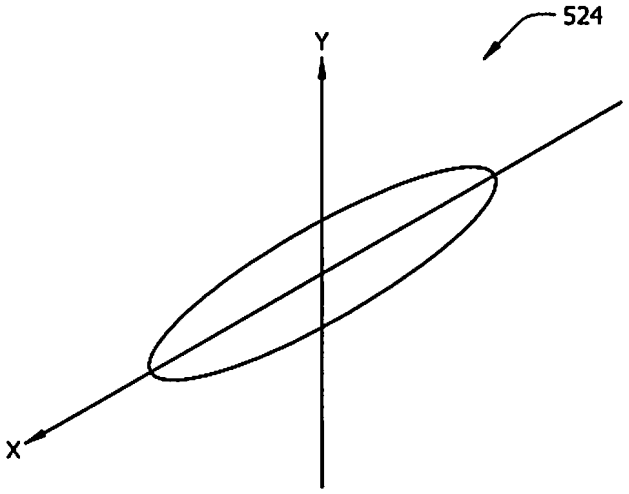


FIG. 5E

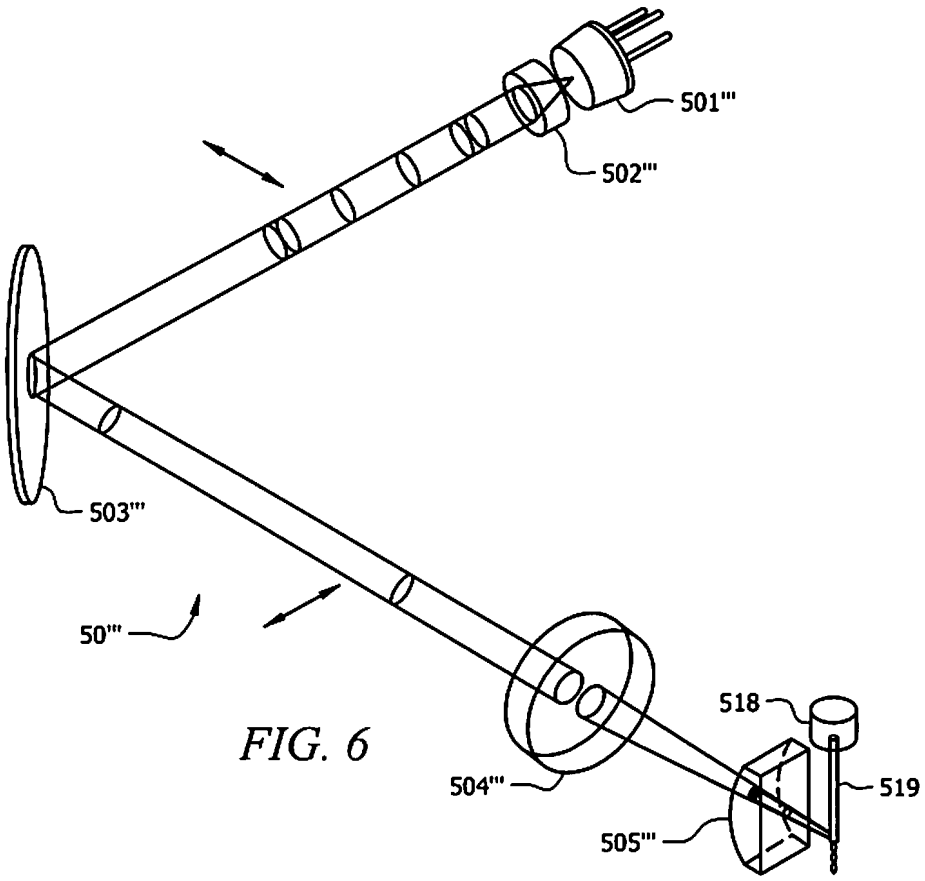


FIG. 6

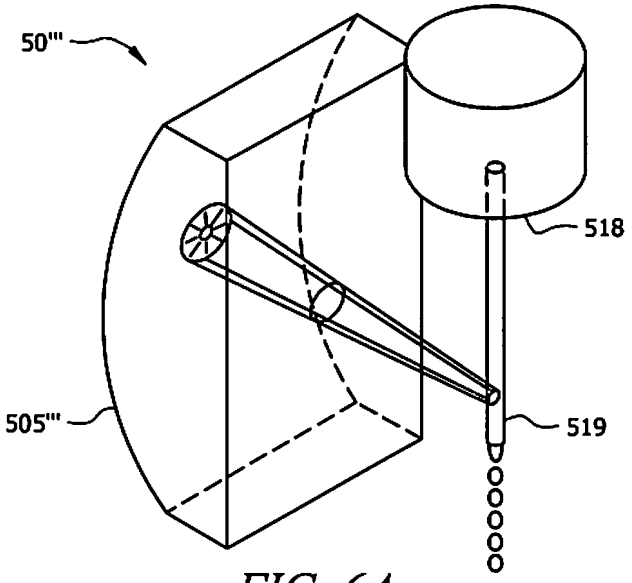


FIG. 6A

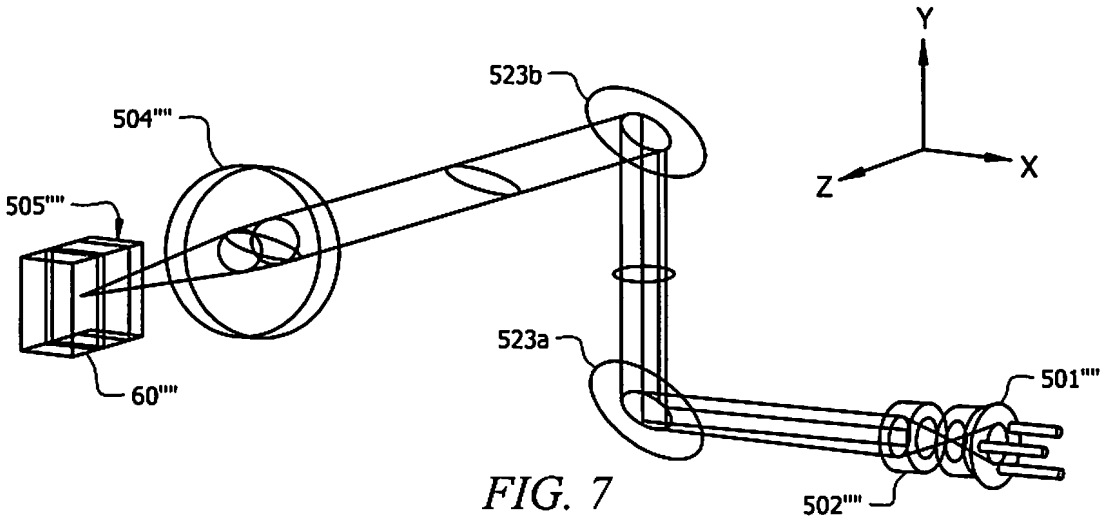


FIG. 7

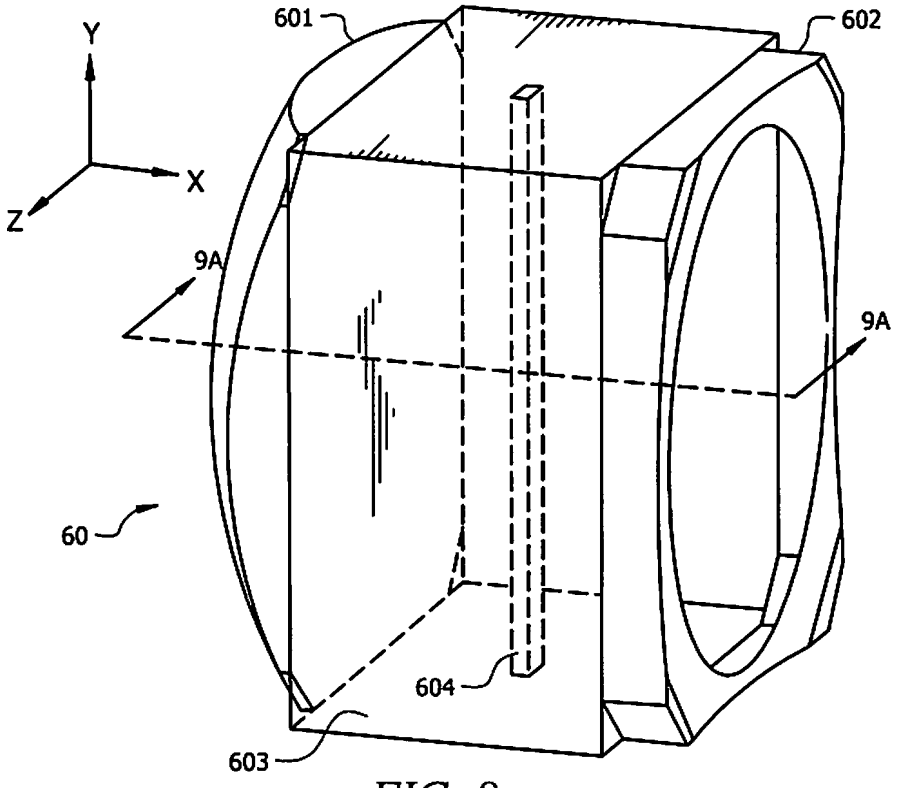


FIG. 8

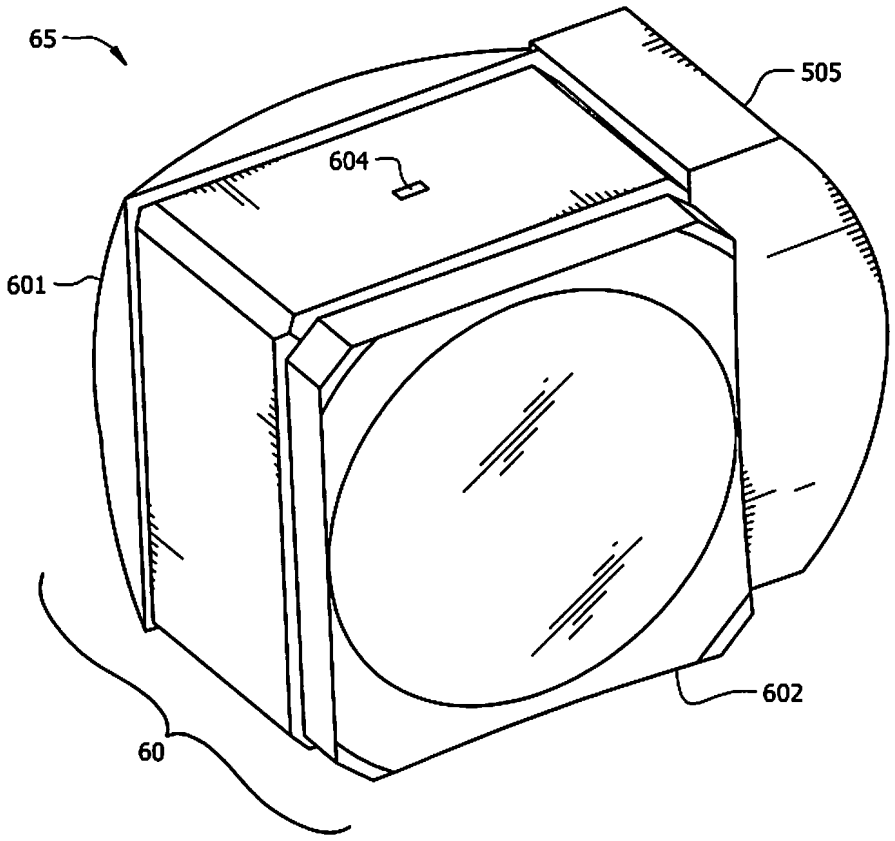


FIG. 8A

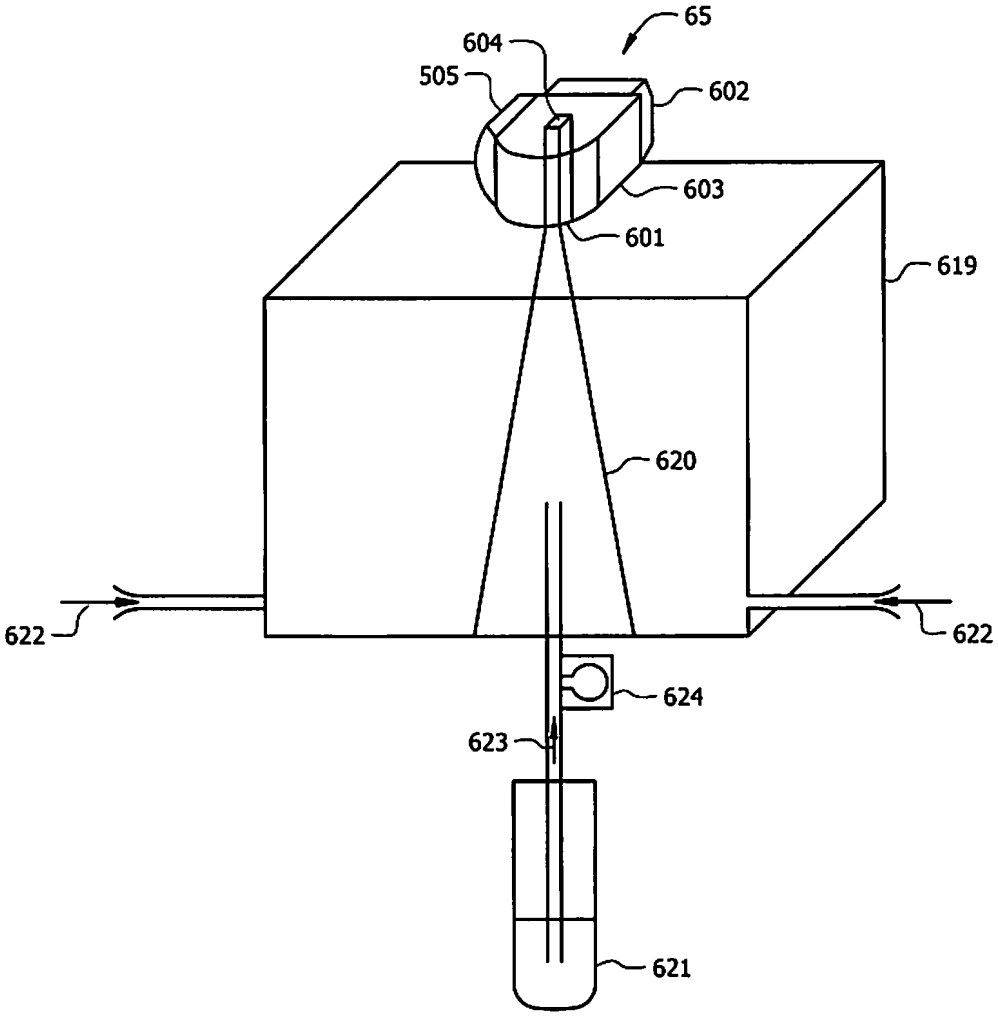


FIG. 8B

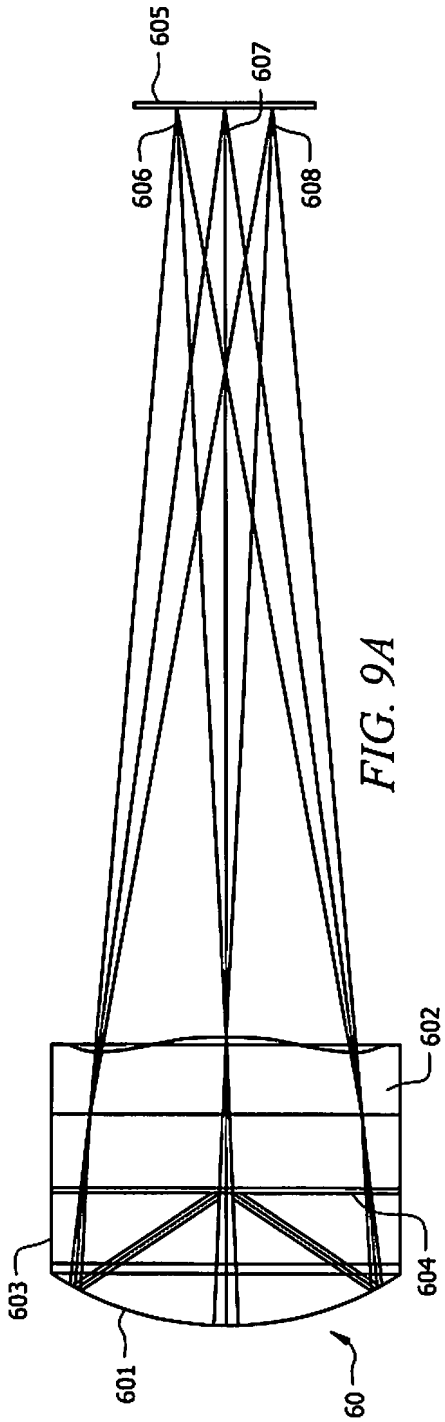


FIG. 9A

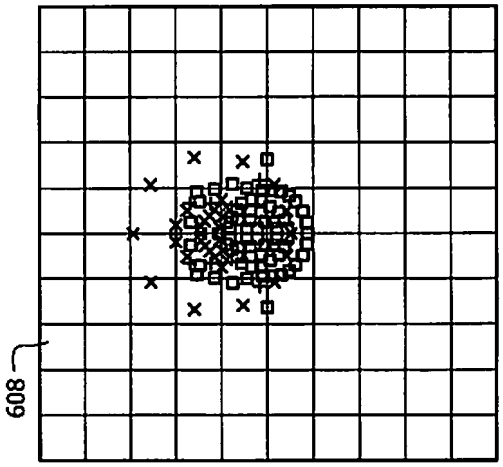


FIG. 9B3

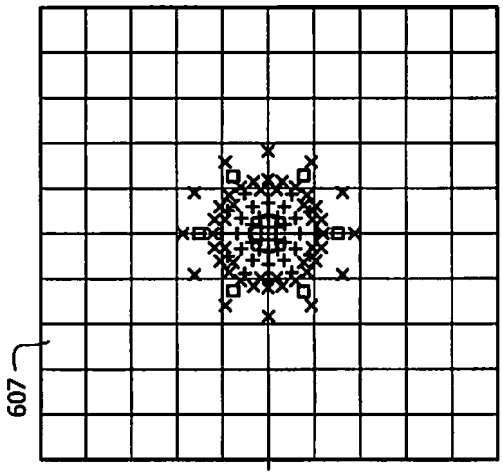


FIG. 9B2

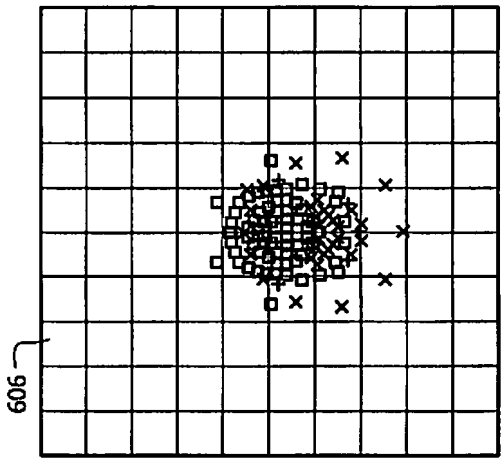


FIG. 9B1

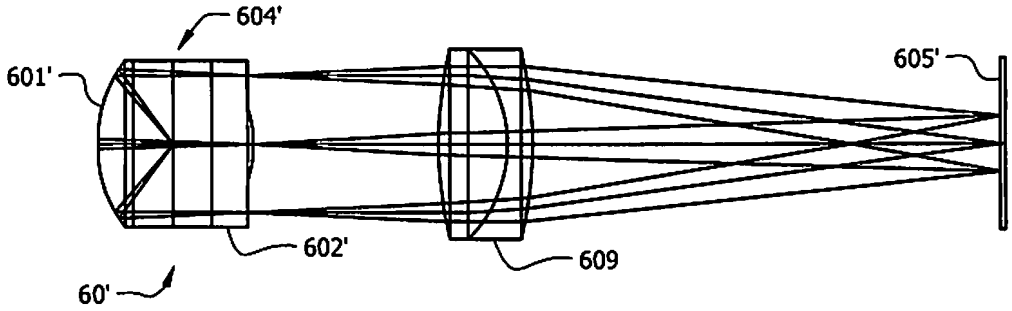


FIG. 10

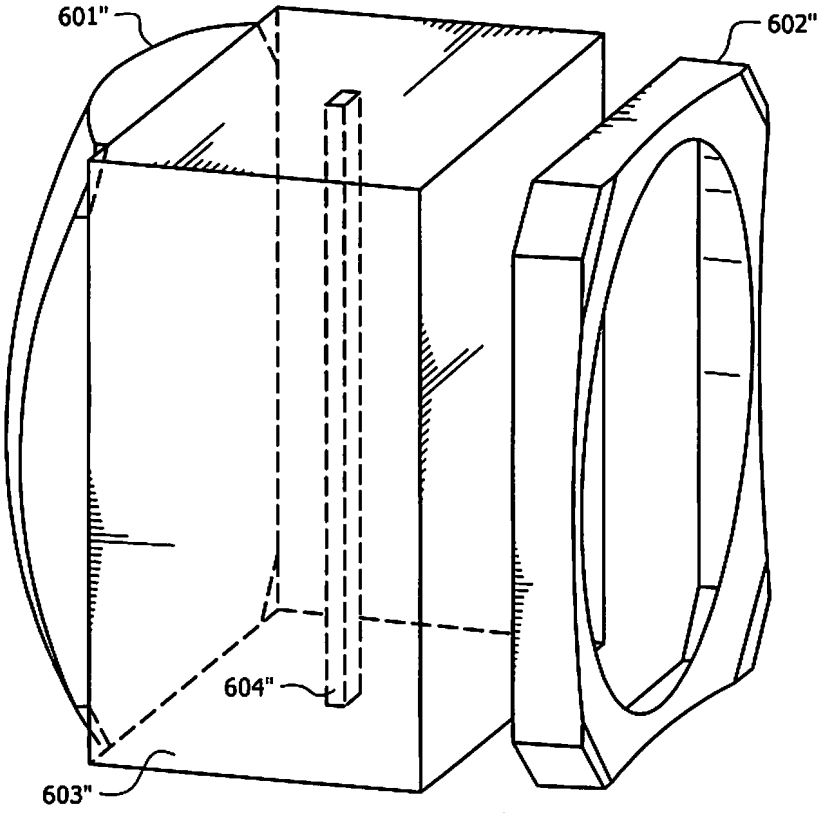


FIG. 11

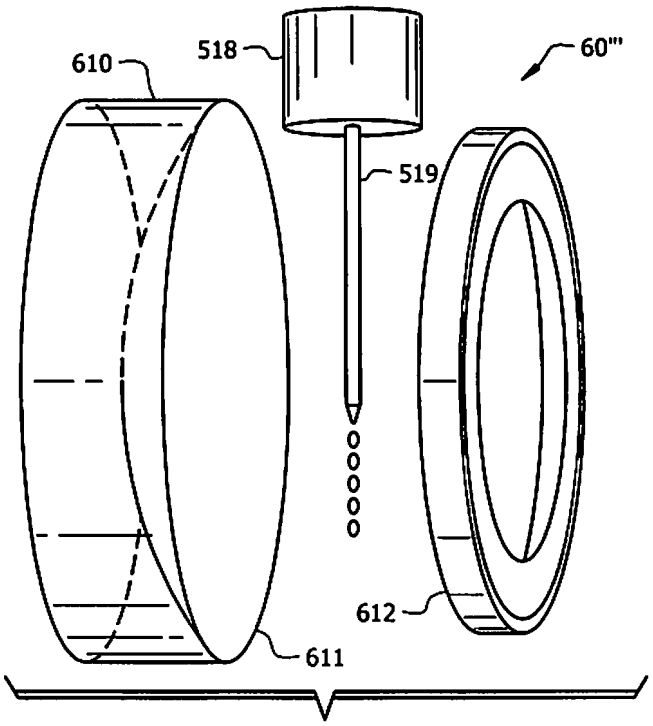


FIG. 12

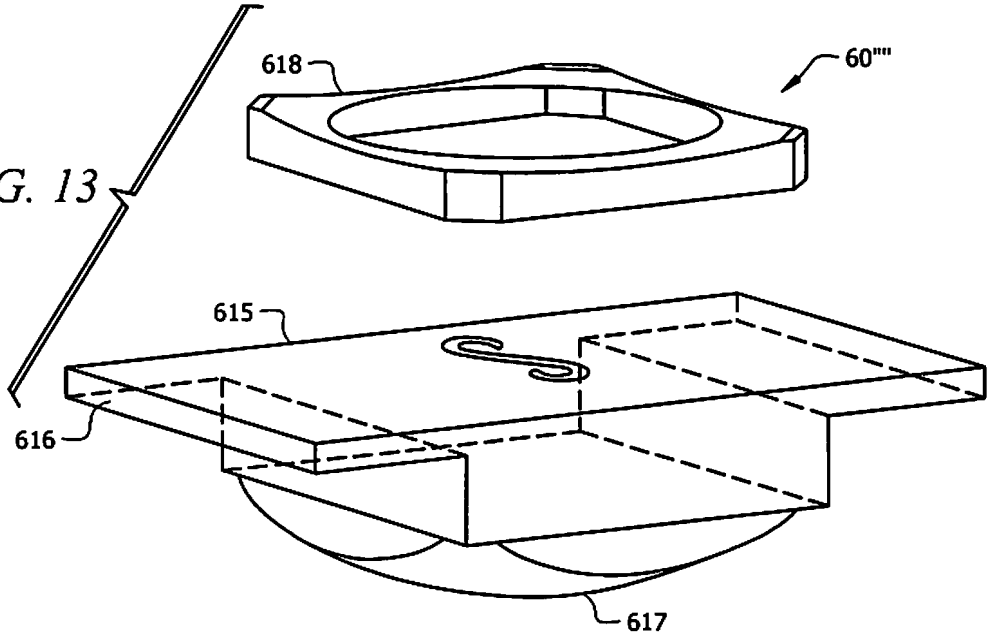


FIG. 13

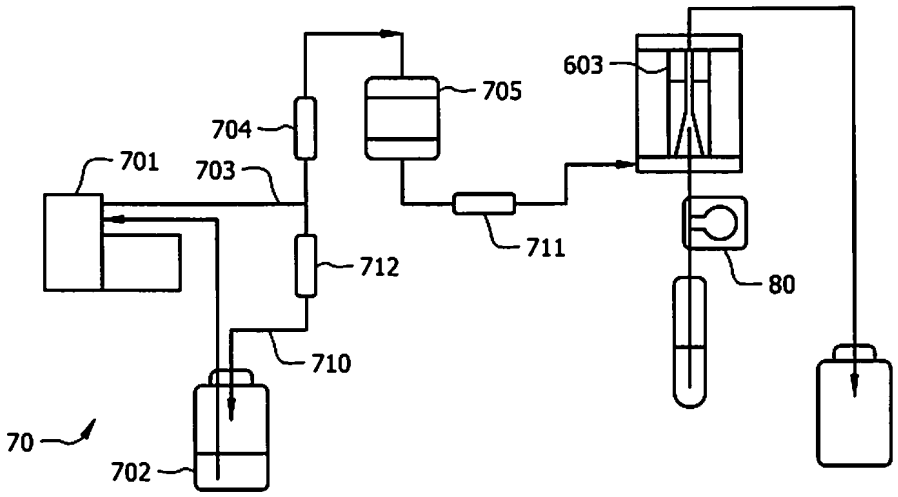

603
705
704
701
703
712
711
80
710
70
702

FIG. 14

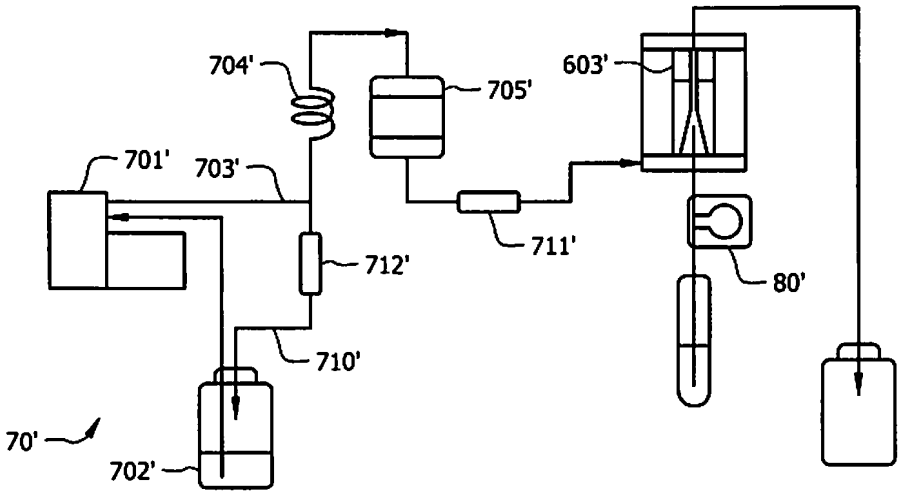

704'
603'
705'
701'
703'
712'
711'
80'
710'
70'
702'

FIG. 15

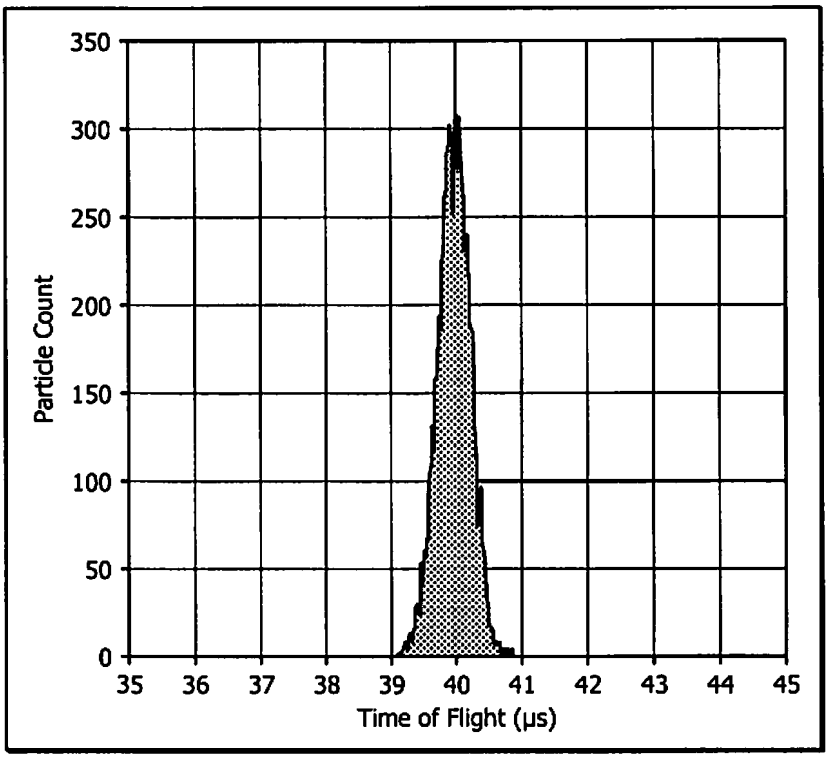


FIG. 16A

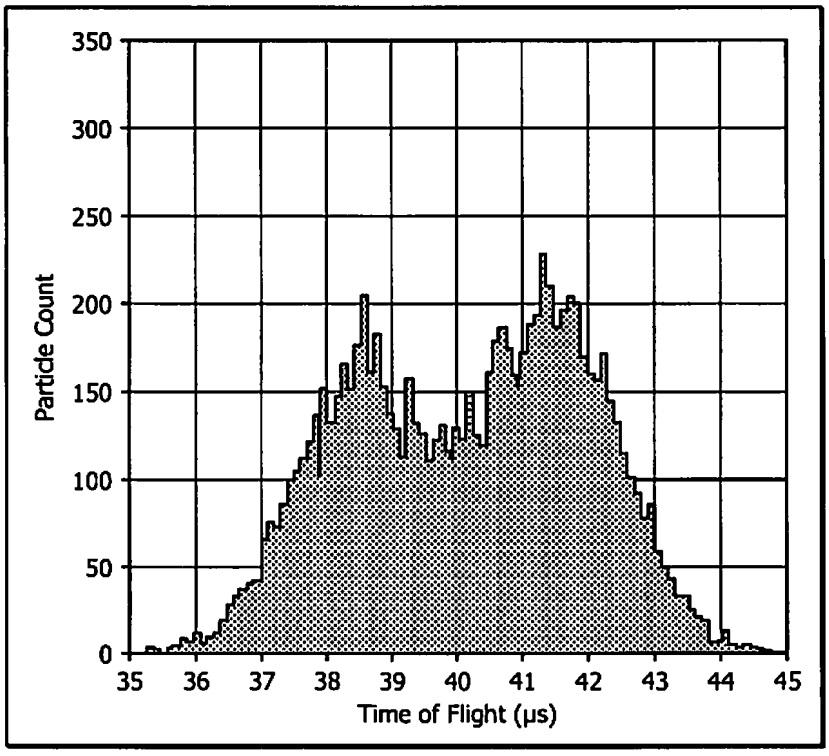


FIG. 16B

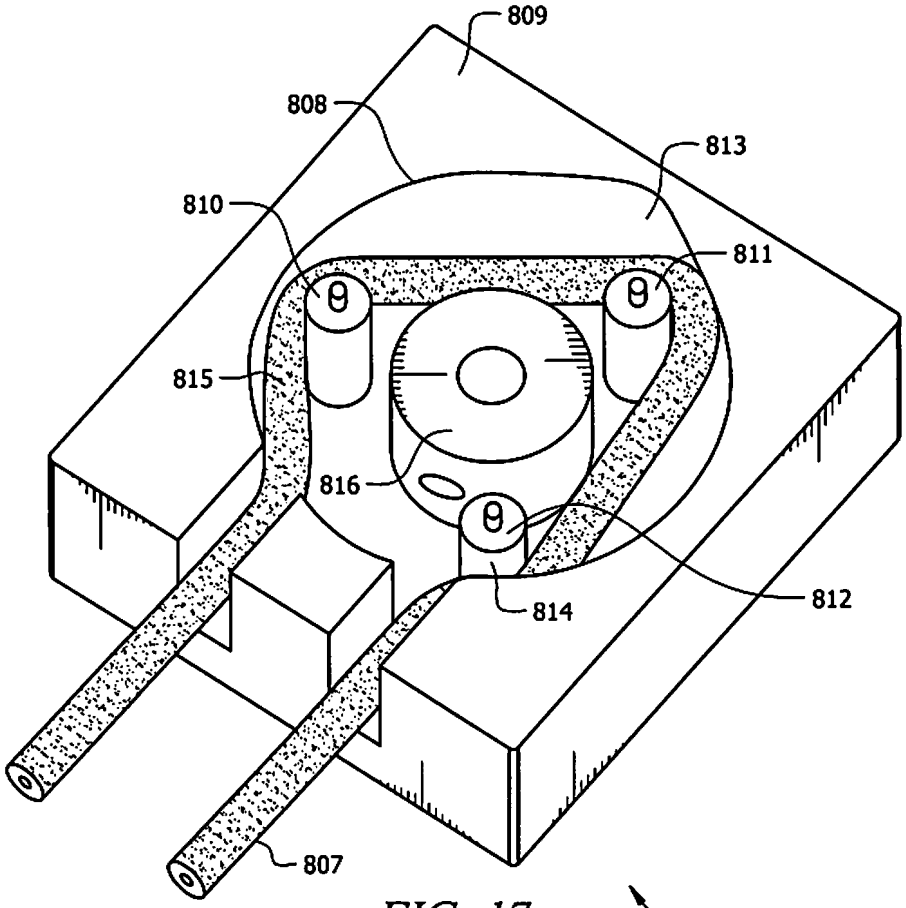


FIG. 17

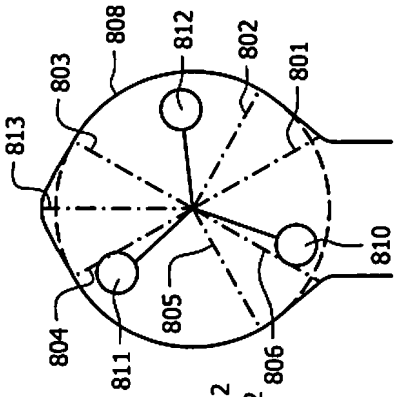


FIG. 18D

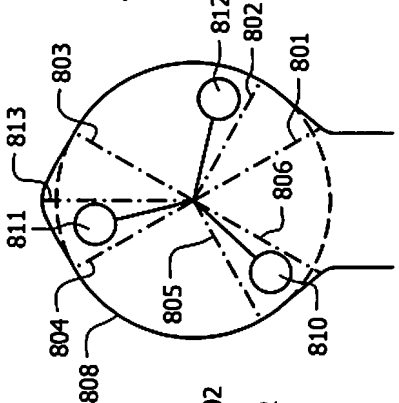


FIG. 18C

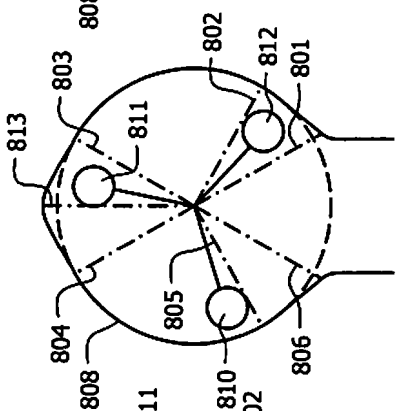


FIG. 18B

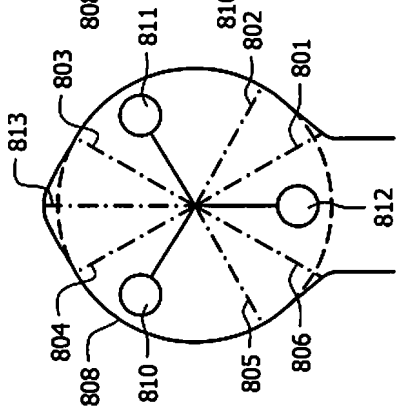


FIG. 18A

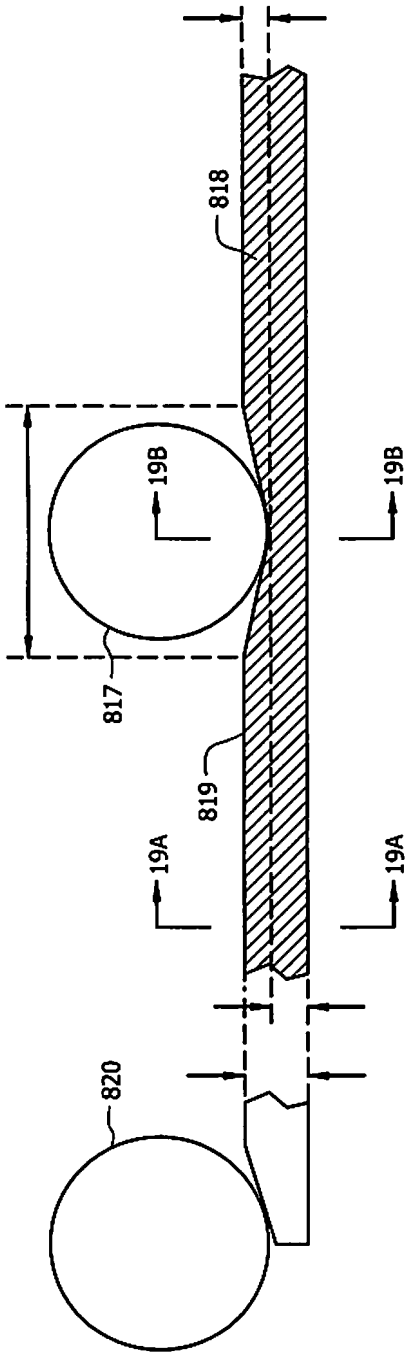


FIG. 19

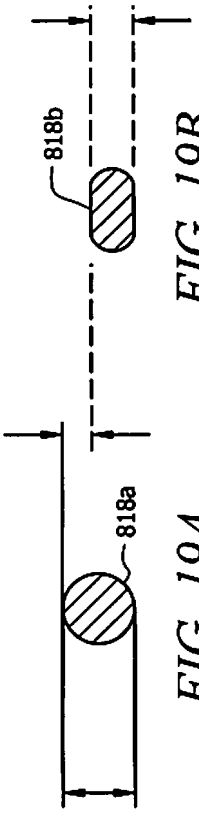


FIG. 19A

FIG. 19B

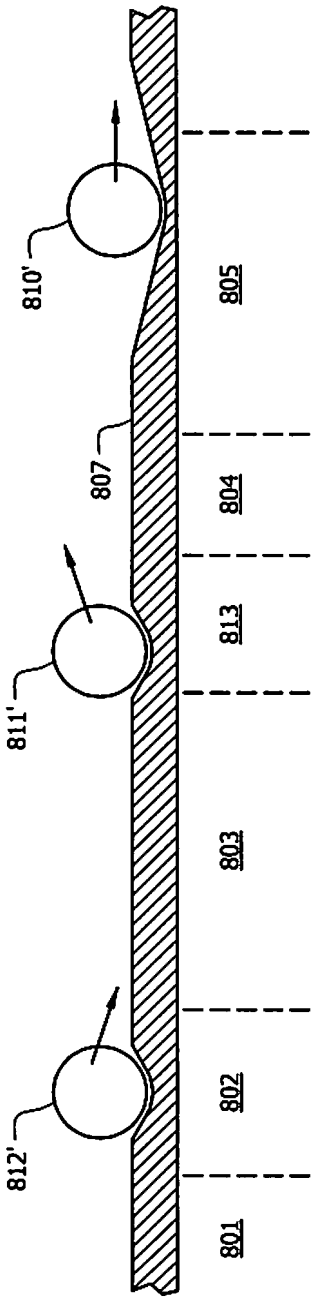


FIG. 20A

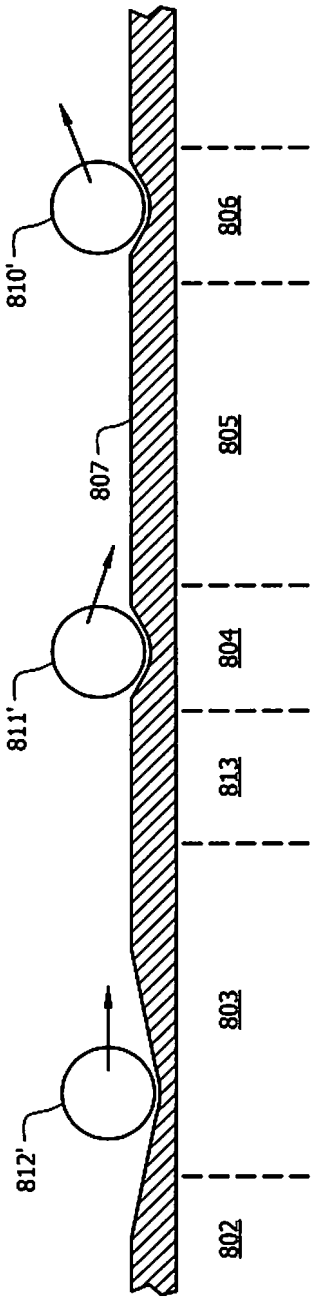


FIG. 20B

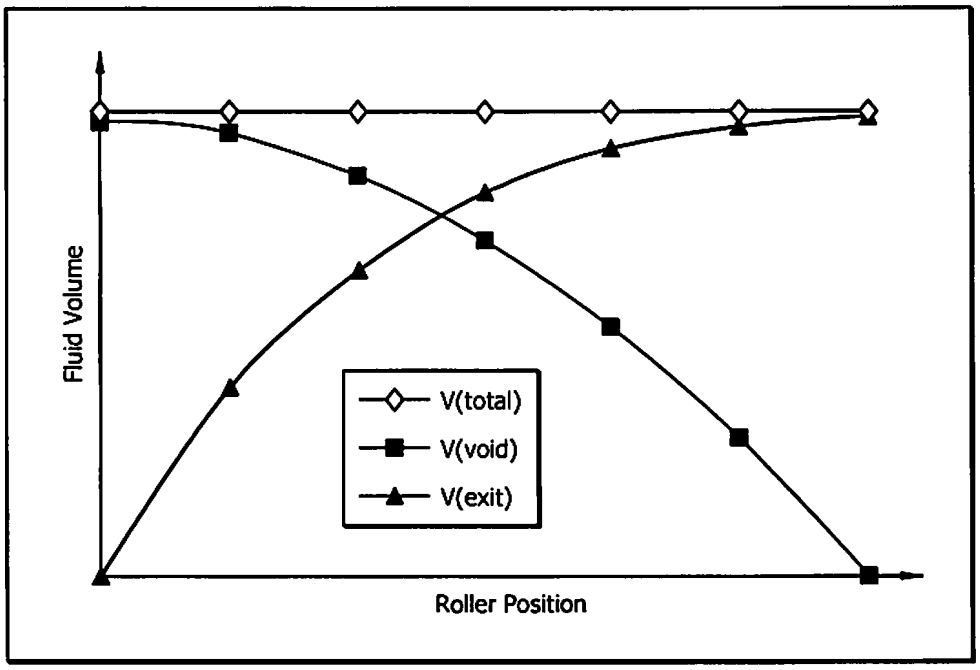


FIG. 21

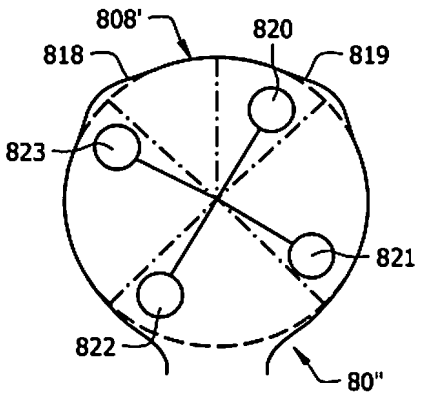


FIG. 22

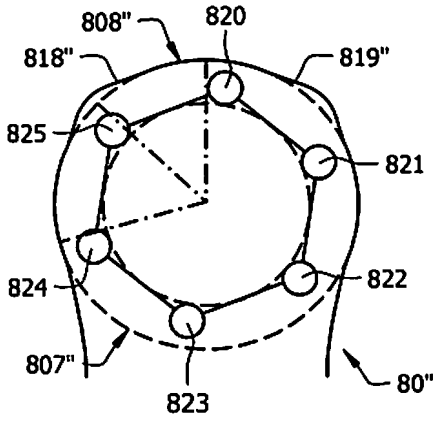


FIG. 23

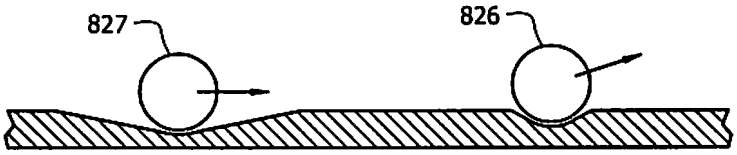


FIG. 24A

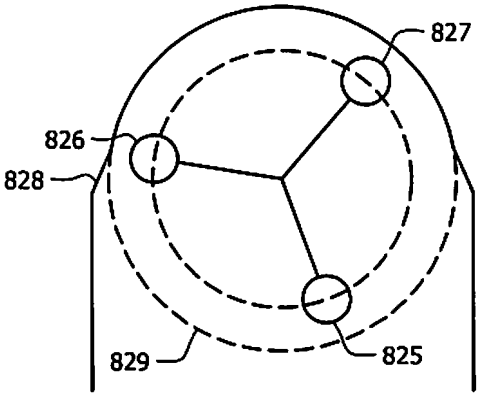


FIG. 24B

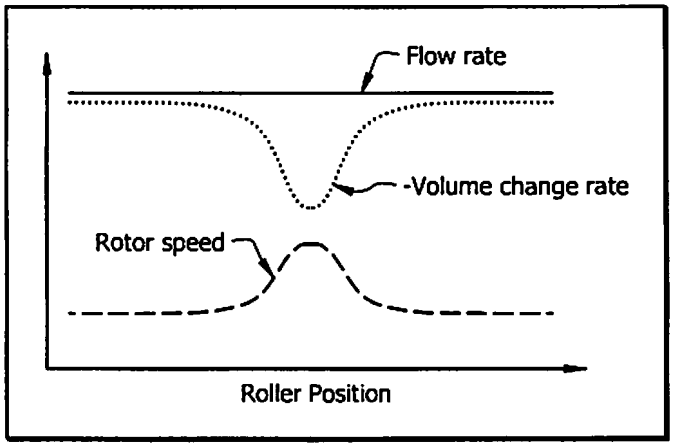


FIG. 24C

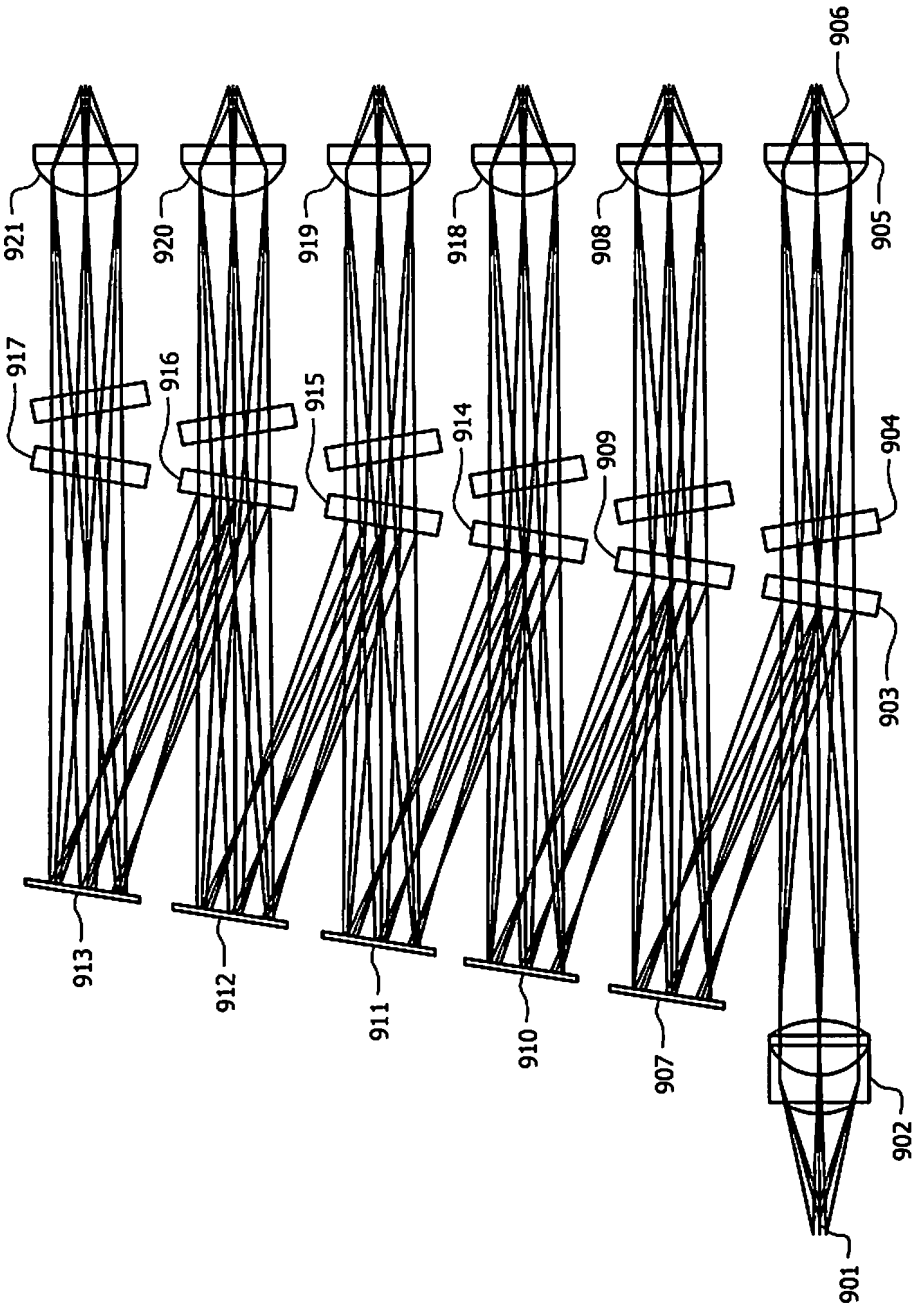

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FIG. 25

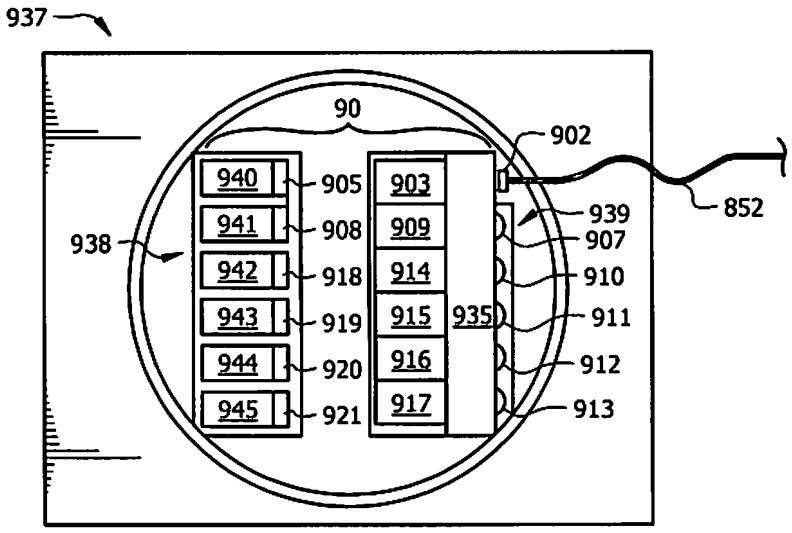


FIG. 25A

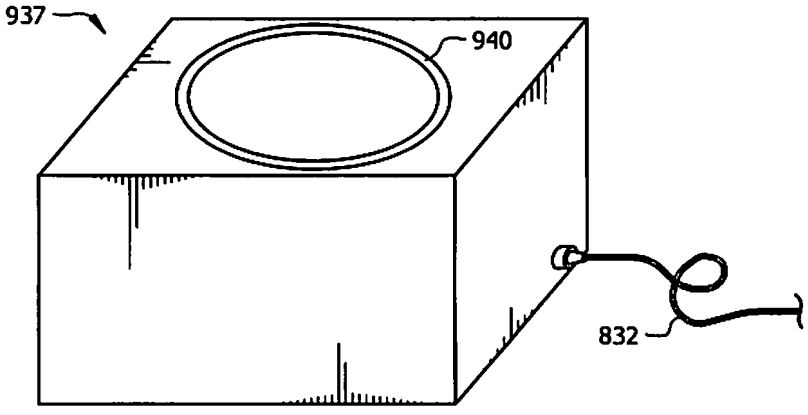


FIG. 25B

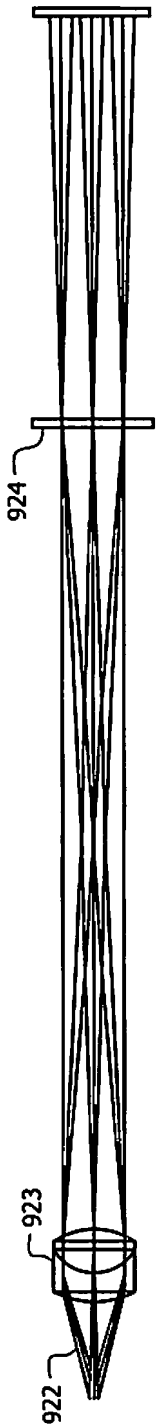


FIG. 26

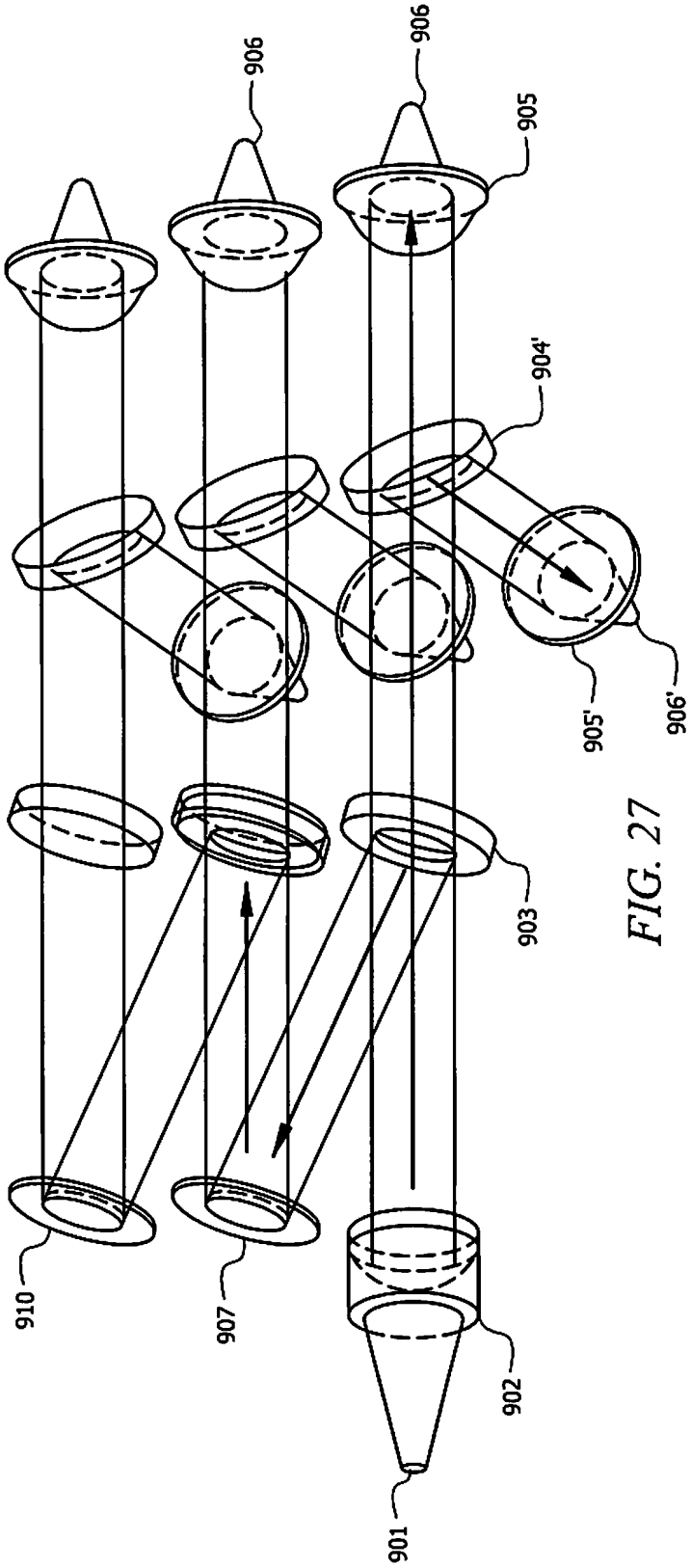


FIG. 27

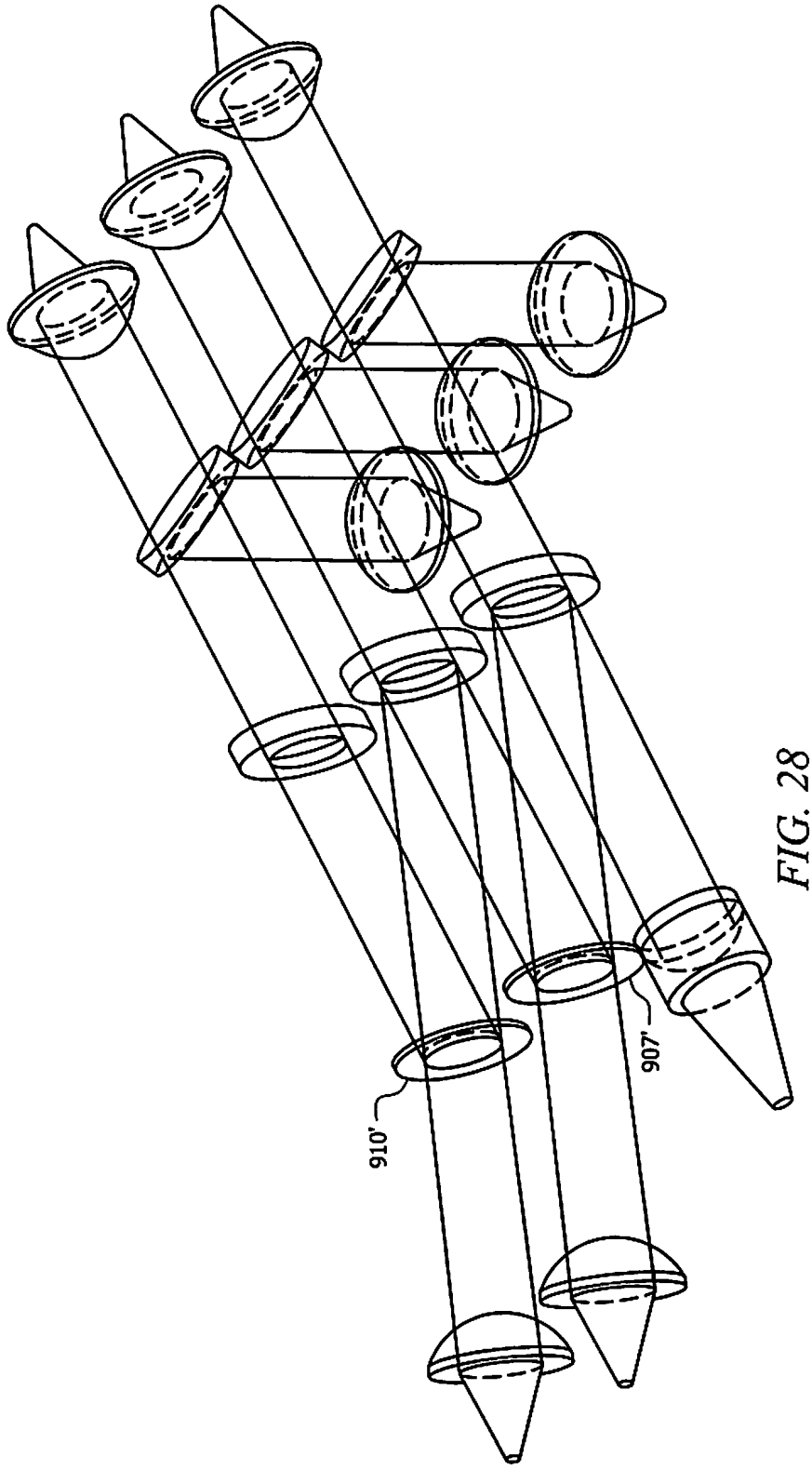


FIG. 28

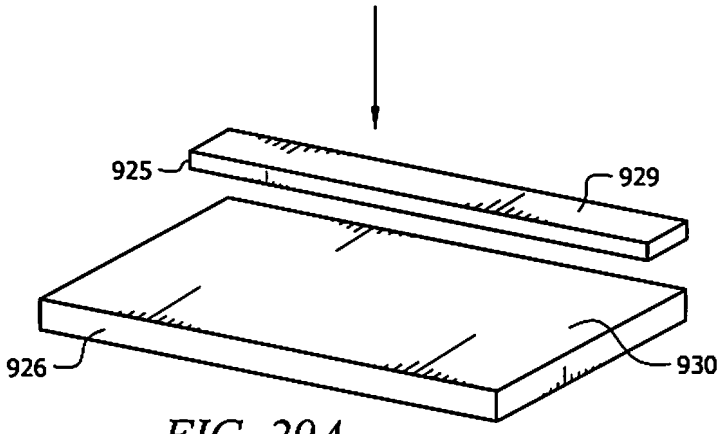


FIG. 29A

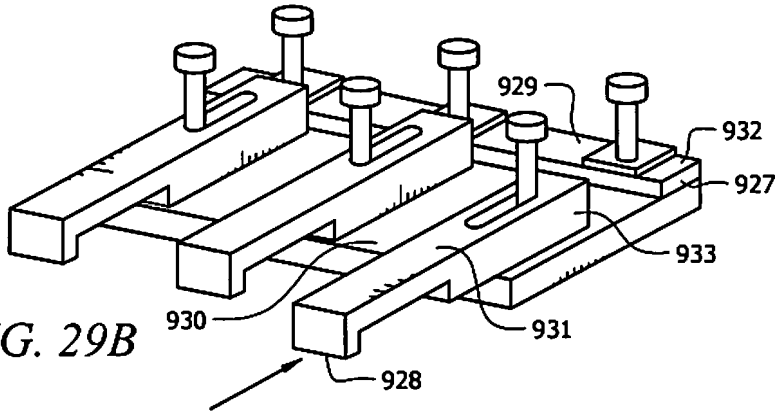


FIG. 29B

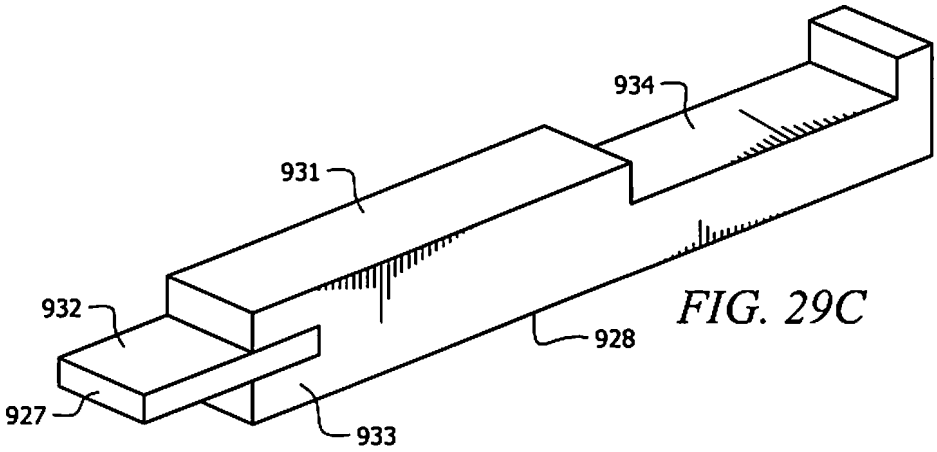


FIG. 29C

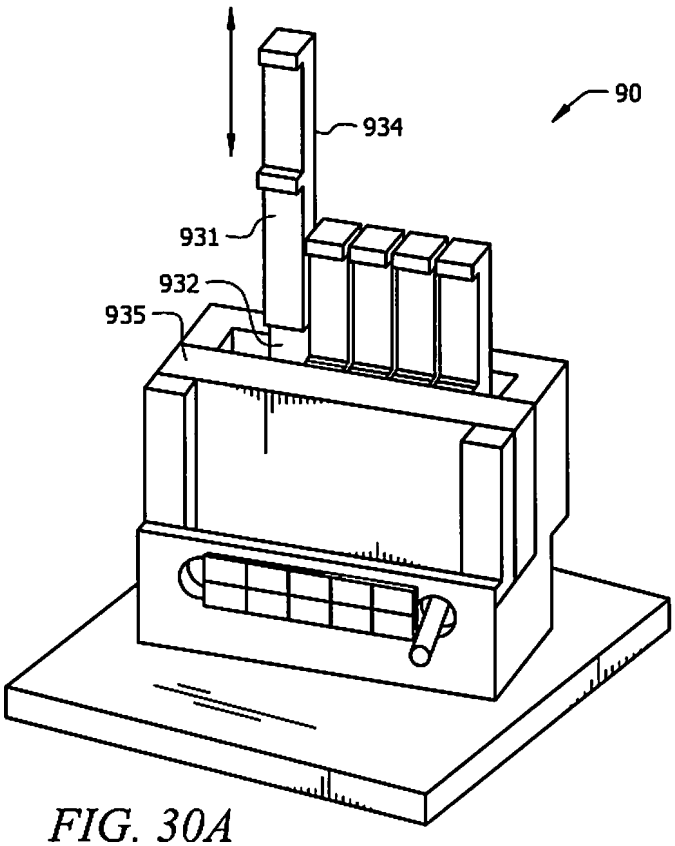


FIG. 30A

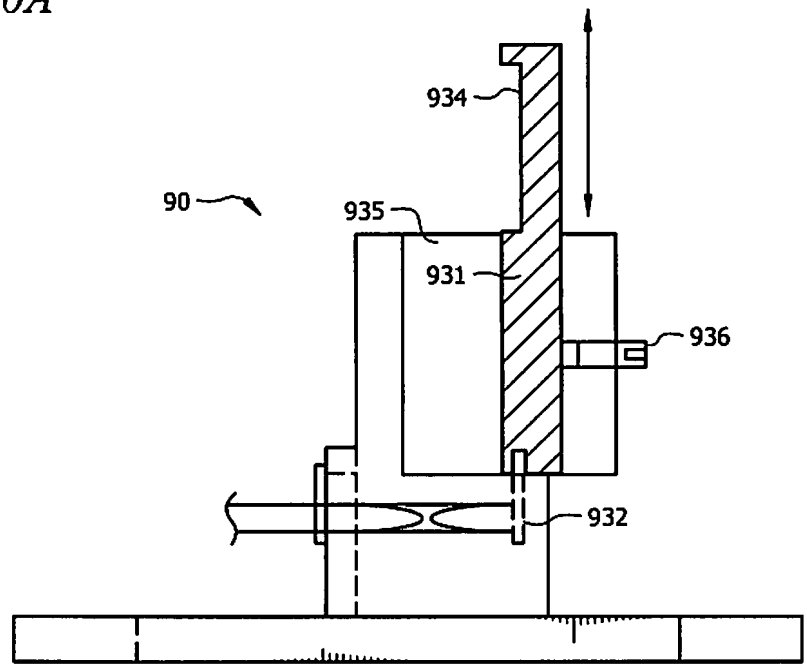


FIG. 30B

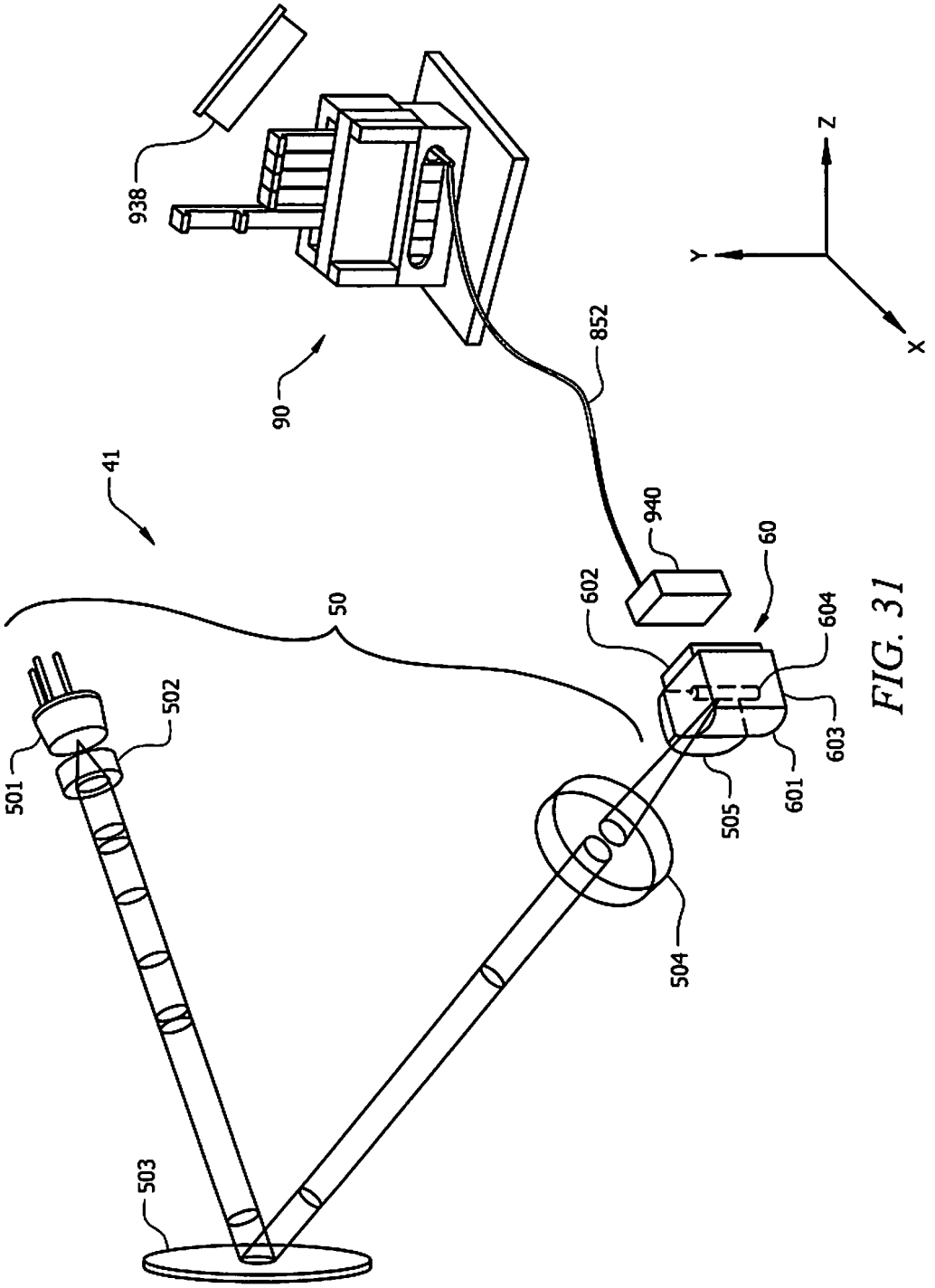


FIG. 31

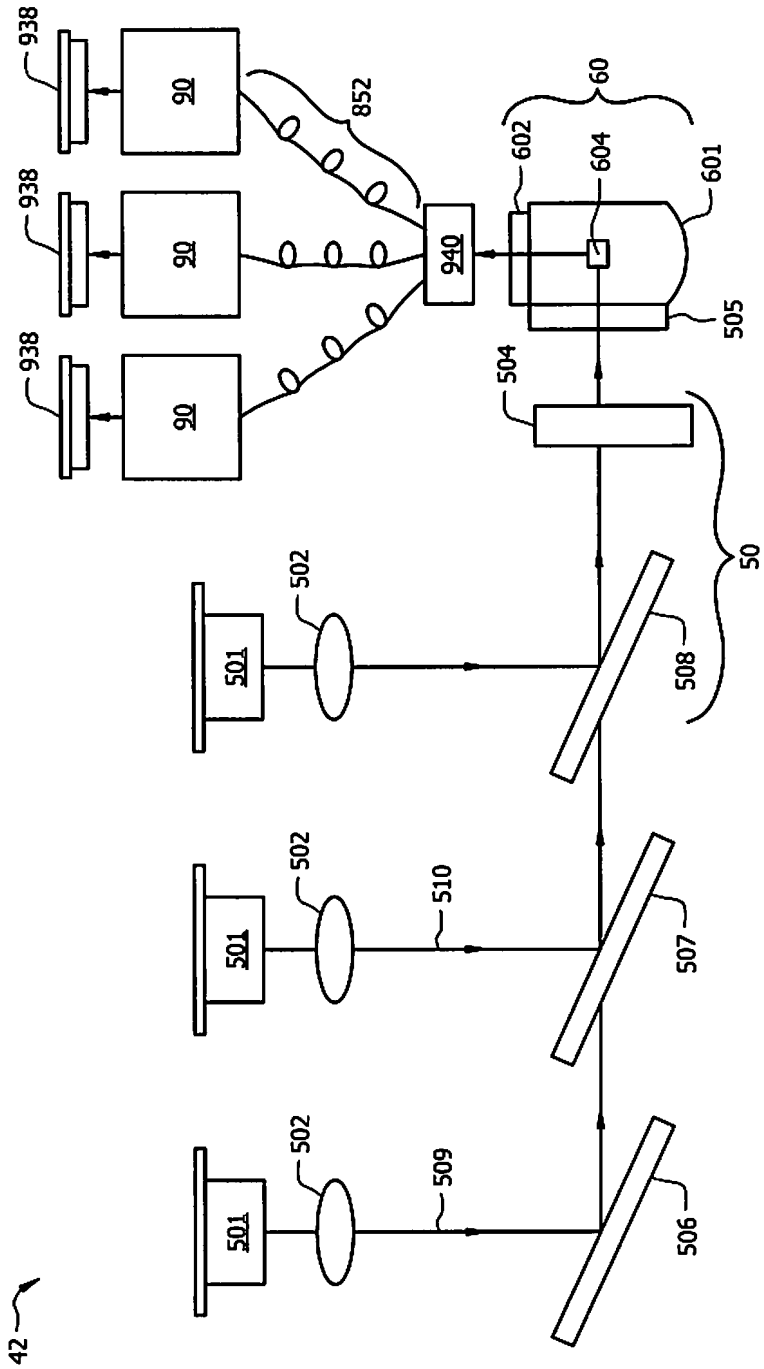

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FIG. 32

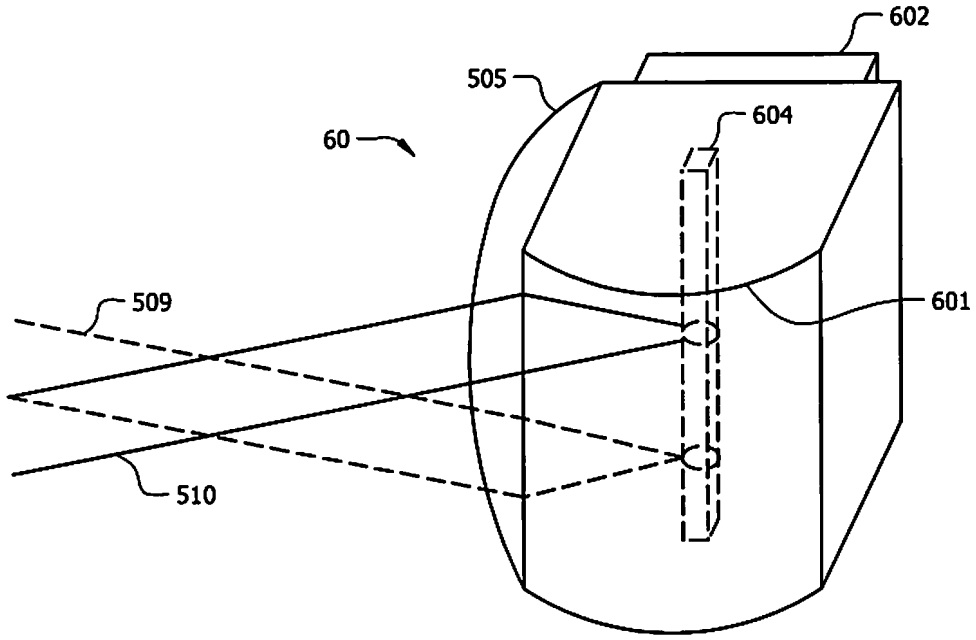


FIG. 33

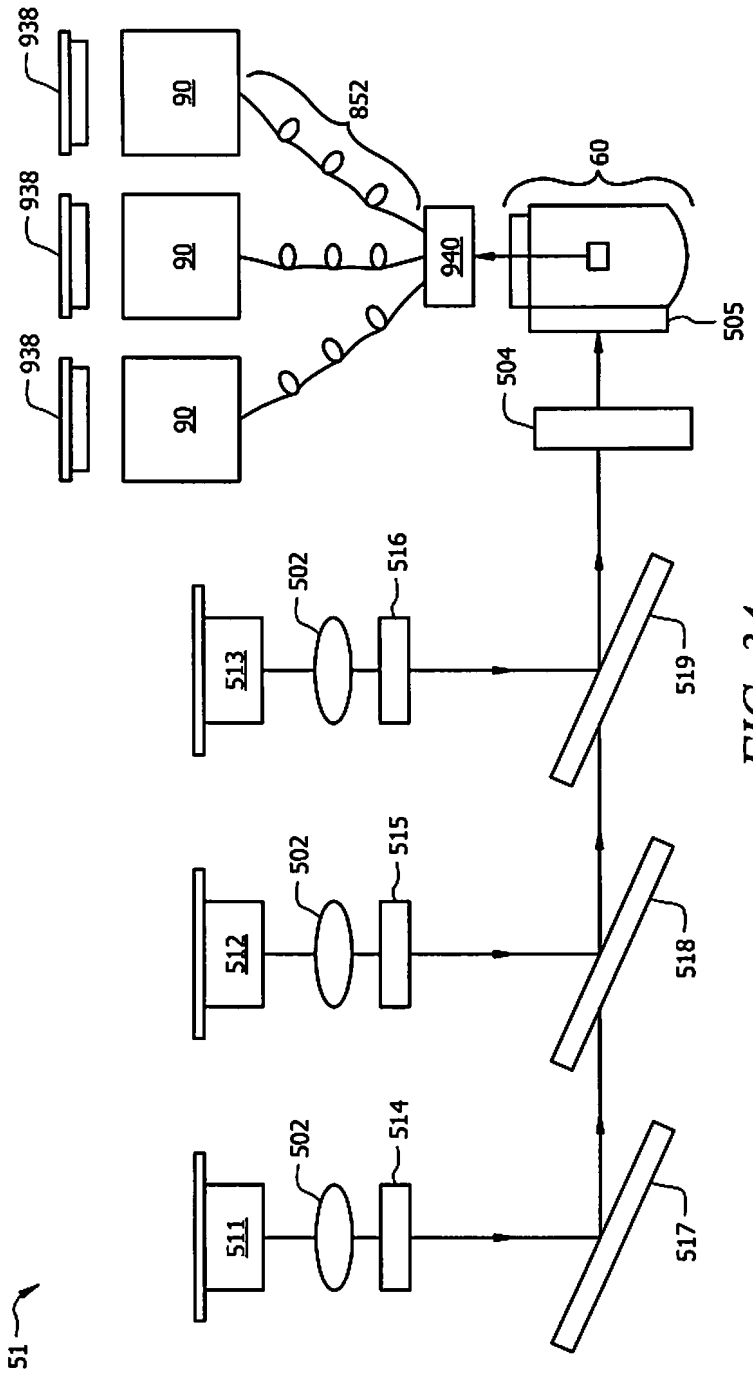

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FIG. 34

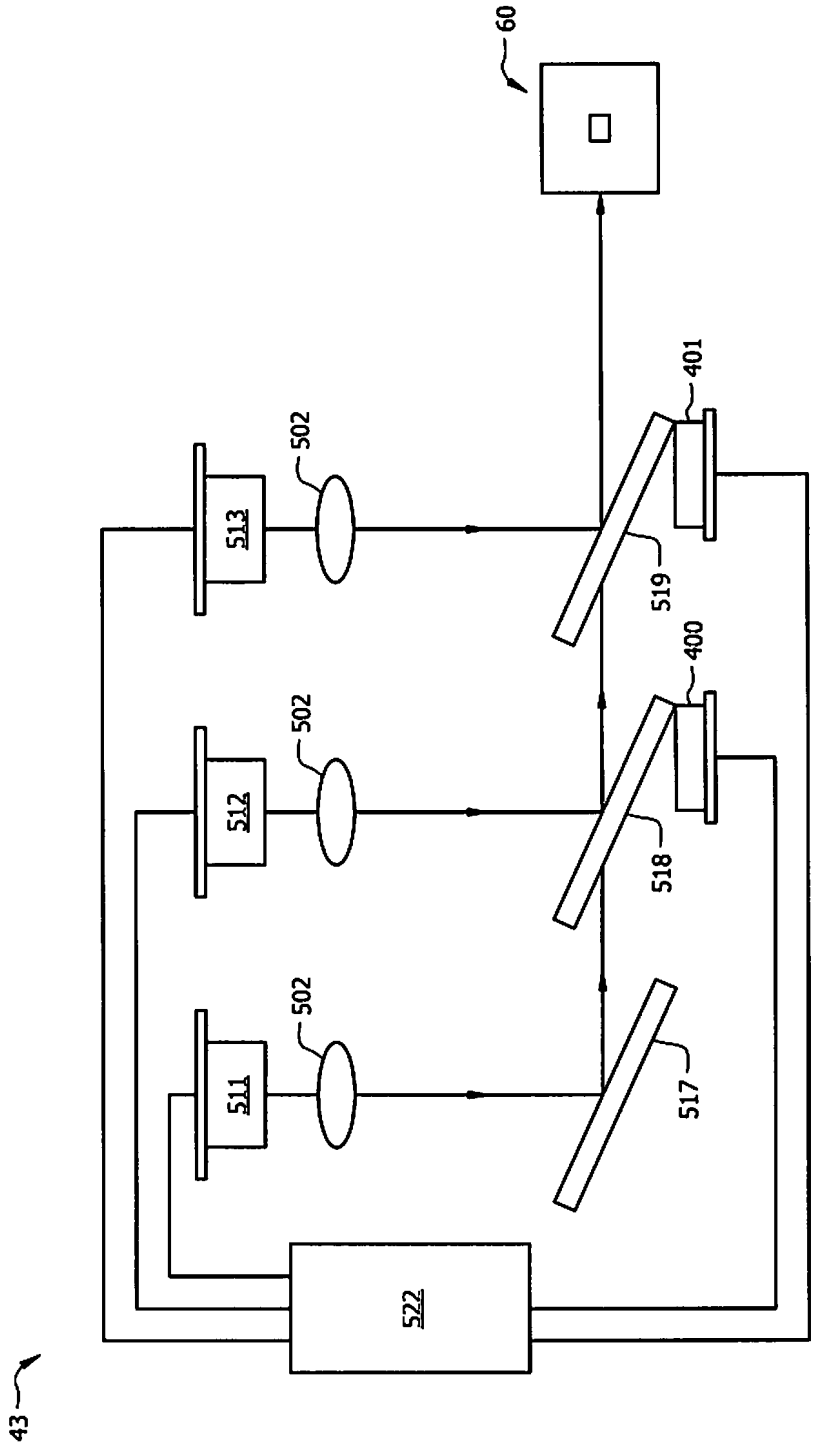


FIG. 35

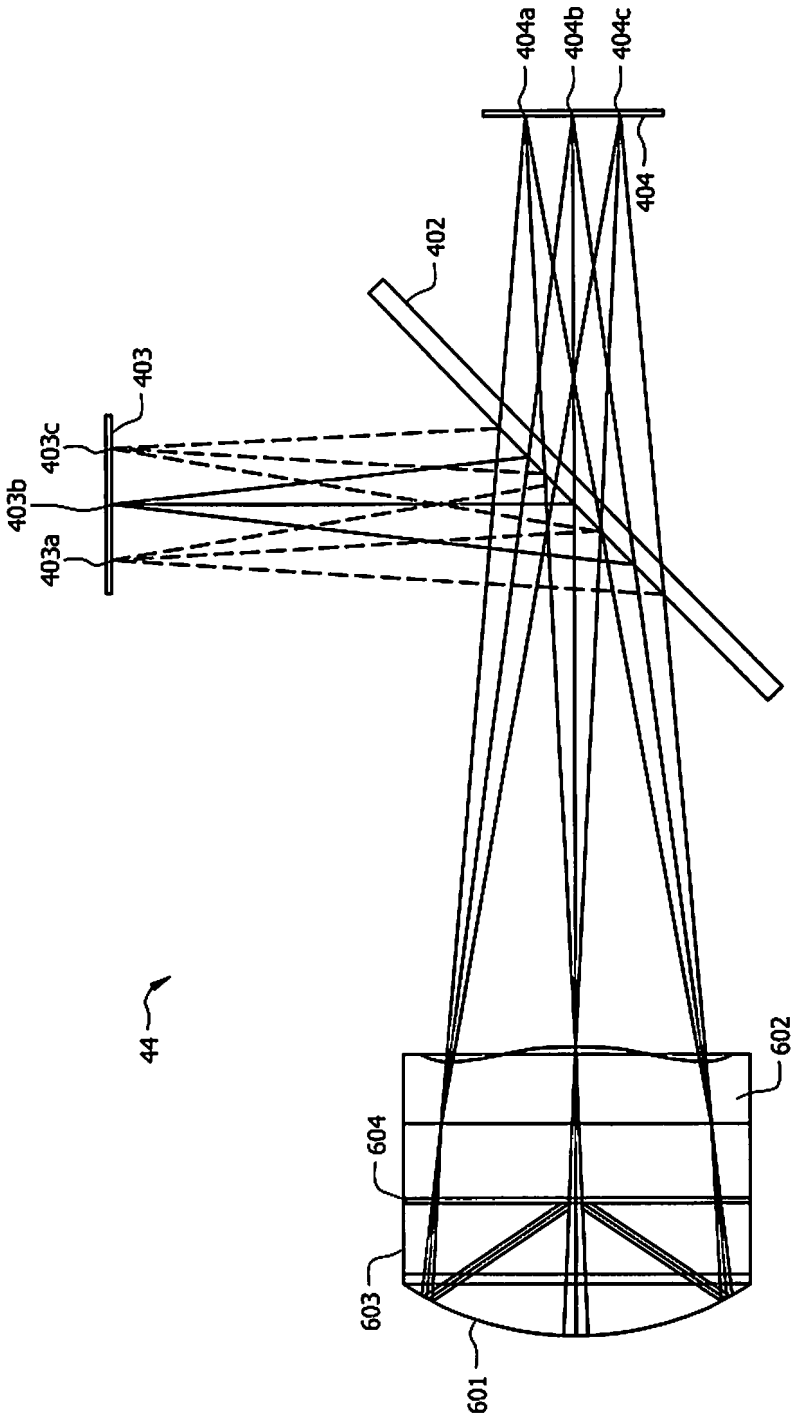


FIG. 36

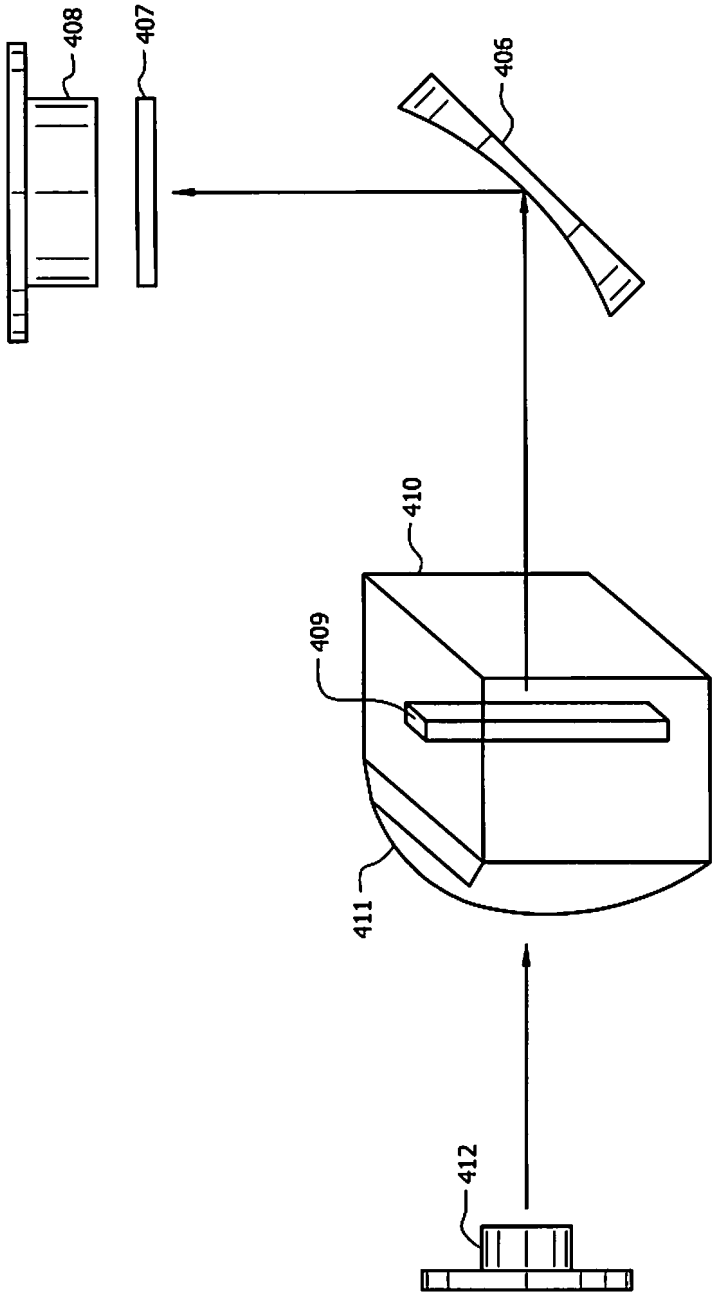


FIG. 37

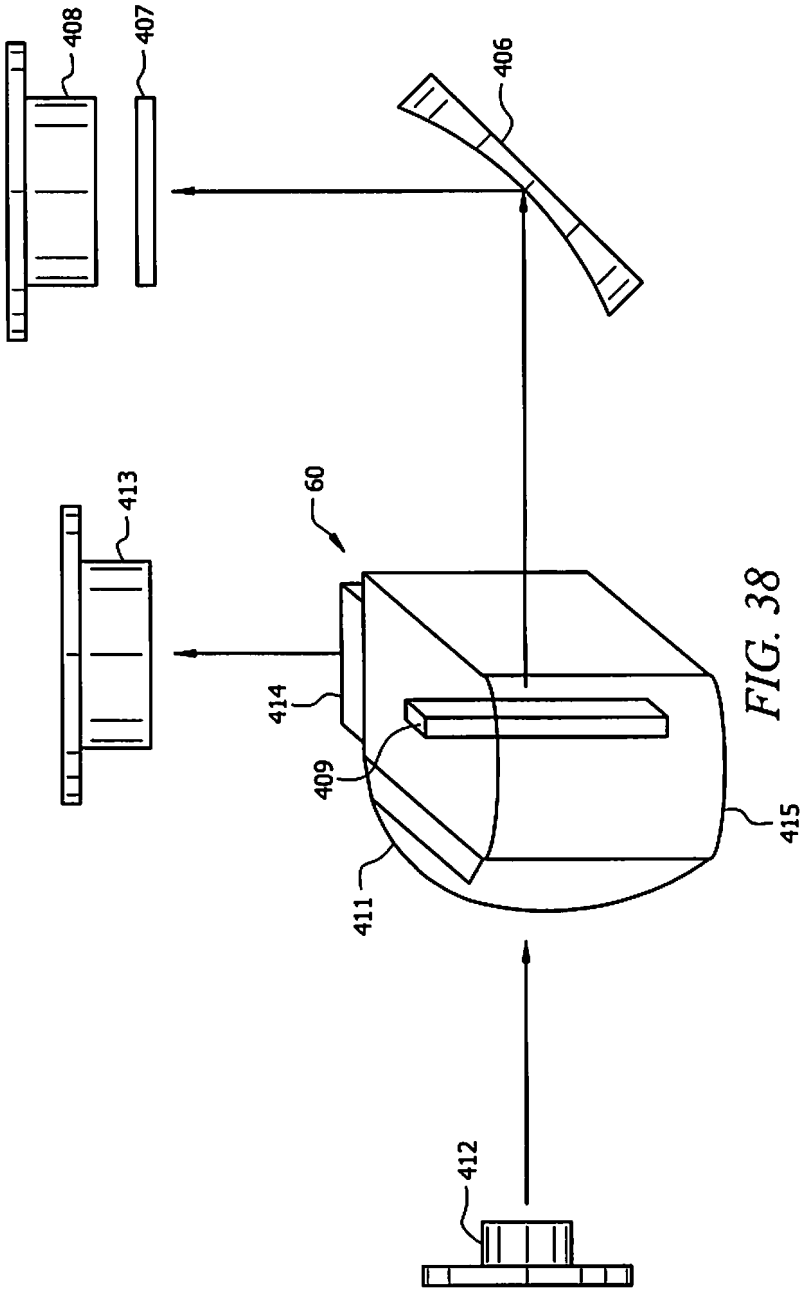


FIG. 38

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 15/638,461	Filing Date 06/30/2017	☐ 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 **06/30/2017**

	(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))	* 26	Minus	** 26	= 0	X $80 =	0
Independent (37 CFR 1.16(h))	* 3	Minus	***3	= 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
eugenia v. hardy

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.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/638,461

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))	26 minus 20 =	* 6			OR	x 80 =	480
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*				x 420 =	0.00
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	2480

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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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/638,461	06/30/2017	2877	2480	BECC.P0002US.CIP.C1	26	3

CONFIRMATION NO. 9847

32425
NORTON ROSE FULBRIGHT US LLP
98 SAN JACINTO BOULEVARD
SUITE 1100
AUSTIN, TX 78701-4255

FILING RECEIPT

OC000000092845076

Date Mailed: 07/19/2017

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: The patent practitioners associated with Customer Number 129381

Domestic Priority data as claimed by applicant

This application is a CON of 14/555,102 11/26/2014
which 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 Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 07/18/2017

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/638,461**

Projected Publication Date: 10/26/2017

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 37, Code of Federal Regulations, 5.11 & 5.15

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List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: IRIS INTERNATIONAL, INC	
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U.S. Patent Documents

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	A1	315667	4/14/85	Serdinko	417	475	10/20/84
	A2	2385495	9/25/45	Brian	250	487.1	8/21/43
	A3	2669709	2/16/54	Glancy et al.	348	784	10/19/60
	A4	2764147	9/25/56	Brunner	126	247	2/23/51
	A5	3199341	08/10/65	Heuer Jr. et al.	73	807	05/28/62
	A6	3411293	11/19/68	Akins	60	454	10/20/66
	A7	3542491	11/24/70	Newman	417	295	5/27/69
	A8	3661460	5/9/72	Elking et al.	356	36	8/28/70
	A9	3726613	4/10/73	von Casimir	417	477.1	10/12/70
	A10	3826593	7/30/74	von Casimir	417	53	5/11/73
	A11	3873204	3/25/75	Friedman et al.	356	39	11/29/72
	A12	3946239	3/23/76	Salzman et al.	250	461.2	1/24/75
	A13	3953727	4/27/76	d'Auria et al.	398	88	1/17/75
	A14	3989381	11/2/76	Fulwyler	356	39	5/5/75
	A15	4073574	2/14/78	Clarke et al.	359	728	10/1/76
	A16	4188543	2/12/80	Brunsting et al.	250	458.1	4/24/78
	A17	4189236	2/19/80	Hogg et al.	356	317	3/20/78
	A18	4244045	1/6/81	Nosu et al.	398	86	1/31/79
	A19	4482994	11/13/84	Ishikawa	398	86	6/7/82
	A20	4515274	5/7/85	Hollinger et al.	209	3.1	12/2/81
	A21	4564342	1/14/86	Weber et al.	417	477.6	7/24/84
	A22	4576556	3/18/86	Thompson	417	477.12	4/2/80
	A23	4623225	11/18/86	Forkner	359	669	6/29/84

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Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A24	4727020	2/23/88	Recktenwald	435	6.11	2/25/85
	A25	4745285	5/17/88	Recktenwald et al.	250	458.1	8/21/86
	A26	4778253	10/18/88	Siga et al.	359	819	3/26/86
	A27	4834630	5/30/89	Godwin	417	475	2/16/88
	A28	4871249	10/3/89	Watson	356	73	7/1/88
	A29	4920275	4/24/90	Itoh	250	574	12/30/88
	A30	4950136	8/21/90	Haas et al.	417	477.7	8/14/89
	A31	4976590	12/11/90	Baldwin	417	53	6/8/88
	A32	4997275	3/5/91	Gaucher et al.	356	72	9/23/88
	A33	5050963	9/24/91	Murakami	359	808	10/9/90
	A34	5142462	8/25/92	Kashima	362	268	4/27/90
	A35	5157917	10/27/92	Liang et al.	60	2261.1	05/20/91
	A36	5177641	1/5/93	Kobayashi et al.	359	820	10/30/90
	A37	5230614	7/27/93	Zanger et al.	417	477.9	6/3/92
	A38	5245318	9/14/93	Tohge et al.	340	611	12/15/92
	A39	5251060	10/5/93	Uenishi et al.	359	328	9/30/92
	A40	5257917	11/02/93	Minarik et al.	417	475	10/02/92
	A41	5299066	3/29/94	Rombult	359	819	8/12/92
	A42	5317162	5/31/94	Pinsky et al.	250	461.2	9/9/92
	A43	5369476	11/29/94	Bowers et al.	399	49	1/28/92
	A44	5373395	12/13/94	Adachi	359	652	5/10/93
	A45	5396487	3/7/95	Abe et al.	359	819	10/31/91
	A46	5467225	11/14/95	Manabe	359	661	11/1/94

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	A47	5470211	11/28/95	Knott et al.	417	477.9	8/11/94
	A48	5475210	12/12/95	Taguchi et al.	250	205	6/8/93
	A49	5548395	8/20/96	Kosaka	356	73	8/31/92
	A50	5583683	12/10/96	Scobey	398	79	6/15/95
	A51	5615200	3/25/97	Hoshino et al.	369	112.04	4/14/95
	A52	5746585	5/5/98	McDunn et al.	417	477.11	12/31/96
	A53	5748372	5/5/98	Kitagawa	359	565	4/17/96
	A54	5777674	7/7/98	Ohmuro	348	338	4/10/95
	A55	5781351	7/14/98	Murakami et al.	359	808	5/31/96
	A56	5786915	7/28/98	Scobey	398	82	10/22/96
	A57	5788927	8/4/98	Farrell et al.	422	63	6/30/96
	A58	5805363	9/8/98	Okuda et al.	359	819	7/17/97
	A59	5850292	12/15/98	Braun	356	419	11/13/97
	A60	5915925	6/29/99	North Jr.	417	36	1/7/97
	A61	5971713	10/26/99	North Jr.	417	36	12/29/98
	A62	6008920	12/28/99	Hendrix	398	79	3/11/98
	A63	6017194	1/25/00	North Jr.	417	36	12/31/98
	A64	6102678	8/15/00	Peclat	417	477.7	4/3/98
	A65	6135734	10/24/00	Isozumi et al.	417	542	9/23/98
	A66	6159686	12/12/00	Kardos et al.	435	6	01/30/98
	A67	6200101	3/13/01	North Jr.	417	36	7/6/99
	A68	6252719	6/26/01	Eichenbaum	359	634	3/19/99
	A69	6315952	11/13/01	Sklar et al.	422	63	6/10/99

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	A70	6510007	1/21/03	Blasenheim	359	659	8/21/01
	A71	6542306	4/1/03	Goodman	359	634	3/16/01
	A72	6572255	6/3/03	Husher	366	132	4/24/01
	A73	6608682	8/19/03	Ortyn et al.	356	419	10/12/01
	A74	6618143	9/9/03	Roche et al.	356	339	10/2/01
	A75	6638481	10/28/03	Sklar et al.	422	63	9/19/01
	A76	6647175	11/11/03	LoRegio et al.	385	24	10/8/01
	A77	6683314	1/27/04	Oostman Jr. et al.	250	461.2	8/28/01
	A78	6713019	3/30/04	Ozasa et al.	422	82.09	3/27/02
	A79	6748133	6/8/04	Liu et al.	385	24	11/26/01
	A80	6767188	7/27/04	Vrane et al.	417	40	8/15/02
	A81	6768593	7/27/04	Jutamulia	359	641	7/24/03
	A82	6788409	9/7/04	Goodwin	356	339	9/3/02
	A83	6794671	9/21/04	Nicoli et al.	250	574	7/17/02
	A84	6813017	11/2/04	Hoffman et al.	356	317	10/19/00
	A85	6839367	1/4/05	Nagamatsu et al.	372	34	3/16/01
	A86	6870679	3/22/05	Randall et al.	359	580	6/10/02
	A87	6870976	3/22/05	Chen et al.	385	14	3/13/01
	A88	6897954	5/24/05	Bishop et al.	356	317	12/20/02
	A89	6941047	9/6/05	Capewell et al.	385	47	3/1/04
	A90	6954722	10/11/05	Parks et al.	702	194	10/17/03
	A91	6975400	12/13/05	Ortyn et al.	356	419	7/28/03
	A92	7038778	5/2/06	Yamauchi	356	419	8/13/02

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	A93	7072540	7/4/06	Szapiel et al.	385	24	4/22/03
	A94	7110192	9/19/06	Sauter et al.	359	754	1/12/05
	A95	7113266	9/26/06	Wells	356	73	3/30/05
	A96	7127356	10/24/06	Nicoli et al.	702	26	5/18/04
	A97	7129505	10/31/06	Oostman Jr. et al.	250	458.1	10/17/03
	A98	7212343	5/1/07	He et al.	359	618	7/11/03
	A99	7260328	8/21/07	Kropp	398	82	2/24/03
	A100	7262838	8/28/07	Fritz	356	73	1/16/04
	A101	7268953	9/11/07	Matthae et al.	359	656	6/7/06
	A102	7305018	12/4/07	Otoma	372	50.1	12/29/05
	A103	7381565	6/3/08	Kurabayashi et al.	436	63	3/20/06
	A104	7385682	6/10/08	Chu et al.	356	73	9/7/06
	A105	7430048	9/30/08	Reel et al.	356	432	2/16/06
	A106	7450229	11/11/08	Ortyn et al.	356	326	5/4/05
	A107	7453915	11/18/08	Imai	372	50.1	12/5/06
	A108	7456960	11/25/08	Cerni et al.	356	336	11/15/05
	A109	7496463	2/24/09	Nicoli et al.	702	104	9/12/06
	A110	7505131	3/17/09	Roth	356	317	3/6/08
	A111	7507588	3/24/09	Mehrpouyan et al.	436	518	4/14/06
	A112	7561267	7/14/09	Luo et al.	356	336	12/22/06
	A113	7580120	8/25/09	Hamada et al.	356	73	4/6/06
	A114	7623243	11/24/09	Kato	356	419	9/13/04
	A115	7645127	1/12/10	Hagen et al.	417	477.12	6/29/06

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	A116	7668422	2/23/10	Kropp	385	47	3/12/04
	A117	7758324	6/20/10	Baumann et al.	417	477.2	5/12/05
	A118	7768120	8/3/10	Takashima	257	706	12/27/07
	A119	7781227	8/24/10	Mehrpouyan et al.	436	523	3/4/09
	A120	7787197	8/31/10	Chen	359	793	9/4/08
	A121	7835000	11/16/10	Graves et al.	356	338	11/3/06
	A122	7876436	1/25/11	Chu	356	338	10/31/07
	A123	7894047	2/22/11	Hamada et al.	356	39	7/24/09
	A124	7952981	3/31/11	Suzuki	369	121	10/23/08
	A125	7981661	7/19/11	Rich	435	286.5	4/17/07
	A126	8009189	8/30/11	Ortyn et al.	348	80	6/3/08
	A127	8049185	11/1/11	Hayashi et al.	250	459.1	2/4/09
	A128	8149525	4/3/12	Do	359	797	3/28/07
	A129	8157547	4/17/12	Vrielink	417	477.3	4/23/07
	A130	8187888	5/29/12	Rich	436	63	7/8/11
	A131	8213472	7/3/12	Hirai et al.	372	36	1/23/08
	A132	8229707	7/24/12	Olson et al.	702	189	7/4/11
	A133	8233146	7/31/12	Chen	356	246	1/5/10
	A134	8253938	8/28/12	Vacca et al.	356	337	5/26/11
	A135	8270098	9/18/12	Fukuta et al.	359	784	2/19/09
	A136	8284402	10/9/12	Frazier et al.	356	419	2/26/10
	A137	8337096	12/25/12	Shen et al.	385	89	11/30/09
	A138	8345237	1/1/13	Tsukii et al.	356	338	12/23/11

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	A139	8405048	3/26/13	Hayashi	250	458.1	2/4/09
	A140	8432541	4/30/13	Rich	356	246	11/4/10
	A141	8436371	5/7/13	Medendorp Jr., et al.	257	88	5/24/07
	A142	8436993	5/7/13	Kaduchak et al.	356	337	2/3/12
	A143	8488244	7/16/13	Li et al.	359	618	2/17/11
	A144	8507279	8/13/13	Ball et al.	436	10	6/2/10
	A145	2004165828	08/26/04	Capewell et al.	385	47	03/01/04
	A146	20020067895	06/06/02	Flanders	385	88	12/01/00
	A147	20020141902	10/3/02	Ozasa et al.	422	82.09	3/27/02
	A148	20030142720	7/31/03	Bradburn et al.	372	108	5/24/02
	A149	20040218184	11/4/04	Joregenson et al.	356	419	2/27/04
	A150	20050084402	4/21/05	Vanek	417	477.3	10/27/04
	A151	20060221325	10/5/06	Wells	356	73	3/30/05
	A152	20060245964	11/2/06	Koslov	417	477.1	6/29/06
	A153	20060256335	11/16/06	Chen	356	369	5/3/06
	A154	20060292021	12/28/06	Tu	417	477.1	11/7/02
	A155	20070124947	6/7/07	Munroe et al.	33	286	10/25/06
	A156	20080024758	1/31/08	Tabata	356	39	7/26/07
	A157	20080241911	10/2/08	Ueno et al.	435	287.1	3/28/08
	A158	20090091746	4/9/09	Fukuda et al.	356	73	10/1/08
	A159	20090190128	7/30/09	Cerni et al.	356	336	11/24/08
	A160	20110014075	1/20/11	Reif et al.	417	477.12	7/1/10
	A161	20110032522	2/10/11	Graves et al.	356	338	10/12/10

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	A162	20120156074	6/21/12	Kusch et al.	417	477.3	2/4/10
	A163	20120274925	11/1/12	Chen et al.	356	73	11/1/12
	A164	20120281204	11/8/12	Hoshishima et al.	356	72	1/13/11
	A165	20120282126	11/8/12	Brandt et al.	417	477.9	11/4/10
	A166	20120287419	11/15/12	Sharpe et al.	356	51	5/11/12
	A167	20130010181	1/10/13	Baba	348	360	3/14/11
	A168	20130020498	1/24/13	Ebi et al.	250	435	7/18/12
	A169	20130050782	2/28/13	Heng et al.	358	494	2/17/12
	A170	20130070243	3/21/13	Goehde	356	338	11/9/12
	A171	20130094102	4/18/13	Baba	359	784	7/13/10
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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)

28461423.1

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.

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP.C1/11706355	Serial No.: 15/638,461
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: IRIS INTERNATIONAL, INC	
	Filing Date: June 30, 2017	Group: Not Yet Assigned

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1-8	*See Page 8-11*	*See Page 10-11*

Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	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
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	B17	JP 05333245	12/17/93	Japan	Japanese (English abstract)
	B18	JP 2004-341204	12/02/04	Japan	Japanese (English Abstract)
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	B20	JP 829726	2/2/96	Japan	Japanese (English translation)
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List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: IRIS INTERNATIONAL, INC	
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U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1-8	*See Page 8-11*	*See Page 10-11*

Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	B32	WO 2011026942	3/10/11	WIPO	English
	B33	WO 2012056217	5/3/12	WIPO	English
	B34	WO 2012177367	12/27/12	WIPO	English
	B35	WO 2013013229	1/24/13	WIPO	English
	B36	WO 2013093035	6/27/13	WIPO	English
	B37	WO 9307471	4/15/93	WIPO	English
	B38	WO 9316368	8/16/93	WIPO	English
	B39	WO 9429695	12/22/94	WIPO	English
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Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
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	C2	Extended European Search Report for EP 13796734.5, mailed March 18, 2016
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	C4	International Search Report and Written Opinion for PCT/US2013/043453 mailed December 20, 2013.
	C5	International Search Report and Written Opinion for PCT/US2014/067682 mailed April 9, 2015.
	C6	Office Action issued in Japanese Patent Application No. 2015-515208, issued May 29, 2017 (English Translation Provided) (Attorney Docket No. BECC.P0002JP)
	C7	Schmidt, B., "Ein lichtstarkes komafreies Spiegelsystem," *Mitteilungen der Haburger Sternwarte in Bergedorf* 7(36):15-17, 1932.

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EXAMINER:	DATE CONSIDERED:

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U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1-8	*See Page 8-11*	*See Page 10-11*

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

	C8	Smiley, C. H., "The Schmidt Camera," *Popular Astronomy* 44:415-421, 1936.

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Electronic Acknowledgement Receipt

EFS ID:	29963470
Application Number:	15638461
International Application Number:	
Confirmation Number:	9847
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	32425
Filer:	Gina N. Shishima/Hannah Wimberley
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US.CIP.C1
Receipt Date:	02-AUG-2017
Filing Date:	30-JUN-2017
Time Stamp:	15:38:48
Application Type:	Utility under 35 USC 111(a)

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		BECCP0002USCIP_C1_IDS.pdf	238151 f50cbe4fbf81d5d828c299d6846b266a94dc8730	yes	13

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	2
	Information Disclosure Statement (IDS) Form (SB08)	3	13
Warnings:			
Information:			
	Total Files Size (in bytes):	238151	

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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: Not Yet Assigned
Serial No.: 15/638,461	Examiner: Not Yet Assigned
Filed: June 30, 2017	Atty. Dkt. No.: BECC.P0002US.CIP.C1/11706355
For: FLOW CYTOMETER	Confirmation No.: 9847

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:

August 2, 2017	/Gina N. Shishima/
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 within three months of the filing date of the subject application, 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.C1/11706355.

This application is a continuation application of Serial No. 14/555,102 , filed November 26, 2014, and is relied upon for an earlier filing date under 35 U.S.C. § 120. In accordance with 37 C.F.R. § 1.98(d), copies of the listed documents are not enclosed as they have been previously cited by or submitted to the Patent and Trademark Office in prior application Serial No. 14/555,102.

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
Austin, TX 78701
(512) 474-5201

Date: August 2, 2017

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/638,461	06/30/2017	Yong Qin Chen	BECC.P0002US.CIP.C1

CONFIRMATION NO. 9847

32425
NORTON ROSE FULBRIGHT US LLP
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PUBLICATION NOTICE

OC000000095036238

Title:FLOW CYTOMETER

Publication No.US-2017-0307506-A1
Publication Date:10/26/2017

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/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

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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.C1	Serial No.: 15/638,461
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	Filing Date: June 30, 2017	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 1*	*See Page 2*

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Exam. Init.	Ref. Des.	Document Number	Date	Name
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	A175	4,673,289	06/16/87	Gaucher
	A176	7,630,147	12/08/09	Kar et al.
	A177	7,889,263	02/15/11	Ortyn et al.

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	B42	JP 2004-500572	01/08/04	Japan	Japanese (English Abstract)
	B43	JP 2008-534960	08/28/08	Japan	Japanese (English Abstract)
	B44	JP 2010-085194	04/15/10	Japan	Japanese (English Abstract)
	B45	JP H03-197641	08/29/91	Japan	Japanese (English Abstract)
	B46	JP H05-119035	05/14/93	Japan	Japanese (English Abstract)
	B47	JP S55-144226	11/11/80	Japan	Japanese (English Abstract)
	B48	JP S61-184427	08/18/86	Japan	Japanese (English Abstract)
	B49	WO 2013/181453	12/05/13	WIPO	English

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U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 1*	*See Page 2*

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C9	Office Action issued in Chinese Patent Application No. 201380039184.6, dated February 12, 2018 (Attorney Docket No. BECC.P0002CN)
	C10	Office Action issued in Japanese Patent Application No. 2015-515208, dated May 02, 2018 (Attorney Docket No. BECC.P0002JP)
	C11	Office Action issued in Japanese Patent Application No. 2016-216891, dated December 04, 2017 (English Translation) (Attorney Docket No. BECC.P0002JP.D1)

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Bibliographic data: JP2004500572 (A) — 2004-01-08

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Inventor(s):

Applicant(s):

Classification: **- international:** ***G01N21/25; G01N21/64; G01N21/76; G01N33/53; G01N37/00; G02B21/00; G02B26/10; G02B6/036;*** (IPC1-7): G01N21/64; G01N21/76; G01N33/53; G01N37/00; G02B26/10; G02B6/22

- cooperative: **G01N21/645**; **G01N21/6452**; **G01N21/6456**; **G01N21/76**; **G02B21/002**; G01N2021/6484

Application number: JP20010568124 20010313

Priority number(s): US20000188873P 20000313 ; WO2001US08043 20010313

Also published as: AU4362701 (A) CA2401511 (A1) EP1264168 (A2) US2002037149 (A1) WO0169302 (A2) more

Abstract not available for JP2004500572 (A)
Abstract of corresponding document: WO0169302 (A2)

The disclosed scanning structure includes an apparatus for light delivery and light receiving from a light-excitable area on a substrate to be measured by the scanning structure. The light delivery and receiving apparatus may include an optical fiber having a proximal end and a distal end which transmits light having a certain wavelength or light with several varying wavelengths to excite the substrate samples. This optical fiber may also simultaneously receive light which may be emitted by fluorescing samples on the substrate. The scanning structure also may further include a holder for the optical fiber that is able to transverse variable distances over the substrate to be measured or examined. Holders may include galvano scanners as well as resonating suspension beams. A light source, e.g., a laser, may be optically coupled to the optic fiber's proximal end. And this light source may be of a certain wavelength, but multiple light sources each having a different wavelength may also be used simultaneously by coupling the light sources into either a single optic fiber through wavelength multiplexers or by placing individual optic fibers carrying differing wavelengths in close proximity to each other. As the light is transmitted down to the substrate through the

optic fiber, the fiber is sufficiently close to the substrate microarray so that it can also receive the emitted fluorescing light.

(19)日本国特許庁(JP)　(12)公表特許公報(A)　(11)特許出願公表番号
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G01N 37/00	G01N 37/00	102	2H050
G02B 6/22	G02B 6/22		

審査請求 未請求　予備審査請求 有　（全 72 頁）　最終頁に続く

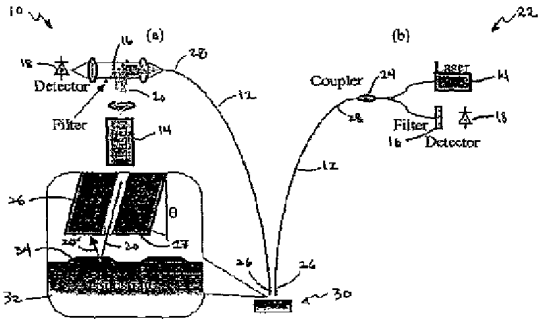
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(54)【発明の名称】光ファイバ走査装置

(57)【要約】
開示される走査構造には、該走査構造で測定される基板上の光励起可能領域に対する光伝達及び受光装置が含まれている。この光伝達及び受光装置には、基端及び先端を有する光ファイバが含まれていてもよい。この光ファイバは、ある波長の光又はいくつかの多様な波長の光を伝達して、基板のサンプルを励起する。また、この光ファイバは、基板上のサンプルに蛍光を発生させることにより放出された光を、同時に受光してもよい。さらに、走査構造には、測定又は検査対象となる基板上を可変の距離を持って横切ることが可能な光ファイバホルダーが含まれていてもよい。ホルダには、ガルバノスキャナや共振懸架ビームが含まれていてもよい。例えばレーザ等の光源が、光ファイバの基端に光学的に連結されてもよい。この光源は、ある波長のものであってもよいが、異なった波長の複数の光源が、波長マルチプレクサで光源を単一の光ファイバに結合するか、あるいは、異なる波長を伝達する個別の光ファイバを互いに近接させて配置することにより、同時に用いられてもよい。光は、光ファイバを通じて基板へと伝達され、光ファイバは、発せ



【特許請求の範囲】
【請求項1】
基板上の光励起可能領域へ光を導くとともに該光励起可能領域から発せられた光を受光する走査構造であって、該走査構造は、第１の波長の光及び第２の波長の光を伝達し、基端及び先端を有する光ファイバを備え、該光ファイバは、前記光励起可能領域が前記第１の波長の光を受光したときに発生する前記第２の波長の光の少なくとも一部を受光するように構成された走査構造。
【請求項2】
前記第１の波長の光及び前記第２の波長の光を伝達する追加的な複数本の光ファイバをさらに備え、前記の追加的な各光ファイバは、基端及び先端を有するとともに、前記基板の前記光励起可能領域に充分近接した状態で移動して、該光ファイバが、前記光励起可能領域が前記第１の波長の光を受光したときに発生する前記第２の波長の光の少なくとも一部を捉えるように構成された請求項１に記載の走査構造。
【請求項3】
前記光ファイバの先端と前記追加的な光ファイバの先端とが、固定された関係で保持されている請求項２に記載の走査構造。
【請求項4】
前記光ファイバの前記基端に光学的に連結されて、前記第２の波長の光を受光する検出器をさらに備えた請求項１～３のいずれか一項に記載の走査構造。
【請求項5】
前記光ファイバが前記基板を走査するように該基板を移動させるべく構成された基板ホルダをさらに備えた請求項１～４のいずれか一項に記載の走査構造。
【請求項6】
前記光ファイバが前記基板の前記光励起可能領域を移動するのに充分である第１の位置及び第２の位置間の距離を移動する光ファイバホルダをさらに備えた請求項１～５のいずれか一項に記載の走査構造。
【請求項7】
前記光励起可能領域を励起するために前記第１の波長の光を発する光源をさらに備え、該光源は、前記第１の波長の光が前記光ファイバの先端から出射するように、該光ファイバの基端に光学的に連結されている請求項１～６のいずれか一項に記載の走査構造。
【請求項8】
基板上の光励起可能領域へ光を導くとともに該光励起可能領域から発せられた光を受光する走査構造であって、該走査構造は、
ａ）基端及び先端を有し、第１の波長の光及び第２の波長の光を伝達する光ファイバと、
ｂ）前記光励起可能領域が前記第１の波長の光を受光したときに、前記光励起可能領域から前記第２の波長の光が発せられる前記基板の前記光励起可能領域を移動するのに充分である第１の位置及び第２の位置間の距離を移動する光ファイバホルダと、
ｃ）前記光励起可能領域を励起するために前記第１の波長の光を発する光源であって、前記第１の波長の光が前記光ファイバの先端から出射するように、該光ファイバの基端に光学的に連結された光源とを備え、
ｄ）前記光ファイバは、マイクロアレイに充分近接して、該光ファイバが、前記光励起可能領域により生じた前記第２の波長の光を、該光ファイバの先端で受光し、該第２の波長の光を該光ファイバの基端へ伝達するように、前記ホルダに取り付けられ、
ｅ）前記ホルダは、前記光源が前記第１の波長の光を発しているときに、前記第１の位置及び前記第２の位置間を移動するように構成されている
走査構造。
【請求項9】
前記走査構造は、先端部分及び基端部分を有するビームをさらに備え、前記先端部分は、前記ホルダを備え、前記ビームは、該ビームの基端で、マウントに取り付けられるととも

に、該ビームの先端部分が前記第１の位置及び前記第２の位置間を移動するように構成された請求項１～８のいずれか一項に記載の走査構造。
【請求項10】
前記ビームは、ガルバノメータの可動ビームである請求項９に記載の走査構造。
【請求項11】
前記ビームは、前記第１の位置及び前記第２の位置間を、該ビームの共振周波数で移動する請求項９に記載の走査構造。
【請求項12】
前記ビームの共振周波数が圧電的に駆動される請求項１１に記載の走査構造。
【請求項13】
前記ビームの共振周波数が磁気的に駆動される請求項１１に記載の走査構造。
【請求項14】
前記ビームの基端は、該ビームの基端が動かないように、前記マウントに取り付けられた請求項９に記載の走査構造。
【請求項15】
前記ホルダは、前記第１の位置及び前記第２の位置間を水平移動するように構成され、前記走査構造は、前記ホルダが前記第１の位置及び第２の位置の少なくとも一方に達したときに、該ホルダを垂直方向に反発させる磁石をさらに備えた請求項８～１４のいずれか一項に記載の走査構造。
【請求項16】
前記光源は、前記第１の波長の光を発するレーザを備えた請求項７～１５のいずれか一項に記載の走査構造。
【請求項17】
前記走査構造は、第１の分岐チャネル及び第２の分岐チャネルを有する光学フィルタをさらに備え、該光学フィルタは、該第１の分岐チャネルで、前記レーザからの前記第１の波長の光を受光するように構成されるとともに、該第２の分岐チャネルを通じて、前記受光可能領域からの前記第２の波長の光を伝達するように構成された請求項１～１６のいずれか一項に記載の走査構造。
【請求項18】
前記走査構造は、前記第１の位置及び前記第２の位置で規定される線に対して概ね垂直な方向に向って、基板を移動させる基板平行移動ステージをさらに備えた請求項８～１７のいずれか一項に記載の走査構造。
【請求項19】
前記ホルダは、前記光ファイバが基板に対して垂直以外の角度になるようにしておく請求項８～１８のいずれか一項に記載の走査構造。
【請求項20】
前記光ファイバの先端を規定する表面は、前記基板と実質的に平行である請求項８～１９のいずれか一項に記載の走査構造。
【請求項21】
前記光ファイバは、毛管ファイバをさらに備えた請求項１～２０のいずれか一項に記載の走査構造。
【請求項22】
前記光ファイバは、第１の屈折率の内側コア及び該内側コアの外側にあり第２の屈折率の外側コアを備えたダブルコアファイバであり、前記第１の屈折率は、前記第２の屈折率よりも大きい請求項１～２０のいずれか一項に記載の走査構造。
【請求項23】
基板上の光励起可能領域へ光を導くとともに該光励起可能領域から発せられた光を受光する走査構造であって、該走査構造は、
ａ）複数本の光ファイバであって、各光ファイバは、対応する基端及び対応する先端を有して、予め選択された複数の異なる波長の光を伝達し、各波長は各光ファイバに対応して

いる複数本の光ファイバを備え、
ｂ）前記複数本の光ファイバは、第１の波長の光及び第２の波長の光を伝達する第１の光ファイバを少なくとも具備し、
ｃ）前記複数本の光ファイバが、前記光励起可能領域が少なくとも前記第１の波長の光を受光したときに、該光励起可能領域から少なくとも前記第２の波長の光が発せられる前記基板の前記光励起可能領域に沿って移動するのに充分な距離を有する第１の位置及び第２の位置間を移動する前記複数本の光ファイバ用のホルダと、
ｄ）前記各光ファイバに対応した複数の光源であって、前記光励起可能領域を励起するために前記第１の波長の光を少なくとも発し、少なくとも前記第１の波長の光が少なくとも前記第１の光ファイバの先端から出射するように、前記複数本の光ファイバの対応する基端に光学的に連結された複数の光源とを、前記走査構造が備え、
ｅ）前記複数本の光ファイバは、マイクロアレイに充分近接して、各光ファイバが、前記光励起可能領域により生じた少なくとも前記第２の波長の光を、各光ファイバの先端で受光し、この少なくとも第２の波長の光を少なくとも前記第１の光ファイバの基端へ伝達するように、前記ホルダに取り付けられ、
ｆ）前記ホルダは、前記複数の光源が少なくとも前記第１の波長の光を発しているときに、前記第１の位置及び前記第２の位置間を移動するように構成されている
走査構造。
【請求項24】
先端部分及び基端部分を有するビームをさらに備え、前記先端部分は、前記ホルダを備え、前記ビームは、そのビームの基端で、マウントに取り付けられるとともに、そのビームの先端部分が前記第１の位置及び前記第２の位置間で移動するように構成された請求項２３に記載の走査構造。
【請求項25】
前記ビームは、ガルバノメータの可動ビームである請求項２４に記載の走査構造。
【請求項26】
前記ビームは、前記第１の位置及び前記第２の位置間を、そのビームの共振周波数で移動する請求項２４に記載の走査構造。
【請求項27】
前記ビームの共振周波数が圧電駆動される請求項２６に記載の走査構造。
【請求項28】
前記ビームの共振周波数が磁気駆動される請求項２６に記載の走査構造。
【請求項29】
前記ビームの基端は、該ビームの基端が動かないように、前記マウントに取り付けられた請求項２４に記載の走査構造。
【請求項30】
前記ホルダは、前記第１の位置及び前記第２の位置間を水平移動するように構成され、前記走査構造は、前記ホルダが前記第１の位置及び第２の位置の少なくとも一方に達したときに、該ホルダを垂直に反発させる磁石をさらに備えた請求項２３～２９のいずれか一項に記載の走査構造。
【請求項31】
前記複数の光源は、少なくとも前記第１の波長の光を発する複数のレーザを備えた請求項２３～３０のいずれか一項に記載の走査構造。
【請求項32】
前記走査構造は、少なくとも第１の分岐チャネル及び第２の分岐チャネルを有する複数の光学フィルタをさらに備え、該光学フィルタは、その第１の分岐チャネルで、前記複数のレーザからの少なくとも前記第１の波長の光を受光するように構成されるとともに、その第２の分岐チャネルを通じて、前記光励起可能領域からの少なくとも前記第２の波長の光を伝達するように構成された請求項２３～３１のいずれか一項に記載の走査構造。
【請求項33】

前記複数本の光ファイバのうちの少なくとも１本は、複数の毛管ファイバをさらに備えた請求項２３～３２のいずれか一項に記載の走査構造。

【請求項34】

前記複数本の光ファイバのうちの少なくとも１本は、第１の屈折率を有する内側コア及び該内側コアの外側にある第２の屈折率を有する外側コアを備えたダブルコアファイバであり、前記第１の屈折率は、前記第２の屈折率よりも大きい請求項２３～３３のいずれか一項に記載の走査構造。

【請求項35】

前記複数本の光ファイバの先端は、複数のファイバ読取ヘッドを備え、各先端は、予め選択されたバンドル構成を有している請求項２３～３４のいずれか一項に記載の走査構造。

【請求項36】

前記複数本の光ファイバの先端は、複数のファイバ読取ヘッドを備え、各先端は、線状に隣接している請求項２３～３４のいずれか一項に記載の走査構造。

【請求項37】

基板上の光励起可能領域へ光を導くとともに該光励起可能領域から発せられた光を受光する走査構造であって、該走査構造は、

ａ）複数本の光ファイバであって、各光ファイバは、対応する基端及び対応する先端を有して、予め選択された異なる複数の波長の光を伝達し、各波長は各光ファイバに対応している複数本の光ファイバを備え、

ｂ）前記複数本の光ファイバは、少なくとも第１の波長の光及び少なくとも第２の波長の光を伝達し、

ｃ）前記複数本の光ファイバの対応する先端のそれぞれを、主基端及び主先端を有する少なくとも１本の主光ファイバに、光学的に結合する複数のマルチプレクサと、

ｄ）前記主光ファイバが、前記光励起可能領域が少なくとも前記第１の波長の光を受光したときに、該光励起可能領域から少なくとも前記第２の波長の光が発せられる前記基板の前記光励起可能領域に沿って移動するのに充分な距離を有する第１の位置及び第２の位置間を移動する前記主光ファイバ用のホルダと、

ｅ）前記複数の各光ファイバに対応した複数の光源であって、前記光励起可能領域を励起するために前記第１の波長の光を少なくとも発し、少なくとも前記第１の波長の光が少なくとも１つの前記マルチプレクサを通じて前記主光ファイバの先端から出射するように、前記複数本の光ファイバの対応する基端に光学的に結合された複数の光源とを備え、

ｆ）前記主光ファイバは、マイクロアレイに充分近接して、前記光励起可能領域により生じた少なくとも前記第２の波長の光を、前記主光ファイバの先端で受光し、前記少なくとも第２の波長の光を、前記複数のマルチプレクサを通じて、前記複数本の光ファイバの基端へ伝達するように、前記ホルダに取り付けられ、　ｇ）前記ホルダは、前記複数の光源が少なくとも前記第１の波長の光を発しているときに、前記第１の位置及び前記第２の位置間を移動するように構成されている

走査構造。

【請求項38】

先端部分及び基端部分を有するビームをさらに備え、前記先端部分は、前記ホルダを備え、前記ビームは、該ビームの基端で、マウントに取り付けられるとともに、該ビームの先端部分が前記第１の位置及び前記第２の位置間で移動するように構成された請求項３７に記載の走査構造。

【請求項39】

前記ホルダは、前記第１の位置及び前記第２の位置間を水平移動するように構成され、前記走査構造は、前記ホルダが前記第１の位置及び第２の位置の少なくとも一方に達したときに、当該ホルダを垂直に反発させる磁石をさらに備えた請求項３７～３８のいずれか一項に記載の走査構造。

【請求項40】

前記複数の光源は、少なくとも前記第１の波長の光を発する複数のレーザを備えた請求項

３７～３９のいずれか一項に記載の走査構造。
【請求項41】
前記走査構造は、少なくとも第１の分岐チャネル及び第２の分岐チャネルを有する複数の光学フィルタをさらに備え、該光学フィルタは、その第１の分岐チャネルで、前記複数のレーザからの少なくとも前記第１の波長の光を受光するように構成されるとともに、その第２の分岐チャネルを通じて、前記光励起可能領域からの少なくとも前記第２の波長の光を伝達するように構成された請求項３７～４０のいずれか一項に記載の走査構造。
【請求項42】
前記主光ファイバは、毛管ファイバをさらに備えた請求項３７～４１のいずれか一項に記載の走査構造。
【請求項43】
前記主光ファイバは、第１の屈折率を有する内側コア及び該内側コアの外側にある第２の屈折率を有する外側コアを備えたダブルコアファイバからなり、前記第１の屈折率は、前記第２の屈折率よりも大きい請求項３７～４１のいずれか一項に記載の走査構造。
【請求項44】
前記光ファイバは、該光ファイバの先端における径が、該光ファイバの全長に亘っての径と同じである請求項１～４３のいずれか一項に記載の走査構造。
【請求項45】
被検体の表面へ光を導くとともに該表面からの光を受光する走査構造であって、前記被検体は、前記表面が第１の波長の光を受光すると第２の波長の光を発するものであり、前記表面は、（ｉ）第１の方向に配列されたプローブの１次元のマイクロアレイであって、前記プローブが第２の方向に幅を有しているマイクロアレイ、又は（ｉｉ）基板の第１の方向及び第２の方向に沿って配列されたプローブの２次元のマイクロアレイを有し、前記走査構造は、
ａ）前記表面の少なくとも一部に、前記第１の波長の光を照射する光源と、
ｂ）先端が前記表面に充分に近接して、前記表面で発せられた前記第２の波長の光を捉えるように配置された導光ロッドと、
ｃ）前記導光ロッドを、前記表面上で第１の位置から第２の位置まで前記第２の方向に沿って移動させるように構成されたキャリアであって、前記第１の位置と前記第２の位置との距離は、（ｉ）前記１次元のマイクロアレイについては、少なくともプローブの幅、又は（ｉｉ）前記２次元のマイクロアレイについては、前記第２の方向に最も離れたプローブ間の距離となっているキャリアとを、備えた走査構造。
【請求項46】
前記導光ロッドの基端が、前記光源により発生した前記第１の波長の光の少なくとも一部を受光し、その光を該導光ロッドの先端へ伝達するように、前記導光ロッドが構成されている請求項４５に記載の走査構造。
【請求項47】
前記導光ロッドは、光ファイバを備えた請求項４５又は４６に記載の走査構造。
【請求項48】
前記走査構造は、第２の導光ロッドをさらに備え、該第２の導光ロッドが、前記光源からの前記第１の波長の光を該第２の導光ロッドの基端にて受光するように構成されるとともに、前記光が、前記基板の表面の近傍にある該第２の導光ロッドの先端から出射するように構成された請求項４５～４７のいずれか一項に記載の走査構造。
【請求項49】
前記第２の導光ロッドは、光ファイバを備えた請求項４８に記載の走査構造。
【請求項50】
前記基板は、実質的に円形であり、該基板の光励起可能領域に対して垂直な回転軸を規定している請求項１～４９のいずれか一項に記載の走査構造。
【請求項51】
被検体の表面へ光を導くとともに該表面からの光を受光する走査構造であって、前記被検

体は、前記表面が第１の波長の光を受光すると第２の波長の光を発するものであり、前記表面は、第１の方向に配列されたプローブの１次元のマイクロアレイであって、前記プローブが第２の方向に幅を有しているマイクロアレイを有し、前記走査構造は、
ａ）前記表面の少なくとも一部に、前記第１の波長の光を照射する光源と、
ｂ）先端が前記表面に充分に近接して、前記表面で発せられた前記第２の波長の光を捉えるように固定的に配置された導光ロッドと、
ｃ）前記導光ロッドを、前記表面上で第１の位置から第２の位置まで前記第１の方向に沿って移動させるように構成されたステージであって、前記第１の位置及び前記第２の位置間の距離は、少なくとも前記第１の方向に沿って配列されたプローブ間の距離になっているステージとを、
備えた走査構造。
【請求項52】
前記導光ロッドの基端が、前記光源により発生した前記第１の波長の光の少なくとも一部を受光し、該光を該導光ロッドの先端へ伝達するように前記導光ロッドが構成された請求項５１に記載の走査構造。
【請求項53】
前記導光ロッドは、光ファイバを備えた請求項５１又は５２に記載の走査構造。
【請求項54】
前記走査構造は、前記基板上の前記光励起可能領域からの前記第２の波長の光を受光するように構成され、前記光励起可能領域には、約１０マイクロメータ乃至約５００マイクロメータの径を有する該領域内に乾式サンプルが含まれている請求項１～５３のいずれか一項に記載の走査構造。
【請求項55】
前記走査構造は、前記基板上の前記光励起可能領域からの前記第２の波長の光を受光するように構成され、前記光励起可能領域には、約１０マイクロメータ乃至約５００マイクロメータの径を有する該領域内に湿式サンプルが含まれている請求項１～５３のいずれか一項に記載の走査構造。
【請求項56】
前記走査構造は、前記基板上の前記光励起可能領域からの前記第２の波長の光を受光するように構成され、前記光励起可能領域には、約１００マイクロメータ乃至約１０００マイクロメータの径を有する該領域内に湿式サンプルが含まれている請求項１～５３のいずれか一項に記載の走査構造。
【請求項57】
前記光ファイバ又は前記導光ロッドは、第１の光励起可能領域からの前記第２の波長の光を受光するために充分に小さい径であるものの隣接した第２の光励起可能領域からは受光しない請求項５４～５６のいずれか一項に記載の走査構造。
【請求項58】
第１のターゲット分子と第１のプローブとの会合及び第２のターゲット分子と第２のプローブとの会合の存在を検出する方法であって、前記第１のプローブは、基板表面の第１の部分上にあって前記第１のターゲット分子と相補的な第１の分子を複数含み、前記第２のプローブは、基板表面の第２の部分上にあって前記第２のターゲット分子と相補的な第２の分子を複数含み、前記プローブは、前記基板上の複数のプローブのうちの１つであり、これらプローブは、（ｉ）前記基板に沿って配列されたプローブの１次元のマイクロアレイであって、前記基板の第２の方向に沿って配列された複数のプローブを有し、前記複数の各プローブは、前記基板上の第１の方向に幅を有する１次元のマイクロアレイ、又は（ｉｉ）前記基板の第１の方向及び第２の方向に沿って配列されたプローブの２次元のマイクロアレイであって、前記第１の方向に沿って配列された複数のプローブ及び前記第２の方向に沿って配列された複数のプローブを有する２次元のマイクロアレイのいずれかとなっており、前記ターゲット分子は、第１の波長の光が照射されたときに第２の波長の光を発する成分を有し、前記方法は、

ａ）前記基板表面の前記第１の部分に、前記第１の波長の光を照射し、
ｂ）光検出器と前記表面の前記第１の部分との前記第１の方向に沿った相対移動により、前記第２の波長の光の存在を検出して、前記第１のターゲット分子が前記第１のプローブと会合しているかどうか判別し、
ｃ）前記基板を前記第２の方向へ進め、
ｄ）前記基板表面の前記第２の部分に前記第１の波長の光を照射し、前記光検出器を前記表面の前記第２の部分に亘って前記第１の方向へ移動させることにより、前記第２の波長の光の存在を再び検出して、前記第２のターゲット分子が前記第２のプローブと会合しているかどうか判別する
行為を含む方法。
【請求項59】
前記光検出器は、第１の光ファイバを備えた請求項５８に記載の方法。
【請求項60】
前記照射の行為は、前記第１の光ファイバの基端に前記第１の波長の光を照射して、前記第１の波長の光を前記第１の光ファイバの先端から射出し、前記基板の前記第１の部分及び前記第２の部分を照射することを含む請求項５９に記載の方法。
【請求項61】
前記照射の行為は、第２の光ファイバの基端に前記第１の波長の光を照射して、前記第１の波長の光を前記第２の光ファイバの先端から射出し、前記基板の前記第１の部分及び前記第２の部分を照射することを含む請求項５９に記載の方法。
【請求項62】
走査装置により蛍光部分の存在を検出する方法であって、前記方法は、前記光ファイバの先端から出射する第１の波長の光で前記蛍光部分を励起して、第２の波長の光を放出させるように、前記走査装置のホルダの光ファイバの先端を位置ずけて、前記光ファイバの前記先端を前記蛍光部分に充分に近接させて、前記光ファイバの前記先端が前記第２の波長の光を受光して、前記第２の波長の光を前記光ファイバの前記基端へ伝達させる方法。
【請求項63】
前記方法は、前記光ファイバの前記先端を、該先端が前記蛍光部分の存在する表面に対して垂直とならないように配置する請求項６２に記載の方法。
【請求項64】
走査装置で用いられるリニアステージの位置を検出する方法であって、
ａ）対応する第１の信号を有する第１のビーコンを、前記ステージ上の第１の位置に配置し、
ｂ）前記第１の信号よりも弱い第２の信号が対応している第２のビーコンを、前記ステージ上の第２の位置に少なくとも配置し、該第２の位置は、前記第１のビーコンから所定の距離にあるとともに前記第１のビーコンから前記第２のビーコンへの線が第１の方向を規定するようになっており、
ｃ）前記ステージを前記第１の方向へ移動させ、
ｄ）前記ステージを移動させている間に、検出器により前記第１の信号、そして前記第２の信号を検出し、
ｅ）検出した前記第１の信号及びより弱い前記第２の信号を、前記ステージの対応する位置に関連づける
行為を含む方法。
【請求項65】
前記検出器は、リニアＣＣＤである請求項６４に記載の方法。
【請求項66】
前記第１のビーコン及び第２のビーコンは、光ファイバを備えた請求項６４又は６５に記載の方法。
【請求項67】
前記所定の距離は、前記リニアＣＣＤよりも短い請求項６５又は５４に記載の方法。

【請求項68】
走査構造を、高いスループットのスクリーニング用に構成された基板に用いる方法であって、前記走査構造は、光源及び検出器を有し、前記方法は、前記光源からの光を前記基板に照射し、該基板から受光した光を前記検出器で検出する方法。
【請求項69】
前記基板には、１平方センチメートル当たり少なくとも４００個のマイクロウェルが含まれる請求項６８に記載の方法。
【請求項70】
前記基板には、１平方センチメートル当たり少なくとも１０００個のマイクロウェルが含まれる請求項６８に記載の方法。
【請求項71】
前記基板には、液体内のプローブが含まれる請求項６８～７０のいずれか一項に記載の方法。
【請求項72】
前記基板から受光される前記光は、サンプルの蛍光により発生される請求項６８～７１のいずれか一項に記載の方法。
【請求項73】
前記基板から受光される前記光は、化学ルミネセンスにより発生される請求項６８～７１のいずれか一項に記載の方法。
【請求項74】
前記基板から受光される前記光には、前記光源により供給された光における少なくとも１つの波長が欠けている請求項６８～７１のいずれか一項に記載の方法。
【発明の詳細な説明】
【０００１】
〔関連出願の参照〕
本発明は、２０００年３月１３日に出願された米国仮出願番号６０／１８８，８７３による権利を主張し、参考としてここにその全てが盛り込まれている。
【０００２】
〔技術分野〕
本発明は、ＤＮＡマイクロアレイ、タンパク質マイクロアレイ、及び化合物マイクロアレイのような、マイクロプレート（ｍｉｃｒｏｐｌａｔｅ）におけるマイクロアレイ（ｍｉｃｒｏａｒｒａｙｓ）又はマイクロウェル（ｍｉｃｒｏｗｅｌｌｓ）を、走査するための装置及び方法に関する。
【０００３】
〔背　景〕
マイクロアレイとは、基板上の所定の位置に固定された生物学又は化学サンプル（「プローブ」）のスポットの列である。各スポットには、単一の生物学的物質又は化学物質のいくつかの分子が含まれている。マイクロアレイは、１つ又はそれ以上の生物学的又は化学的サンプルを含んだ液体（「ターゲット」）に浸される。通例、ターゲットは、マイクロアレイ上で１つ又はそれ以上の相補的なプローブと互に影響し合う。特に、ＤＮＡマイクロアレイでは、プローブがオリゴヌクレオチド又はｃＤＮＡ菌株（ｓｔｒａｉｎｓ）であり、ターゲットが蛍光又は放射性ラベルがなされたＤＮＡサンプルである。ターゲット中の分子鎖は、プローブのマイクロアレイ中の相補的な分子鎖とハイブリッドを形成する。
【０００４】
ハイブリッド化されたマイクロアレイは、マイクロアレイ・リーダにより検査される。マイクロアレイ・リーダは、放射性ラベルの存在を検出するか、あるいは、レーザや他のエネルギー源を用いて蛍光ラベルを励起することにより、該蛍光ラベルを刺激し、光を放射させる。マイクロアレイ・リーダは、マイクロアレイにおけるラベルの放射の位置及び強度を検出する。プローブは、マイクロアレイにおける予め定められた既知の位置に配置されているので、液体中の一連のターゲットは、蛍光又は放射線が検出された位置及び蛍光又は放射線の強度により、識別される。

【０００５】
従って、マイクロアレイ・リーダは、マイクロアレイ技術の装置における重要な部分の１つである。現在、２種類のマイクロアレイ・リーダが利用可能である。第１種のものは、その一例が米国特許第５，３２４，６３３号に記述されており、走査顕微鏡の原理に基づくものである。それによると、2以上の波長のレーザビームが組み合わされ、マイクロアレイ上の１つのスポットに集束されて、蛍光ラベルを励起する。組み合わされたレーザビームは、２軸の平行移動ステージ上でマイクロアレイを動かすか、あるいは、顕微鏡のレンズを一方の軸で動かし、マイクロアレイ基板を他方の軸で動かすことにより、マイクロアレイ全体を各点毎に高い空間解像度（～１０μｍ）で走査する。第２種のものは、その一例がＷＯ００／１２７５９号に示されており、ＣＣＤ画像装置を用いて、マイクロアレイからの蛍光の放出を、小領域毎に一度に検出してゆく。アークランプのような広帯域の光源が、蛍光の励起に用いられている。
【０００６】
走査顕微鏡技術では、コストに大きく影響するのは、その光学系である。これは、光学系が、波長の異なる複数のレーザビームを結合して正確に並べ、その後、別々の検出チャネルへと再び分割しなければならないためである。顕微鏡のレンズ及びスライドキャリアの双方とも、かさばって重く、高速に動かすことができない。このことにより、走査スピードが制限される。
【０００７】
ＣＣＤ方式のシステムでは、画像装置及びレンズの双方がコストに大きく影響する。ここでは、励起光は、エネルギー密度を大幅に減少させて、広範囲に拡がってしまう。この減少を補償するために、露光時間を数十分の１秒まで延ばさなければならない。露光時間が長いので、ＣＣＤ画像装置は、妥当なＳＮ比を維持するために、冷却されている必要がある。冷却されている大型のこのようなＣＣＤ画像装置は、現在のところ非常に高価である。さらに、このシステムの光学レンズは、色収差及び広い視野に亘る像の歪曲が補正されていなければならない。このことにより、走査顕微鏡の各点毎のシステムにおけるレンズに比べて、コストが非常に上昇する。そのうえ、スライドの全領域（２５ｍｍ×７５ｍｍ）に対応するのに充分な解像度と大きさを持ったＣＣＤ画像装置は、現在のところ存在しない。ＣＣＤ方式のシステムでは、機械的な走査が、これまで通り必要とされる。これにより、マイクロアレイ・リーダが低速になる。現在、ほとんどの市販のマイクロアレイ・リーダは、２５ｍｍ×７０ｍｍのスライドの走査を完了するのに、５分以上を要する。これは、多くの用途に対しておそすぎる。
【０００８】
光ファイバは、近接場走査顕微鏡法に用いられてきている（Ｄ．Ｗ．Ｐｏｈｌ，“Ｓｃａｎｎｉｎｇ ｎｅａｒ－ｆｉｅｌｄ ｏｐｔｉｃａｌ ｍｉｃｒｏｓｃｏｐｙ”ｉｎ Ａｄｖａｎｃｅｓ ｉｎ Ｏｐｔｉｃａｌ ａｎｄ Ｅｌｅｃｔｒｏｎ Ｍｉｃｒｏｓｃｏｐｙ １２，Ｃ．Ｊ．Ｒ．Ｓｈｅｐｐａｒｄ ａｎｄ Ｔ．Ｍｕｌｖｅｙ，Ｅｄｓ．（Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ，Ｌｏｎｄｏｎ，１９９０）；Ｍ．Ｈ．Ｐ．Ｍｏｅｒｓ，Ｗ．Ｈ．Ｊ．Ｋａｌｌｅ，Ａ．Ｇ．Ｔ．Ｒｕｉｔｅｒ，Ｊ．Ｃ．Ａ．Ｇ．Ｗｉｅｇａｎｔ，Ａ．Ｋ．Ｒａａｐ，Ｊ．Ｇｒｅｖｅ，Ｂ．Ｇ．Ｄｅ Ｇｒｏｏｔｈ，Ｎ．Ｆ．ｖａｎ Ｈｕｌｓｔ，Ｊ．Ｍｉｃｒｏｓｃｏｐｙ １８２，ｐ．４０，（１９９６））。ここでは、光エネルギーは、直径がサブマイクロメータにまで縮小されて、光ファイバ先端に導かれる。ファイバ先端は、被検面のナノメータの数十分の１以下のところに置かれる。そして、ファイバ先端は、小領域（通常１０ｍｍ角）を走査し、被検面からの直接散乱光を受光する。（Ｃｈｕｃｋ，開示されている発明で、ファイバのコアが集光を最大化するには大きすぎ、集光された光は、被検体からの蛍光のように、間接的に放出されたものである。）
一方、光ファイバ・リーダ・ヘッドは、ＡＢＩのＴａｑｍａｎリーダ（ＵＳ５５８９３５１）のようないくつかの機器で、マイクロタイタープレート（ｍｉｃｒｏｔｉｔｅｒ ｐｌａｔｅ）から放出された蛍光を受光するのに用いられてきた。ここで、リーダの空間解

像度は、数ミリメートルのオーダである。励起光と受光された光とは、バンドルにおける別々のファイバで伝達される。光学ヘッドは、被検体から比較的大きく隔てられている。

【０００９】

〔概　要〕

以下に充分に記述するように、本発明により、プローブのマイクロアレイ又はサンプル（マイクロウェルに収納された乾式又は湿式）を走査するいくつかのシステム、装置、手段、及び方法が提供される。本開示内容のこの部分により、本発明の顕著な点についての要約が提供されるが、この部分は、本発明を、当該部分について考察された特徴及び実施形態のみに限定するように解釈されるためのものではない。むしろ、本発明には、特許請求の範囲とともに当該部分及びそれに続く部分で考察される全ての装置、システム、及び方法が含まれている。

【００１０】

開示される走査構造には、該走査構造で測定される基板上の光励起可能領域に対する光伝達及び受光装置が含まれている。この光伝達及び受光装置は、基端及び先端を有する光ファイバを備えていてもよい。この光ファイバは、ある波長の光又はいくつかの多様な波長の光を伝達して、サンプルを照射する。これにより、発光が励起されたり１つかそれ以上の波長がサンプルに吸収されたりする。また、この光ファイバは、基板上のサンプルに蛍光を発生させることにより放出されるかあるいは他の方式で生じた、反射又は回折した光を、同時に受光してもよい。さらに、走査構造には、検査対象の基板上を可変の間隔で横切ることが可能な光ファイバホルダが含まれていてもよい。例えば、ホルダには、ガルバノスキャナや共振懸架ビームが含まれていてもよい。基板のサンプルを励起する光を発生させるために、例えばレーザ等の光源が、光ファイバの基端に光学的に結合されてもよい。異なった波長の複数の光源が、波長マルチプレクサで光源を単一の光ファイバに結合するか、あるいは、異なる波長を伝達する個別の光ファイバを互いに近接させて配置することにより、同時に用いられてもよい。光は、光ファイバを通じて基板へと伝達され、光ファイバは、発せられた蛍光をも受光できるように、この基板に充分に近接している。

【００１１】

本発明のある実施形態は、蛍光を発するものとして記述されているが、本発明には、他の形式による光の発生も含まれている。他の形式として、化学ルミネセンス、及び、光が吸収されて吸収スペクトルが生じ、それが走査構造の導光部分（例えばファイバ又はロッド）により伝達されること等がある。このように、基板上の光励起可能領域は、湿式及び乾式のサンプルを含んだ基板の一部である。サンプルは、走査構造の導光部分により基板に伝達された複数の波長のうちのある波長が吸収された領域あるいは、基板（蛍光や化学ルミネセンスのような）により受光した光とは異なる波長の光を発生する。従って、基板上の光励起可能領域で「発生」した第２の波長は、光源により供給されていない波長であってもよく、光源から伝達されて、サンプルや基板により反射又は回折するものの光励起可能領域における基板やサンプルには吸収されていない波長であってもよい。このように、走査構造は、光を検出するか、あるいは、光においてある波長が欠落していることを検出するように、構成されうる。

【００１２】

走査装置の空間解像度は、励起光のエネルギーを伝達するファイバのコアの径にほぼ等しいので、好適なコア径は、５μｍ，１０μｍの空間解像度の走査装置に対して、それぞれ５μｍ，１０μｍとなる。このようなコア径は、通信ファイバで容易に入手可能である。ファイバ先端でコアのサイズを小さくする必要はない。

【００１３】

開示される本走査装置は、通常の正方形又は長方形状の基板上のマイクロアレイの他にも、ＣＤのように回転する基板を読み取ること、及び１次元のマイクロアレイにも適用可能である。

【００１４】

開示される本発明は、マイクロアレイに加え、高いスループットでスクリーニングするこ

との用途において、顕微鏡的リアクションウェルのアレイを読むことにさらに適用可能である。

【００１５】

現在のところ、ＨＴＳからの出力信号は、蛍光、化学ルミネセンス、あるいは吸光度であってもよく、これらは、「マイクロプレート・リーダ」と称する装置を用いて検出される。本発明は、現在のＤＮＡマイクロアレイと同等のサイズ及び密度（例えば、約５００ウェル／ｃｍ^{2}よりも高密度、好ましくは約１０００ウェル／ｃｍ^{2}よりも高密度、より好ましくは約２０００ウェル／ｃｍ^{2}よりも高密度、さらに好ましくは約５０００ウェル／ｃｍ^{2}よりも高密度）のマイクロウェルアレイ内の溶液からのこのような信号を読み取る走査装置に関する。マイクロウェルの内径は、約１００マイクロメータから約１０００マイクロメータであり、好ましくは約５００マイクロメータ未満であり、より好ましくは２００マイクロメータ未満である。ある特定の実施形態では、本発明の光ファイバ走査装置は、マイクロウェル内の溶液からの信号の読取装置に適用されている。例えば、光ファイバが基板となす角度は、垂直であってもよく、反射を防ぎながらも光が存在するかどうかを検出できるような垂直に近い角度であってもよい。ファイバ径は、マイクロウェルの径に基づいて選択されてもよい。走査前に読取装置が待機する時間は、サンプル及びプローブ（例えば、オリゴヌクレオチド、タンパク質、又は反応物）にサンプルと会合又は反応させるのに必要な反応又は会合時間に基づいて選択されてもよい。光ファイバによって読取装置が、スクリーニングシステムに統合可能となる柔軟性が提供される。

【００１６】

〔発明の詳細な説明〕

本発明では、多くの基本的な光学部材が、励起光を伝達することに、あるいは蛍光の放出を受光することにも、用いられてもよい。このような光学部材として、導光ロッド及び光ファイバのような部材が、含まれていてもよい。光学部材として光ファイバを用いることが好ましく、以下に開示する実施形態では、このような光ファイバを例として用いることが考察される。しかしながら、本発明はこれに限定されるものではない。

【００１７】

図１（ａ）に、本発明の一実施形態としての走査構造１０が、図示されている。この図において、レーザ１４から射出された励起レーザ光２０は、光ファイバ１２の基端２８に入る前に、フィルタ１６により反射される。光ファイバ１２は、励起光２０を導いて、当該光ファイバ１２の先端２６から、マイクロアレイ・サンプル３０上の例えばＤＮＡプローブ等のサンプルを照射する。マイクロアレイ・サンプル３０は、上述の如く、基板３２及びプローブ３４から構成されていてもよい。フィルタ１６は、光の予め選択した波長又は帯域を反射しつつ、同時に、反射光における所望の波長又は帯域を通過できるように、選択的に設計されていてもよい。プローブ３４から発せられた蛍光２０´は、同ファイバ１２により受光され、フィルタ１６を通過した後、検出器１８へ導かれる。本実施形態１０における励起光２０及び蛍光２０´は、フィルタ１６によって分離される。フィルタ１６は、所定の波長の光を反射させる一方で、特定の波長を透過可能としている。図１（ａ）に示された特定の実施形態では、フィルタ１６が、励起光２０を反射するように設計されていてもよい。また、図１（ａ）におけるレーザ１４と検出器１８の位置を交換することにより、このシステムに、蛍光を反射するフィルタが用いられてもよい。

【００１８】

図１（ｂ）には、同システム設計の代替的な実施形態２２が図示されている。ここでは、かさばっていた光ビーム分割及び結合構造が、光ファイバカプラ２４で置き換えられている。この記述の全体に亘り、別々の実施形態における構造で同一の番号が付されたものは、その機能が類似している。また、カプラ２４は、特定の波長を他の分岐ファイバへと選択的に結合する波長多重（ＷＤＭ：ｗａｖｅｌｅｎｇｔｈ　ｄｏｍａｉｎ　ｍｕｌｔｉｐｌｅｘｅｒ）であってもよい。好ましくは、励起光を遮断するために、検出器１８の前に、追加のフィルタ１６が配置されてもよい。

【００１９】

さらなる他の実施形態では、レーザの代わりに、励起光２０の光源として発光ダイオード（ＬＥＤ）が用いられる。単一のＬＥＤ、又は複数のＬＥＤで各ＬＥＤが別々の波長の光を発するものを、基板３２に直に近接して配置してもよい。このようにしても、光ファイバ１２が蛍光２０´を集光可能であり、光ファイバの基端２８にあるレーザ１４を置換可能である。

【００２０】

図２に示すように、光ファイバの先端２６から出射する光３６は、ファイバの開口数（ＮＡ）で規定される固有の角度で発散する。光ビームがファイバで導かれるために、光ビームは、以下の両条件を満たさなければならない。１）光ビームは、ファイバの開口数で規定されるコアの領域に入射すること、そして、２）光ビームは、ファイバの軸に対して、ＮＡよりも小さい角度をなしていること。例えば、矢で示された光ビーム３８は、上記の両条件を満たしているので、コア領域４２に導かれうる。しかし、光ビーム４０は、上記条件の一方のみを満たしているだけなので、導かれることなく、コア領域４２ではなくクラッド４４に入射する。

【００２１】

図１の（ａ）及び（ｂ）に図示された実施形態において、ビームの発散及び導光条件により、ファイバのコアに非常に近い領域へ発せられた光が集光される。ファイバの集光能力は、２つの要素で規定される。すなわち、１）コアのサイズ、及び２）ＮＡである。これら２つのパラメータが大きくなるほど、ファイバ１２の集光能力が高くなる。しかしながら、コアのサイズが大きくなると、システムの空間解像度が低下するおそれがある。ファイバ先端１２と基板３２との間隔がd_c／２ＮＡ以内であれば、空間解像度は、ファイバのコアのサイズにほぼ等しい。ここで、d_cは、ファイバのコア４２の直径とする。基板３２がファイバ先端１２からd_c／２ＮＡの間隔より大きく離れていれば、蛍光を受光する効率が著しく低下する。標準的な通信ファイバは、そのコア径が約９μｍであり、ＮＡ＝０．１２である。従って、本発明の走査構造を構成するのに標準的な通信ファイバが用いられると、基板表面がファイバ端面２７から３８μｍ以内に保たれていれば、約１０μｍの空間解像度が達成可能となる。ＮＡが０．６６のファイバが報告されている。ファイバ端面２７を基板の約７μｍ以内に保つことにより、集光能力を約２７倍に引き上げる可能性が存在する。

【００２２】

ファイバ１２を基板３２に近接させておくように、いくつかの方法を用いうる。

【００２３】

本発明の走査構造の第１実施形態では、ファイバ先端１２と基板３２表面との間隔がリアルタイムで検出され、この間隔が能動的に制御される。間隔の大きさは、干渉計を用いた光学干渉のような通常の方法で検出され、干渉計から取得した信号に応答して動作する例えば圧電アクチュエータ（米国特許第６，１４２，４４４号記載のようなもの）に、光ファイバを取り付けることにより、制御される。

【００２４】

本発明の第２の走査構造は、基板近傍でファイバ先端が高速動作することにより発生するエアクッション上に、マイクロアレイ基板３２全体に亘ってファイバ１２の先端を空気力学的に「浮ばせる」ことができるように、構成されている。これは、例えば、フロッピーディスクドライブ（ＴＭ）、ハードドライブ、及びＣＤ－ＲＯＭドライブに使用されている技術である。フロッピーディスクドライブ（ＴＭ）の読取ヘッドは、厚さが２マイクロメートルのエアギャップで浮遊する。エアギャップは、回転するフロッピーディスク（ＴＭ）及び読取ヘッド間に発生したいわゆる「地面効果」により空気力学的に形成される。読取ヘッドとフロッピーディスク（ＴＭ）との相対動作により、真空が形成されて読取ヘッドがフロッピーディスク（ＴＭ）表面へ引き付けられる。しかし、読取ヘッドが表面に近接してゆくと、読取ヘッド及び表面間のギャップにおいて圧力が充分に増大し、読取ヘッドはディスク表面に接触しない。

【００２５】

この「地面効果」が、本発明の走査装置に適用されてもよい。ファイバ先端が基板表面において充分に早く動作して、ファイバ及び基板間の相対動作により、ファイバ先端は、基板表面の数マイクロメータ以内に引き付けられる。図３に示すように、ファイバ先端は、読取ヘッド５０内に収容されていてもよい。読取ヘッド５０は、基板３２とともにベンチュリを形成するような形状を有しており、それにより気流に地面効果を発生させる。以下に詳述するように、読取ヘッドは、１本の光ファイバが、該ファイバを基板表面全体に亘って移動するホルダに取り付けられたものであってもよく、あるいは、読取ヘッドは、光ファイバの束がホルダに取り付けられたものであってもよい。図３に示すように、読取ヘッドにおける基板に対向した表面は、側面から見てパラボラ状の形状を有していてもよい。あるいは、図１や図２に示すように、読取ヘッドは、平坦な表面を有していてもよい。読取ヘッドすなわちファイバ先端が、反対方向へ移動する際に、方向を変えるために減速してマイクロアレイを走査するので、速度が地面効果を得るのに必要な速度より小さければ、読取ヘッドやファイバ先端が、基板又は基板のホルダの一部に接触するおそれがある。

【００２６】

磁気及び空気力学的浮揚を併用した走査構造４６では、読取ヘッドやファイバ先端が走査中に方向を変えるために減速する時に、読取ヘッドやファイバ先端が基板や基板ホルダに接触するのを、防止することができる。図３に示すように、走査構造４６のマイクロアレイ基板３２は、極性の方向が同じの一対の磁石４８で支持されている。光ファイバが組み込まれた読取ヘッド５０は、永久磁石が組み込まれて形成されるか、あるいは、それ自体が磁化されて（磁性材料で形成される場合）、その極性が支持磁石４８の対と同じになっており、読取ヘッド及び磁石４８間で斥力が発生する。基板３２の中央の位置１では、読取ヘッド５０は、比較的速く動作し、空気力学的に浮揚する。読取ヘッド５０は、基板３２の端へと位置２から位置３へ移動するにつれて、おそくなってゆく。おそくなると、空気力学的な浮揚力が減少するが、読取ヘッド５０は、磁気支持部４８からの磁力により支持され、当該読取ヘッドがマイクロアレイ基板３２や磁石４８に接触しないように浮揚した状態が保たれる。

【００２７】

本発明の走査構造の第３の実施形態は、特に、表面が粗い基板上のマイクロアレイのラベルを励起して検出するのに適している。但し、その用途は、表面の粗い基板上のマイクロアレイに限定されるものではない。図４に示す走査構造５２では、読取ヘッド５０を一定な状態で空気力学的に浮揚させておくために、光ファイバ５６間に毛管５４が組み込まれていてもよい。複数本のファイバが用いられる場合には、それらは、任意に束ねられるか、あるいは、高速又は冗長性を持たせてマイクロアレイを走査できるように、周知の空間配列で連続的に又は規則正しい配列に束ねられる。毛管ファイバ５４を通じて、気体を下方へ吹きつけることにより、極めて薄い気体クッション５８が、読取ヘッド５０及び基板３２間に形成される。空気や窒素のような不活性な気体であれば、何でも使用可能である。ファイバ方式の読取ヘッド５０は極めて軽いため、読取ヘッド５０を基板３２上に浮揚させて、両者を数マイクロメータのオーダの小さな間隔に保つのには、小さな正の圧力で充分なはずである。正の圧力の大きさは、読取ヘッド５０の個々の設計に依存する。

【００２８】

信号対雑音比の向上、複数波長での励起及び受光、並びに走査機構といったいくつかの特徴について、以下、考察する。

【００２９】

〔信号対雑音比の向上〕

ファイバ１２の長さ方向の軸が、基板３２の表面に対して実質的に垂直であると、元の励起光２０のうちのある部分は、ファイバ１２の端面２７を出て基板３２で反射され、そして、ファイバ１２へと戻ってくる。これにより、信号中にバックグラウンドノイズが生じる。図１の（ａ）及び（ｂ）に示すように、このバックグラウンドノイズは、システム中のフィルタ１６により抑制しうる。フィルタ１６は、それがなければ検出器１８が検出し

てしまう望ましくない波長の光を反射することにより、バックグラウンドノイズを抑制するように作用する。しかしながら、発せられた蛍光が比較的小さいために、フィルタが抑制するにもかかわらず、かなりのレベルのノイズがなおも存在するおそれがある。より高い信号対雑音比（ＳＮ比）を有する走査構造では、基板３２の表面に対して角度θで傾いたファイバ１２が、含まれている。この角度は、図１及び図５に示すように、ファイバ１２のＮＡよりもわずかに大きい。この構成により、反射された励起光は、光ファイバで検出器へと導かれるのではなく、ファイバの壁面を透過するようになる。

【００３０】

ＳＮ比がより高い走査構造の代替的な実施形態には、基板３２の表面と実質的に平行に端面２７が研磨されたファイバ１２が、含まれている。このように研磨すると、全ての光（励起光２０を含む）が、直接、ファイバ端面２７及びマイクロアレイ基板３２で反射され、ファイバ１２の軸とＮＡより大きい角度で交差するので、この光がファイバコア４２を通じて検出器１８へ導かれることがない。一方、蛍光２０´は、あらゆる方向へ発せられ、端面が研磨されていないファイバに受光されるのと同じ割合の光２０´が、その信号レベルを変化させずにファイバ１２に受光される。その結果、システムのＳＮ比は、著しく向上する。

【００３１】

ＳＮ比が向上した他の走査構造には、ダブルコアファイバ６０が用いられている。図５に示すように、ダブルコアファイバ６０は、２つの同軸のコア６２，６４を有し、コア６２の屈折率は、コア６４の屈折率よりも大きくなっている。ダブルコアファイバ６０の相対屈折率の縦断面図を、図５に示す。この図において、ピーク７０は、インナーコア６２の相対屈折率に相当し、ピーク７２は、アウターコア６４の相対屈折率に相当し、ピーク７４は、ファイバ６０のクラッドの相対屈折率に相当する。

【００３２】

ダブルコアファイバ６０を備えた走査構造は、レーザからの光をインナーコア６２を通じて伝達する一方、基板からアウターコア６４へ入射した光については、１００％に近い結合比（ｃｏｕｐｌｉｎｇ　ｒａｔｉｏ）を有する。このように、ダブルコアファイバは、コアセレクティブカプラ（ｃｏｒｅ－ｓｅｌｅｃｔｉｖｅ　ｃｏｕｐｌｅｒ）として機能する。

【００３３】

アウターコア６４は、インナーコア６２よりも低い屈折率を有するので、インナーコア６２に対してクラッドのように機能する。従って、ダブルコアファイバが図１の（ｂ）のシステムに用いられると、例えばレーザから発せられた励起光２０は、臨界角より小さな角度でインナーコア６２に入射し、励起光は、実質的に、インナーコア６２内に閉じ込められる。

【００３４】

アウターコア６４内の光は、このダブルコアファイバにより外側に結合されることになる。インナーコア６２の臨界角より大きな角度でアウターコア６４に入射した光は、インナーコア６２へと内部反射することがないので、主にアウターコア内６４で見い出され、インナーコア６２には実質的にレーザからの光だけに任せた状態となる。

【００３５】

さらに、インナーコア６２は、該インナーコア６２から出射した後の光ビーム６８の発散が小さくなるように、小さなＮＡを有していてもよい。一方、発せられた蛍光６６の大部分は、アウターコア６４に入射して、ファイバ６０を遡って検出器へと伝達する。このような方法で、集光効率が著しく向上し、そのために、ＳＮ比が向上することになる。さらに、アウターコア６４は、インナーコア６２よりもはるかに大径に形成可能であるため、集光された光の強度は、ファイバ１２の端面２７と基板３２との間隔に決定的に依存するということがより少なくなる。このため、機器設計の許容度及び自由度がより高くなる。

【００３６】

ダブルコアファイバ６０を製造する一方法として、化学気相成長法（ＣＶＤ）がある。こ

れによると、例えばシラン及びO_2をともなった気体のＧｅ等のドーパントを、通常のファイバ形成チューブ（プリフォーム）の内面に第１の濃度で蒸着させ、層を形成する。次に、ドーパント（第１の濃度のドーパントと同じか又は同様の通常のドーパントからなる）を、第１の濃度よりも高い第２の濃度で、前記層上にドープしてもよい。その後、プリフォームを伸ばしてファイバ６０を形成する。

【００３７】

〔複数波長での励起及び受光〕

通常、本発明の走査構造は、少なくとも２つの別々の波長の光を伝達する。２つの波長の光とは、ある波長の励起光、及び、蛍光マーカから発せられた他の波長の蛍光である。現在知られている技術として、５つまでの別々の励起及び／又は受光波長を有する走査装置がある。現状のマイクロアレイ走査装置では、波長の異なる全ての光ビームが、顕微鏡の対物レンズに通じた単一の光路に集約される。通常の走査装置で用いられている複数の大きな光学コンポーネントには、これらの大きな光学コンポーネントの運搬及び移動させるために、高精度のアライメント及び複雑な構成が必要とされる。高精度で大きな光学コンポーネントにより、現行の走査装置はかさばって高価なものとなっている。さらに、集約された光路を有する現行の光学検出器では、ある波長を他のものから分離することが、困難であることが多い。２つ以上のレーザが同時に稼動しているときには、ＳＮ比が悪化する。現行の多くのマイクロアレイ走査装置では、走査速度を大幅に低下させてしまうという犠牲を払いながら、一度に一つのレーザに切り替えることにより、この問題の発生を防止している。

【００３８】

図６に、本発明の走査構造７６を示す。ここでは、複数の波長に対応した複数の光源$14_{\lambda 1}$～$14_{\lambda n}$が、波長多重（ＷＤＭ）78_1，78_nを用いて、単一の光ファイバ１２に集約されている。この走査構造は単純である。これは、特に、現行の走査装置で現在使用されている例えばレンズ及びミラーアセンブリのための複雑な支持構造を必要としない光ファイバ１２の柔軟性によるものである。ＷＤＭ78_1，78_nは、例えば、プレーナ又は溶融型カプラの作製することにおいて電気通信業界で周知の技術を用いて、作製される。

【００３９】

本発明の一実施形態では、複数波長の光ビームが、マイクロアレイ基板３２全体に亘って同時に走査されるが、これら光ビームは、正確に同じ位置にある必要はない。図７に、代替的な実施形態８０を示す。これによると、１つの波長が、他の波長からより容易に、速度の犠牲を払うことなく分離されている。図７に示すように、各波長の光$14_{\lambda 1}$～$14_{\lambda n}$が、別々であっても比較的近接した位置を照射する一方、同期して走査することを可能にする応用例に応じていくつかの所望の構成に、光ファイバ１２を配列してもよい。束になった光ファイバは、例えば、ファイバが挿入されてその順序を保つように束ねるガイドプレートを使用して、あるいは使用せずに、形成されてもよい。ここに言及された全ての論文、特許、及び特許出願は、その全体が一体に組み入れられる。光ファイバ１２は、極めて軽量で、細く正確な直径なので、各波長毎に別々のファイバを用いて、同期したマルチスポット走査が実現する。ここで、個々のファイバ１２がマイクロアレイ状に束ねられて、光ファイバ走査ヘッドを形成してもよい。このようなマイクロアレイは、規則的な配列又はランダムなバンドルとなるように、束ねられてもよい。バンドル中のファイバの本数は任意であるが、多くの例で、その本数は１０よりも少ない。システムにおける各ファイバの先端の相対位置が既知となるため、どちらの変形例も実現可能である。ファイバ端が極めて近接しているにもかかわらず、全てのファイバ１２が、同一のスポットを照射するように集束される必要はない。但し、そのようになされても、さしつかえない。逆に、各光ファイバ１２は、別々のスポットを同時に照射し、必要に応じて各スポットから同時に蛍光を集光するように、配列されてもよい。

【００４０】

〔走査機構〕

光ファイバ１２は、極めて軽いので、上記の走査ヘッド５０は、非常に高速に移動し、それにより、マイクロアレイを読み取る速度が増大する。図８に、走査装置８２の実施形態を示す。ここでは、光ファイバ１２の読取ヘッド５０が、通常のガルバノスキャナ（Ｇａｌｖａｎｏ　ｓｃａｎｎｅｒ）８４によりｙ方向に往復移動する。ガルバノスキャナ８４は、光ファイバ１２及び読取ヘッド５０を保持した懸架ビーム（ｓｕｓｐｅｎｓｉｏｎ　ｂｅａｍ）８６を、所望の角度αに亘って、基板３２の幾何学的構成に応じた所望の周波数で、移動させるように設定されている。走査装置は、Ｘステージ位置決め機構を備えていてもよい。Ｘステージ位置決め機構は、基板を読取ヘッドの下側を移動させるとともに、光ファイバ１２がＸ軸上のどの位置を読み取っているのかを確定する情報を提供する。光ファイバ１２のＹ軸上の位置は、ガルバノスキャナ８４に組み込まれた角度エンコーダにより検出される。ガルバノスキャナ８４が懸架ビーム８６をＹ方向に移動させると、基板３２は、通常の移動ステージ８８によりＸ方向に段階的に移動する。

【００４１】

図９に、走査装置９０の代替的な実施形態を示す。この実施形態では、ガルバノスキャナ８４及び懸架ビーム８６が、共振アクティベータ９２及び共振懸架ビーム９４に置き換えられている。ここでは、光ファイバ読取ヘッド５０は、共振懸架ビーム９４がその共振周波数で共振アクティベータにより駆動されることにより、Ｙ方向に往復するように振動する。共振懸架ビーム９４近傍に配置された圧電素子のような任意個数の通常の共振アクティベータ９２装置により、あるいは、共振懸架ビーム９４の両側に配置された磁気素子により駆動されて、共振が発生する。どちらの実施形態であっても、読取ヘッド５０は、基板３２の端部又は外側で止まるように設定される。基板３２の端部や外側には、プローブが存在していない。読取ヘッド５０及び基板３２間の接触（それにより汚染を防止）は、基板３２上に読取ヘッド５０を浮揚させるのに用いた空気力学的浮揚機構を実装すること等による通常の、あるいは、上述のいくつかの方法により、防止される。

【００４２】

図８及び図９に示す個々の実施形態では、走査ヘッド５０は、Ｙ方向における湾曲した経路を通る。このことは、未処理のデータにおける画像の画素が、ほとんどの画像ファイルのような矩形の格子状になっていないことを意味する。しかしながら、各画素の正確な位置が登録されているかぎり、この走査装置で生成される画像ファイルは、通常のソフトウェアによって標準的な画像ファイルに変換可能である。どちらの実施形態においても、Ｘ位置は、移動ステージ８８の通常の位置エンコーダにより記録されてもよい。図８に示す実施形態では、Ｙ位置は、ガルバノスキャナ８４が角度αに亘って掃引することにより生じる角変位から算出されてもよい。図９に示す実施形態では、Ｙ位置は、いくつかの異なった方法のうちの任意のものにより、算出されてもよい。１つの方法として、共振懸架ビーム９４の両側表面上の既知の位置における変形歪力を、通常の歪ゲージを用いることにより測定して記録する方法がある。２番目の方法では、新規の光学方式位置測定装置が用いられる。これについては後ほど詳述する。この方法は、このような測定の目的に適合したものである。第３の方法として、共振周期をリアルタイムで測定し、経時的な振動の両極値間の読取ヘッド５０の位置を算出する方法がある。

【００４３】

図１０（ａ）乃至図１０（ｃ）には、ＣＣＤアレイ及び光ファイバビーコンが組み込まれた単純な位置検出装置９６の代替的な実施形態が示されている。この実施形態は、スライドする大きさおよび移動速度が多様であったとしても、実施可能である。

【００４４】

図１０（ａ）乃至図１０（ｃ）に示すように、光ファイバ９８，１００が、移動ステージ１０６の移動部分に、ビーコンとして実装されている。固定ステージ１０４は、移動ステージ１０６とは異なり、静止している。その代わりに、ステージ１０４が動いて、ステージ１０６が固定されていてもよい。第１のビーコン９８は最も明るく、第２のビーコンはより暗くというようになっている。また、リニアＣＣＤアレイ１０２が、ステージの固定部分１０４又はステージの移動部分１０６に実装されていてもよい。近接した２つのビー

コン９８，１００の間隔は、リニアＣＣＤアレイ１０２よりもわずかに短い。移動開始時に、リニアＣＣＤアレイ１０２は、そのピクセルアレイの一端において、単一の明るいスポットを検出する。図１０の（ａ）に示すように、このスポットの位置は、移動ステージ１０６の相対位置を示している。図１０の（ｂ）、図１０の（ｃ）に順に示すように、ステージ１０６が移動してゆきながら第１のビーコン９８がリニアＣＣＤ１０２の範囲を越える手前で、このスポットがリニアＣＣＤの他端へ移動してゆくと、第２のビーコン１００が移動してきて、位置監視が継続される。全範囲に亘る移動ステージ１０６の位置を絶対位置で監視可能とするのに必要であれば、ビーコンは、いくつ用いられてもよい。

【００４５】

ＣＣＤの長さと比較したビーコンの分離間隔の一例として、ピクセルアレイに沿ったスポットの正確な位置は、中心軌跡アルゴリズム（ｃｅｎｔｒｏｉｄ ａｌｇｏｒｉｔｈｍ）を用いて、少なくともピクセルのピッチの約１／５０まで算出されてもよい。例えば、通常の２０４８ピクセルでピッチが２４μｍの代表的で安価なＣＣＤアレイ１０２を考慮すると、リニアＣＣＤアレイ１０２の有効な長さは、約４９ｍｍである。２つのファイバビーコンを例えば約４０ｍｍ間隔で実装すると、位置検出装置９６は、移動ステージ１０６の位置を、約８０ｍｍの範囲に亘って０．４８μｍの解像度で監視可能となる。これは、マイクロアレイ走査装置のためには、充分過ぎるものである。

【００４６】

図１１に示すように、位置検出装置９６は、１次元のマイクロアレイ１０８の実施形態に用いられてもよい。ここで、特許出願中の米国特許出願番号６０／２４４，４１８（名称「Ｇｅｎｅ Ｔｈｒｅａｄ」，発明者：Ｓｈｉｐｉｎｇ Ｃｈｅｎ，Ｙｕｌｉｎｇ Ｌｕｏ，ａｎｄ Ａｎｔｈｏｎｙ Ｃｈｅｎ，出願日：２０００年１０月３０日）に記述されているように、１次元のマイクロアレイ１０８は、移動ステージ１０６上に位置していてもよい。この米国特許出願番号６０／２４４，４１８は、その全てがここに一体に組み入れられる。１次元のマイクロアレイ１０８上には、ハイブリッド化のためのサンプルが含まれた標準的なプローブ１１０が配列されていてもよい。その代わりに、プローブは、所望の応用例に応じた様々な構成で配置されてもよい。いくつかの例として、プローブは、直線状１１２にあるいは斜め１１４に配置されてもよい。どのような場合であっても、プローブの位置は、上述の方法及び装置のいずれかを用いて、監視され読み取られる。

【００４７】

その代わりに、本発明の走査装置の一実施形態では、１次元のマイクロアレイ１０８が当該読取ヘッド５０の下側を移動する一方で、読取ヘッド５０は、固定された状態で、走査可能となっていてもよい。これにより、１次元だけのプローブ１１０がステージ１０６上で一方向に移動しているため、読取ヘッドを振動させる必要がなくなる。

【００４８】

また、位置検出装置９６を調整するのに、ビーコン９８，１００をその相対的なピーク強度で識別するシステムが用いられてもよい。図１０の（ａ）乃至図１０の（ｃ）に示すように、ＣＣＤアレイは、該アレイ上の位置の関数になった相対的な信号強度を出力する。所与の信号強度が、所与のビーコンに対応している。例えば、第１のビーコン９８は、最も明るいビーコンであるため、最も明るい信号９８´に対応し、第２のビーコン１００は、第１のビーコン９８よりも暗いビーコンであり、より暗い信号１００´に対応し、以下同様である。従って、ＣＣＤアレイからの出力は、ビーコンをその相対強度で監視することにも用いられうる。その代わりに、スポットの大きさやビーコンにおけるファイバの本数により、個々のビーコンが識別されてもよい。例えば、第１のビーコン９８が、一本のファイバを備えていて、ＣＣＤ上に単一のスポットを形成し、第２のビーコン１００が、近接配置された２つの光ファイバを備えていて、近接した２つのスポットを形成するというようにである。なお、第２のビーコン１００の２つのファイバは、第１のビーコン９８と第２のビーコン１００との間隔に比べて、極めて小さな間隔で互いに離れていてもよい。例えば、隣接した第１及び第２のビーコン９８，１００が４０ｍｍ離れていて、第２のビーコン１００の２つのファイバが０．１ｍｍといった小さな間隔で離れていてもよい。

【００４９】
さらに、Ｘ方向における必要な走査範囲が約３０ｍｍよりも小さい場合に、単一のビーコンが、ＣＣＤアレイ又はアナログ位置検出装置とともに用いられてもよい。例えば、アナログ位置検出装置は、ストリップ上の光のスポット位置に比例した２端子における差動電圧出力が得られる連続フォトレジスタ・ストリップであってもよい。
【００５０】
図１２に、回転位置検出機構１１６を備えた走査装置の実施形態を示す。ここでは、マイクロアレイは、回転する基板１２２上に形成されている。基板１２２は、上記の特許出願中の米国特許出願番号６０／２４４，４１８に記載されているように、ＣＤ－ＲＯＭディスクに類似した形状を有している。本実施形態では、マイクロアレイ基板１２２は、回転ステージ１２０により連続的に回転し、その一方で、１つの光学読取ヘッド１１８又は複数のヘッドが段階的に径方向へ移動して、マイクロアレイ基板１２２表面の全体を走査してもよい。この設計の利点は、成熟したＣＤ－ＲＯＭ設計及び製造能力による多くの利点が、システムコストを削減可能とすることにある。基板が連続的に高速で回転しうるので、通常の読取機構によるよりも高速の読取速度が達成可能である。走査装置の本実施形態には、上述のように、基板１２２における光学又は磁気マーカが組み込まれてもよい。これにより、読取ヘッドの位置が指示されるようになる。
【００５１】
走査装置には、上述のような光ファイバを有する読取ヘッド１１８の他にも、コンパクトディスク（ＣＤ）リーダに見られるものに似た大きな光学レンズに基づく読取ヘッドが組み込まれてもよい。このように構成されることにより、現行のＣＤ－ＲＯＭドライブの成熟した技術の利点が存分に活かされうる。
【００５２】
マイクロアレイ走査装置が高いスループットの読取装置として用いられている場合、このシステムにおけるマイクロアレイ基板は、マイクロ反応ウェル（ｍｉｃｒｏ　ｒｅａｃｔｉｏｎ　ｗｅｌｌ）に置き換えられてもよい。マイクロ反応ウェルは、流体で満たされている。全ての型のマイクロアレイ走査装置が、この用途に適合可能であろう。特に、開示されている発明がこの用途に用いられた場合、光ファイバは、励起光のエネルギーをウェルに導き、発せられた光を同時に集光する。ウェルアレイは、一方向に移動してもよい。この際、読取ヘッドは、他の方向へ移動する。走査を完全なものにするため、通常、当該他の方向は直交方向である。一方、アレイが固定されていて、読取ヘッドにより両方向への走査運動が得られてもよい。読取ヘッドは、開示されている発明では光ファイバ式であるため、光源から独立して移動可能である。このことにより、コンパクトで経済的な構造設計を実現するための自由度が追加される。
【００５３】
本発明のどの実施形態においても、走査構造は、基板上の乾式領域又は湿式領域からの光を受光できるように構成されている。本発明のある好適な実施形態では、当該領域の直径は、約１０マイクロメータ乃至約５００マイクロメータである。他の好適な実施形態では、当該領域は、直径が約１００乃至１０００マイクロメータのマイクロウェルである。通常、当該領域は既知のスポット形成技術により形成され、また、ウェル内の液体は断面が円形であるため、当該領域は、直径を有するものとして記述されているが、円形の形状に限定されるものではない。
【００５４】
走査構造は、特定のサイズのいくつかの方式の領域からの第２の波長の光を受光するように、構成されうる。例えば、ファイバ又はロッドは、この領域からの光を読み取るのに適した直径になっている。（すなわち、ファイバ又はロッドが所与の領域からの信号を読み取っている時にはいつでも２以上の領域からはみ出すほどには、この直径は大きくない。）検出器は、既知の技術によりタイミングが調節され、読み取られるべき領域を横切る走査構造の導光部分に対応した、所与の時刻及び／又は所与の位置で信号を読み取る。走査速度は、アレイ密度に対応させて変更されてもよい。また、これらの技術の任意のものが

組み合わされて、用いられてもよい。
【００５５】
基板の光に反応する領域は乾式であっても湿式であってもよい。遺伝子又はタンパク質のマイクロアレイは、マイクロウェルが反応や会合（ａｓｓｏｃｉａｔｉｏｎ）の生成物を含むことがある（高いスループットの薬品のスクリーニングで発生するように）ので、走査装置を使って読み取られてもよい。このようなマイクロアレイとしては、ＷＯ９９／５５４６０、ＷＯ９９／５５４６１、ＥＰ０９５５０８４、ＷＯ００／５３７３６、ＷＯ００／５３７３９、２００１年１月１０日出願の米国特許出願番号０９／７５８，８７３、２００１年２月２２日出願の米国特許出願番号０９／７９１，９９８、２００１年２月２２日出願の米国特許出願番号０９／７９１，４１０、及び２００１年２月２２日出願の米国特許出願番号０９／７９１，４１１（これらのうち最後の４件は、本出願人による発明）に、記述されているものがある。これらの全てがここに一体に組み入れられる。
【００５６】
本発明の走査構造は、上述したような高いスループットのために構成された基板と組み合わされて用いられてもよい。この走査構造は、光源及び検出器を備えている。基板は、光源からの光を受光し、検出器は、基板からの光を受光して、１つ又はそれ以上の波長の光が存在するかどうかを検出する。オリゴヌクレオチドを含んだマイクロアレイ上のハイブリッド化を検出するのに用いられる他の走査装置が、同様に使用されてもよい。このようなマイクロアレイは、上述の背景の部分で考察されている。例えば、これらのマイクロアレイには、液体サンプルの処理のために、１平方センチメートル当たり少なくとも１００、４００、又は１０００のマイクロウェルが含まれている。
【００５７】
本発明は、特定の実施形態を参照して記述されているが、この記述は、本発明の適用例に過ぎず、これにより何らの制限がなされるものではない。様々な改良及び開示された実施形態の特徴の組み合わせが、通常の技術を有する者に今や明らかとなり、これらは、本発明の範囲に属するものである。ここに考察又は言及された全ての参考文献は、その全体が一体に組み入れられる。
【図面の簡単な説明】
【図１】（ａ）及び（ｂ）はプローブ・マイクロアレイを走査するために用いられる本発明の実施形態を示す図である。
【図２】光ファイバがその先端面のコアの部分に極めて近接した領域を励起し、照射された領域の一部からの光が同ファイバに受光されうることを示す図である。
【図３】読取ヘッド支持用に磁気的及び空気力学的浮揚を組み合わせた実施形態を示す図である。
【図４】読取ヘッド支持用に薄い気体クッションを発生させるための実施形態を示す図である。
【図５】一様でない屈折率のダブルコア光ファイバを示す図である。
【図６】複数の異なった波長を単一の光路にまとめた走査装置の実施形態を示す図である。
【図７】異なった波長の個々のファイバを束ねるか近接させて構成した走査装置の実施形態を示す図である。
【図８】ガルバノスキャナを利用した走査装置の実施形態の側面図及び平面図である。
【図９】共振懸架ビームを利用した走査装置の実施形態の側面図及び平面図である。
【図１０】移動ステージの固定ステージに対する移動、及び強度の異なる複数のビーコンを用いてそれを測定することを示す図である。
【図１１】図１０（ａ）、図１０（ｂ）、及び図１０（ｃ）の移動ステージを利用した１次元のマイクロアレイ上に配置された様々な複数のプローブを示す図である。
【図１２】回転する基板を備えた走査装置の実施形態を示す図である。

【図1】

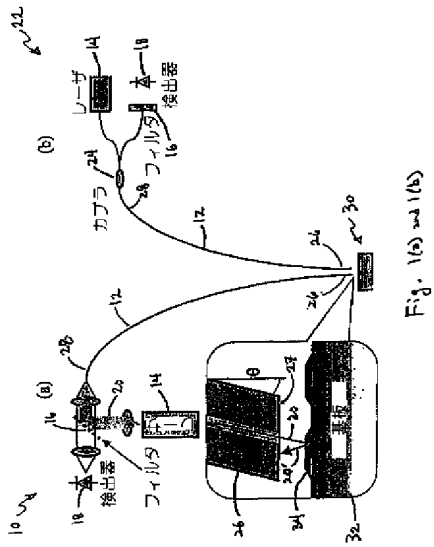


Fig. 1(a) and 1(b)

【図2】

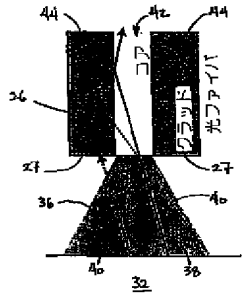


Fig. 2

【図3】

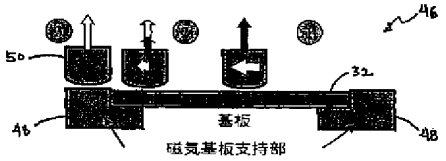


Fig. 3

【図4】

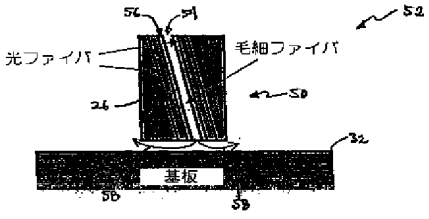


Fig. 4

【図5】

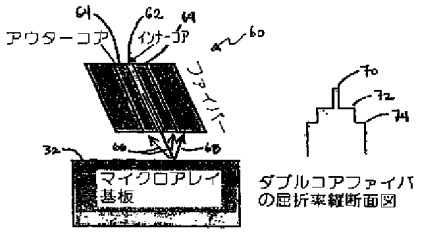


Fig. 5

【図8】

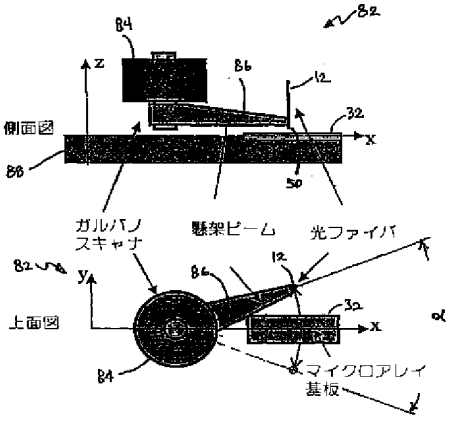


Fig 8

【図9】

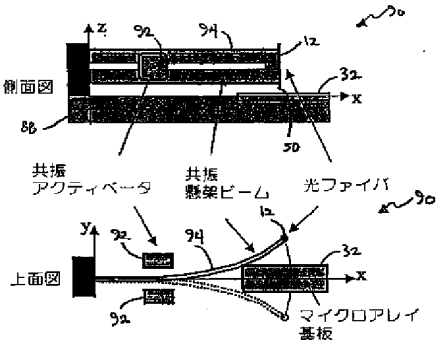
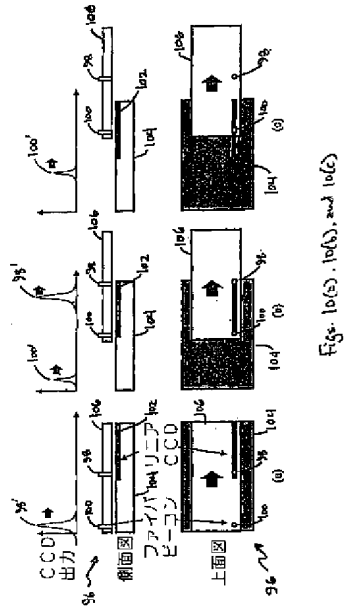


Fig. 9

【図10】



Figs. 10(a), 10(b), and 10(c)

【図12】

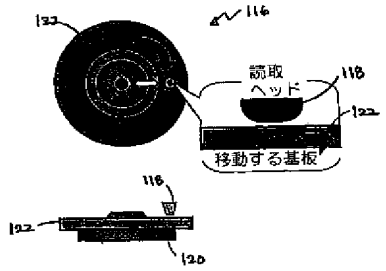


Fig. 12

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WO 01/69302 A2

(54) Title: FIBER OPTIC SCANNER

(57) Abstract: The disclosed scanning structure includes an apparatus for light delivery and light receiving from a light-excitable area on a substrate to be measured by the scanning structure. The light delivery and receiving apparatus may include an optical fiber having a proximal end and a distal end which transmits light having a certain wavelength or light with several varying wavelengths to excite the substrate samples. This optical fiber may also simultaneously receive light which may be emitted by fluorescing samples on the substrate. The scanning structure also may further include a holder for the optical fiber that is able to transverse variable distances over the substrate to be measured or examined. Holders may include galvano scanners as well as resonating suspension beams. A light source, e.g., a laser, may be optically coupled to the optic fiber's proximal end. And this light source may be of a certain wavelength, but multiple light sources each having a different wavelength may also be used simultaneously by coupling the light sources into either a single optic fiber through wavelength multiplexers or by placing individual optic fibers carrying differing wavelengths in close proximity to each other. As the light is transmitted down to the substrate through the optic fiber, the fiber is sufficiently close to the substrate microarray so that it can also receive the emitted fluorescing light.

FIBER OPTIC SCANNER

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional application 60/188,873 filed Mar. 13, 2000 which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The invention relates to equipment and methods for scanning microarrays or microwells in a microplate, such as DNA microarrays, protein microarrays, and compound microarrays.

BACKGROUND

A microarray is an array of spots of biological or chemical samples ("probes") immobilized at predefined positions on a substrate. Each spot contains a number of molecules of a single biological material or chemical. The microarray is flooded with a fluid containing one or more biological or chemical samples (the "target"), which typically interact with one or more complimentary probes on the microarray. In DNA microarrays in particular, the probes are oligonucleotide or cDNA strains, and the target is a fluorescent or radioactive-labeled DNA sample. The molecular strands in the target hybridize with complimentary strands in the probe microarray.

The hybridized microarray is inspected by a microarray reader, which detects the presence of the radioactive label or which stimulates the fluorescent label to emit light by exciting the label using a laser or other energy source. The reader detects the position and strength of the label emission in the microarray. Since the probes are placed in predetermined and thus known positions in the microarray, target sequences in the fluid are identified by the position at which fluorescence or radiation is detected and the strength of the fluorescence or radiation.

A microarray reader is therefore one of the key pieces of equipment in microarray technology. Currently, there are two types of microarray readers available. The first type, an example of which is depicted in U.S. Pat. No. 5,324,633, is based on scanning microscope principles, where laser beams at two or more wavelengths are combined and focused on a single spot on the microarray to excite the fluorescent labels. The combined laser beam scans across the entire microarray point-by-point at a high spatial resolution (~10 μm) by either carrying the microarray on a two-axis translation stage, or moving the microscope lens in one axis and the microarray substrate on the other. The second type, an example of which is illustrated in WO00/12759, uses a CCD imager to detect the fluorescent emission from the microarray one small region at a time. A broadband light source, such as an arc lamp, is used to excite fluorescence.

For scanning microscope technology, the cost driver is its optical system, as it has to combine and precisely align multiple laser beams at different wavelengths and later re-split them into separate detection channels. Both the microscope lens and the slide carrier are bulky and heavy and cannot be moved very fast, which limits scanning speed.

In the CCD based system, the imager and lens are both the main cost drivers. Here, the excitation light is expanded to a large area causing a great reduction in energy density. The exposure time has to be extended several tens of seconds to compensate for this reduction. Because of the long exposure time, the CCD imager has to be cooled to maintain a reasonable single to noise ratio. Such a cooled, large format CCD imager is very expensive at present. In addition, the optical lens in the system has to be corrected for chromatic aberrations and image distortions over a large field of view, which significantly increases its cost in comparison to the lens in the point-to-point system in a scanning microscope. Furthermore, there is currently no CCD imager with sufficient resolution and format to cover the entire area of a slide (25 mm x 75 mm). Mechanical scanning is still required for CCD based systems, which reduces the speed of the reader. At present, most microarray readers on the market require 5 minutes or more to

complete the scanning of a 25 mm x 70 mm slide, which is too slow for many applications.

Optical fibers have been used in near field scanner microscopy (D.W. Pohl, "Scanning near-field optical microscopy" in *Advances in Optical and Electron Microscopy* 12, C.J.R. Sheppard and T. Mulvey, Eds. (Academic Press, London, 1990); M.H.P. Moers, W.H.J. Kalle, A.G.T. Ruiter, J.C.A.G. Wiegant, A.K. Raap, J. Greve, B.G. De Grooth, N.F. van Hulst, J. Microscopy 182,p. 40, (1996)), where light energy is guided to a fiber tip reduced to sub-micron in diameter. The fiber tip is brought to within several tens of nanometer of the surface to be inspected. Then, the tip scans over a small area (normally within 10mm square) and collects the direct scattering light from the inspected surface. (Chuck, In the disclosed invention, the fiber core is much larger to maximize the light collection and the collected light is indirect emission, such as fluorescence from the object under inspection.)

On the other hand, optical fiber reader head has been use to collect fluorescent emission from microtiter plates in a number of instruments such as ABI's Taqman reader [US5 589 351]. Here the spatial resolution of the reader is in the order of several millimeters.The excitation and receiving lights travel through different fibers in a bundle. The optical head is kept at a relative large distance from the object.

SUMMARY

The invention provides a number of systems, components, means, and methods for scanning probe microarrays or samples (dry or liquid, as contained in microwells) as are more fully described below. This section of the disclosure provides a summary of some salient points of the invention, but this section is not to be interpreted as limiting the scope of the invention to only those features and embodiments discussed in this section. Instead, the invention involves all components, systems, and methods discussed in this and the following sections as well as the appended claims.

The disclosed scanning structure includes an apparatus for light delivery and light receiving from a light-excitable area on a substrate to be measured by the scanning structure. The light delivery and receiving apparatus may be comprised of an optical fiber having a proximal end and a distal end which transmits light having a certain wavelength or light with several varying wavelengths to illuminate the samples and excite light emission or have one or more of the wavelengths absorbed by the samples. This optical fiber may also simultaneously receive light which may be emitted by fluorescing samples on the substrate or light that has otherwise encountered the samples and been reflected or diffracted. The scanning structure also may further include a holder for the optical fiber that is able to traverse variable distances over the examined substrate. Examples of holders may include galvano scanners as well as resonating suspension beams. To generate the light which excites the substrate samples, a light source, e.g., a laser, may be optically coupled to the optic fiber's proximal end. Multiple light sources each having a different wavelength may be used simultaneously by coupling the light sources into either a single optic fiber through wavelength multiplexers or by placing individual optic fibers carrying differing wavelengths in close proximity to each other. As the light is transmitted down to the substrate through the optic fiber, the fiber is sufficiently close to the substrate microarray so that it can also receive the emitted fluorescing light.

Although certain embodiments of the invention are described as causing fluorescence, the invention also includes other forms of light generation such as chemiluminescence and light absorption so that an absorption spectrum is generated and transmitted by the light conducting portion (e.g. fiber or rod) of the scanning structure. Thus, a light excitable area on a substrate is a portion of the substrate containing a wet or dry sample that either generates light of a different wavelength than the light received by the substrate (such as by fluorescence or chemiluminescence) or an area that absorbs one wavelength of multiple wavelengths transmitted to the substrate by the light conduction portion of the scanning structure. Therefore, a second wavelength that is "generated" by the light excitable area on the substrate may be a wavelength that is not provided by

the light source or may be a wavelength that the light source transmits and is reflected or diffracted by the sample or substrate but the substrate or sample in the light-excitable area does not absorb. Thus, a scanning structure can be configured to detect light or to detect the absence of a wavelength of light.

As the spatial resolution of the scanner equals approximately the diameter of the fiber core that transmits the excitation light energy, the preferred core diameter is therefore 5μm, 10μm for scanner with 5μm or 10μm spatial resolutions, respectively. Such core diameters are readily available in communication fibers. There is no need to reduce the core size at the fiber tip.

Aside from microarrays on tranditional square or rectangular substrates, the disclosed scanner can be adapted to read a rotating substrate in the manner of a CD and one dimensional microarrays.

In addition to microarrays, the disclosed invention can further be adapted to read arrays of microscopic reaction wells in high throughput screening applications.

At present, the output signals of HTS, which could be fluorescence, chemiluminescence or absorbance are detected using a device referred as "microplate readers". This invention relates to a scanner that reads such signals from solutions in microwell arrays with size and density comparable to today's DNA microarrays (e.g., more than about 500 wells/cm^2, preferably more than about 1,000 wells/cm^2, more preferably more than about 2,000 wells/cm^2, and even more preferably more than about 5,000 wells/cm^2). The inner diameter of the microwell is from about 100 microns to about 1,000 microns, preferably no more than about 500 microns, and more preferably no more than about 200 microns. In a specific embodiment, the optical fiber scanner of this invention is adapted as a reader of signals from solutions in microwells. For instance, the angle that the optical fiber makes with the substrate may be vertical or near vertical to avoid reflection but also detect the presence or absence of light. The diameter of the fiber may be selected based on the diameter of the microwells. The length of time that the reader waits before scanning may be selected based on the reaction or association time needed for the sample and probe (oligonucleotide, protein, or

reactant, for instance) to associate or react with the sample. The optical fibers provide the flexibility that enables the reader to be integrated into the screening system.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1(a) and 1(b) depict embodiments of the present invention used in scanning probe microarrays.

Figure 2 illustrates that an optical fiber excites an area very close to its core region in the distal end facet, and light from a portion of the illuminated area may be collected by the same fiber.

Figure 3 depicts an embodiment combining magnetic and aerodynamic levitation for read head support.

Figure 4 depicts an embodiment for generating a thin gas cushion for read head support.

Figure 5 depicts a double-core optical fiber having varying refractive indexes.

Figure 6 depicts a scanner embodiment for combining multiple different wavelengths into a single optical path.

Figure 7 depicts a scanner embodiment for bundling or closely configuring separate fibers having light of differing wavelengths.

Figure 8 depicts a side view and a top view of a scanner embodiment utilizing a galvano scanner.

Figure 9 depicts a side view and a top view of a scanner embodiment utilizing a resonating suspension beam.

Figures 10(a), 10(b), and 10(c) depict the progression and measurement of a translation stage relative to a stationary stage using beacons of varying strength.

Figure 11 depicts multiple varying probes disposed upon a one dimensional microarray utilizing the translation stage of Figures 10(a), 10(b), and 10(c).

Figure 12 depicts a scanner embodiment having a rotating substrate.

DETAILED DESCRIPTION OF THE INVENTION

In this invention, many basic optical elements for the delivery of excitation light and possibly also for the collection of the fluorescent emission may be used. Such optical elements may include devices such as light-guiding rods and optical fibers. It may be preferable to use optical fiber as the optical element and the embodiments disclosed in the following discuss the use of such optical fibers as examples; however, the invention is not so limited.

One embodiment of the invention, a scanning structure (10), is illustrated in Figure 1(a) where an excitation laser light (20) emitted from laser (14) is reflected by filter (16) before excitation laser light (20) is coupled into a proximal end (28) of optical fiber (12), which guides excitation light (20) to illuminate the sample, e.g., DNA probes, on microarray sample (30) through distal end (26) of optical fiber (12). Microarray sample (30) may be comprised of substrate (32) and probes (34), as discussed above. Filter (16) may be selectively designed to reflect a pre-selected wavelength or range of light while simultaneously allowing the passing of a desired wavelength or range of reflected light. The fluorescent light (20') emitted from probes (34) may be collected by the same fiber (12) and is guided to detector (18) after passing through filter (16). The excitation light (20) and fluorescent light (20') in embodiment (10) are separated by filter (16), which reflects light at a specific wavelength while allowing a particular wavelength to pass through. In the particular embodiment depicted in Figure 1(a), filter (16) may be designed to reflect excitation light (20). The system can also be adapted to use a filter that reflects the fluorescent light by swapping the positions of laser (14) and detector (18) in Figure 1(a).

Figure 1(b) depicts an alternative embodiment (22) of the same system design where the bulk optic beam splitting and coupling structure is replaced by a fiber optic coupler (24). Structures in different embodiments depicted with the same numbering are used to indicate similar function throughout this description. Coupler (24) can also be a wavelength domain multiplexer (WDM) which selectively couples a particular wavelength to the other fiber branch. An

additional filter (16) may preferably be placed in front of detector (18) to reject the excitation light.

Yet another embodiment utilizes light emitting diodes (LEDs) as a source of excitation light (20) rather than a laser. A single LED or multiple LEDs each emitting light at a different wavelength may be disposed directly adjacent substrate (32) yet still allow optical fiber (12) to gather fluorescent light (20') or replace laser (14) at the optical fiber proximal end (28).

As depicted in Figure 2, light (36) exiting optical fiber distal end (26) will diverge at a characteristic angle defined by the numerical aperture (NA) of the fiber. In order for a light beam to be guided by the fiber, the light beam should satisfy both of the following conditions: 1) the light beam enters the fiber within the core region, defined by the numerical aperture of the fiber; and 2) the light beam intersects the fiber axis at an angle smaller than NA. For example, light beam (38), as represented by an arrow, can be guided into core region (42) because it satisfies both of the above conditions. However, light beams (40) are not guided and enter cladding (44) rather than core region (42) because they only satisfy one of the above conditions.

Because of the beam divergence and guiding conditions, the light emitted in the region very close to the core of the fiber is collected in the embodiments illustrated in Figures 1(a) and (b). The light collection power of the fiber is determined by two factors: 1) the core size; and 2) the NA. The larger these two parameters, the higher the light collection power of fiber (12). However, a large core size may reduce the spatial resolution of the system. The spatial resolution is approximately equal to the size of the fiber core when the distance between fiber tip (12) and substrate (32) is within $d_c/2NA$, where d_c is the diameter of fiber core (42). If substrate (32) is greater than distance, $d_c/2NA$, away from fiber tip (12), the efficiency of fluorescent light collection may be significantly reduced. Standard telecommunication fibers have a core diameter of about 9μm and NA = 0.12. Therefore, if a standard telecommunication fiber is used to form a scanning structure of this invention, a spatial resolution of approximately 10 μm can be achieved if the substrate surface is kept within about 38 μm from fiber facet (27).

Fibers with NAs as large as 0.66 have been reported. The potential exists to boost light collecting power by about 27 times by having fiber facet (27) kept to within about 7 μm of the substrate.

Several measures may be adopted to provide a fiber (12) in close proximity to substrate (32).

A first embodiment of a scanning structure of this invention detects the distance between fiber tip (12) and substrate (32) surface in real time and actively controls this gap. The size of the gap may be detected by conventional methods such as optical interometry using interferometers, and the gap is controlled by attaching the fiber to e.g. a piezoelectric actuator (such as the one described in U.S. Pat. No. 6,142,444) that moves in response to signals received from the interferometers.

A second scanning structure of the invention is configured to allow the tip of fiber (12) to aerodynamically "float" across microarray substrate (32) on a cushion of air created by rapid movement of the fiber tip near the substrate. This is a technology used in floppy disk drives, hard drives, and CD-ROM drives, for instance. The read head of a floppy disk drive is suspended by an air gap a couple of micrometers thick, which is created aerodynamically through a so-called "ground effect" created by the air between the rotating floppy disk and the read head. The relative movement of the read head and floppy disk creates a vacuum that draws the read head to the floppy disk surface, but as the read head nears the surface, sufficient pressure builds within the gap between the read head and the disk surface that the read head does not contact the disk surface.

This "ground effect" may be applied to a scanner of this invention. The fiber tip moves sufficiently rapidly across the surface of the substrate that the relative movement between the fiber and substrate draws the fiber tip to within a few microns of the surface of the substrate. As illustrated in Fig. 3, the fiber tip may be housed in a read head (50) having a shape that, together with substrate (32), forms a venturi through which the air flows to create the ground effect. A read head may be one optical fiber attached to a holder that moves the fiber across the surface of the substrate, or a read head may be a bundle of optical fibers

attached to a holder as discussed in further detail below. The surface of the read head that faces the substrate surface may have a parabolic shape in profile as illustrated in Fig. 3, or the read head may have a flat face as illustrated in Fig. 1 or Fig. 2. If the velocity is less than the velocity needed to create the ground effect, the read head or fiber tip may contact the substrate or a portion of a substrate holder as the read head or fiber tip slows to reverse direction and scan the microarray while traveling in the opposite direction.

A scanning structure (46) having a combined magnetic and aerodynamic levitation can prevent the read head or fiber tip from contacting the substrate or substrate holder as the read head or fiber tip slows to reverse direction during scanning. As illustrated in Figure 3, microarray substrate (32) of scanning structure (46) is supported on a pair of magnets (48) each having a similar polarity direction. The read head (50) with integrated optical fibers may be formed with integrated permanent magnets or may itself be magnetized (when formed of a magnetic material) so that its polarity is similar to the pair of support magnets (48) to provide a repulsive force between the read head and magnets (48). At the middle of substrate (32) at Position 1 where read head (50) moves relatively fast, read head (50) floats aerodynamically. As read head (50) moves toward the edge of substrate (32) from Position 2 to Position 3, read head (50) slows. This slowing reduces the aerodynamic float, but the read head (50) is supported by the magnetic force from magnetic supports (48) to maintain a read head (50) suspension that prevents the read head from contacting the microarray substrate (32) or magnets (48).

A third embodiment of a scanning structure of the invention is particularly well-suited to activate and detect labels of a microarray on a substrate having a rough surface, although its use is not limited to a microarray on a substrate having a rough surface. In scanning structure (52) illustrated in Figure 4, a fiber capillary (54) may be incorporated among optical fibers (56) to maintain a consistent aerodynamic float for read head (50). If multiple fibers are used, they may be bundled together randomly, or they may be placed in a linear or ordered array with known spacings to allow faster or redundant microarray scanning. A very

thin gas cushion (58) may be generated between read head (50) and substrate (32) by blowing a gas down through capillary fiber (54). Any inert gas may be used such as air or nitrogen. Because fiber-based read head (50) is very light, a small amount of positive pressure should be sufficient to float read head (50) over substrate (32) and maintain a small distance on the order of a few microns between them. The amount of positive pressure will depend on the specific design of read head (50).

A number of features such as signal-to-noise ratio improvement, multiple wavelength excitation and collection, and scanning mechanisms are discussed in the following sections.

Signal-to-Noise Ratio Improvement

If an axis along the length of fiber (12) is substantially perpendicular to the surface of substrate (32), a certain amount of the original excitation light (20) will be reflected off a facet (27) of fiber (12) to the substrate (32) and then back to fiber (12), thereby producing background noise in the signal. This background noise may be suppressed by filter (16) in the system, as depicted in Figure 1(a) and 1(b). Filter (16) acts to suppress the background noise by reflecting light of unwanted wavelengths that the detector (18) would otherwise detect. However, because of the relative weakness of the emitted fluorescent light, there may still be a significant level of noise despite the filter suppression. One scanning structure having a greater signal to noise ratio (SNR) includes a fiber (12) which is tilted relative to the surface of substrate (32) by an angle, θ, which angle is slightly larger than the NA of fiber (12), as depicted in Figures 1 and 5. This configuration allows the reflected excitation light to pass through the wall of the fiber instead of being guided to the detector by the optical fiber.

An alternative embodiment of a scanning structure with greater SNR includes a fiber (12) in which its facet (27) is polished so that it is substantially parallel to the surface of substrate (32). This polishing causes any light (including excitation light(20)) directly reflected off a fiber facet (27) and microarray substrate (32) to intersect the fiber (12) axis at an angle larger than the NA thus

preventing this light from being guided to the detector (18) via fiber core (42). On the other hand, fluorescent light (20') is emitted in all directions and the same proportion of light (20') as is captured by a fiber with unpolished facet will be captured by fiber (12) leaving its signal level unaffected. As a result, the SNR in the system can be improved significantly.

Another scanning structure having enhanced SNR employs a double-core fiber (60) which, as depicted in Figure 5, has two concentric cores (62, 64), with the refractive index of core (62) being greater than the refractive index of core (64). A relative refractive index profile of double-core fiber (60) is seen in Figure 5 where peak (70) corresponds to the relative refrective index of inner core (62), peak (72) corresponds to the index of outer core (64), and peak (74) corresponds to the index of the cladding of fiber (60).

A scanning structure having a double-core fiber (60) has a near 100% coupling ratio to light received from the substrate in its outer core (64) while light from the laser travels to the substrate through the inner core (62), and thus the double-core fiber acts as a core-selective coupler.

Outer core (64) acts like cladding to inner core (62) because outer core (64) has a lower refractive index than inner core (62). Consequently, when a double-core fiber is used in the system depicted in Figure 1(b), excitation light (20) from e.g. a laser is launched into inner core (62) at an angle less than the critical angle, and the excitation light is essentially confined to inner core (62).

The light in outer core (64) will be coupled out by this double-core fiber. Light entering outer core (64) at an angle greater than the critical angle for inner core (62) does not undergo internal reflection in inner core (62) and is therefore found primarily in outer core (64), leaving essentially only light from the laser in inner core (62).

Moreover, inner core (62) may have a small NA so that dispersion of the light beam (68) after exiting inner core (62) is small. Most of the fluorescent emissions (66), on the other hand, are collected by outer core (64) and travel back up fiber (60) to the detector. In this way, the light collection efficiency can be increased significantly, which in turn boosts the SNR. In addition, because the

outer core (64) can be made much larger in diameter than inner core (62), the intensity of the collected light is less critically dependent upon the distance between the facet (27) of fiber (12) and substrate (32), providing more tolerance and freedom in the instrument design.

One method for fabricating double-core fiber (60) involves chemical vapor deposition (CVD). This involves depositing a first concentration of a dopant, e.g., Ge in gaseous form with silane and O_2, on the inner surface of a conventional fiber-forming tube (preform) to form a layer. Then, a second concentration of dopant (consisting either of the same dopant as the first concentration or a similar typical dopant) greater than the first concentration may be doped upon the layer, followed by stretching the preform to form fiber (60).

Multiple Wavelength Excitation and Collection

A scanning structure of the invention typically carries at least two separate wavelengths of light, the excitation light having one wavelength and the fluorescent light emitted by the fluorescent markers having another wavelength. Scanners having up to five separate excitation and/or collection wavelengths are currently known in the art. In current microarray scanners, all light beams at different wavelengths are combined into a single optical path through an objective lens of a microscope. The bulk optical components used in a conventional scanner require precision alignment and complicated structural configurations to carry and move a number of the bulk optical components. The high precision and bulk optical components make current scanners bulky and expensive. Further, it is often difficult to separate one wavelength from another in existing optical detectors having a combined optical path. The SNR suffers when more than one laser is activated simultaneously. Many existing microarray scanners avoid this problem by switching on one laser at a time but at the cost of a much slower scanning speed.

Figure 6 depicts a scanning structure (76) of the invention in which multiple light sources ($14_{\lambda 1}$ to $14_{\lambda n}$) having multiple corresponding wavelengths are combined into a single optic fiber (12) through the use of Wavelength

Division Multiplexers (WDM) (78_1, 78_n). The scanning structure is simple, especially since the flexibility of optical fiber (12) eliminates the need for complex supporting structures for e.g. lens and mirror assemblies as are currently used in existing scanners. WDMs (78_1, 78_n) are formed using techniques well-known in the telecommunications industry to form planar or fused fiber couplers, for instance.

In one embodiment of the invention, multiple wavelength light beams may be scanned simultaneously across microarray substrate (32), but the light beams need not be in exactly the same location. Figure 7 depicts an alternative embodiment (80) which isolates one wavelength from another more easily without paying a speed penalty. As depicted in Figure 7, optic fibers (12) may be arranged in a number of desired configurations depending upon the application to allow for each wavelength light ($14_{\lambda 1}$ to $14_{\lambda n}$) to scan in synchronization while illuminating a separate yet relatively closely-spaced location. Bundled optic fibers may be formed with or without the use of, e.g., a guide plate into which the fibers are inserted and then bundled to preserve their order. All articles, patents, and patent applications mentioned herein are incorporated by reference in their entirety. Because optical fibers (12) are extremely light-weight and have a very small, precise diameter, synchronized multi-spot scanning can be achieved by using separate fibers for each wavelength. Here, the individual fibers (12) may be bundled in a microarray to form a fiber optic scanning head. Such a microarray may be bundled in an ordered array or in a random bundle. The number of fibers in a bundle may be any number, but in many instances the number will be less than ten. Either variation is feasible since the relative positions of the distal ends of each fiber (12) will be known in the system. Despite the close proximity of the fiber ends, all of the fibers (12) need not be focused to illuminate the same spot, although this may be done. Rather, each of the fibers (12) may be arranged so that they illuminate and optionally also gather fluorescent light from multiple spots simultaneously.

Scanning Mechanism

Because optical fibers (12) are very light, scanning head (50) described above can be moved very quickly, thereby increasing the microarray read speed. Figure 8 depicts an embodiment of a scanning apparatus (82) where read head (50) of optical fiber (12) is moved back and forth in the Y-direction by a conventional galvano scanner (84). Galvano scanner (84) may be set to move suspension beam (86), which holds optic fiber (12) and read head (50), through a desired angle, α, and at a desired frequency depending upon the geometric configuration of substrate (32). The scanning apparatus can have an X-stage positioner, which moves the substrate beneath the read head and also provides information to determine where along the X axis the optic fiber (12) is reading. The optical fiber (12) position in the Y direction is determined by an angular encoder incorporated in the galvano scanner (84). As galvano scanner (84) moves suspension beam (86) in the Y direction, substrate (32) may be step-moved in the X-direction by a conventional translation stage (88).

Figure 9 depicts an alternative embodiment of a scanning apparatus (90) where galvano scanner (84) and suspension beam (86) are replaced by resonance activators (92) and resonating suspension beam (94). Here, optical fiber read head (50) is oscillated back-and-forth in the Y-direction as resonating suspension beam (94) is forced to its resonant frequency by resonance activators (92). Resonance may be actuated by any number of conventional resonance activator (92) devices such as a piezo device adjacent to resonating suspension beam (94) or by a magnetic device on each side of resonating suspension beam (94). In either embodiment, read head (50) may be set to stop at the edge or outside substrate (32), where there is no probe. Preventing contact (and thereby preventing contamination) between read head (50) and substrate (32) may be avoided by any number of conventional or previously-mentioned methods such as by the implementation of the aerodynamic suspension mechanism used to float read head (50) over substrate (32).

With the particular embodiments depicted in Figures 8 and 9, scanning head (50) travels in a curved path in the Y-direction. This implies that image pixels in the row data will not be in a square grid as in most image files.

However, as long as the precise position of each pixel is registered, the image file generated with this scanner can be converted into a standard image file by conventional software. The X-position in either embodiment may be registered by a conventional position encoder on translation stage (88). For the embodiment depicted in Figure 8, the Y-position may be calculated from the angular position generated by galvano scanner (84) as it sweeps through angle, α. For the embodiment depicted in Figure 9, the Y-position may be calculated by any one of several different methods. One method involves measuring and recording the strain at known locations on the surfaces of each side of resonating suspension beam (94) by the use of conventional strain gauges. A second method involves a new optically based position measurement device, described further below, which may be adapted for such measurement purposes. A third method involves measuring the resonant period in real-time and then calculating the position of read head (50) between the two extremes of the oscillation through time.

Figures 10(a) to 10(c) illustrate an alternative embodiment for a simpler position sensing device (96) incorporating a CCD array and fiber optic beacons. This embodiment may be feasible despite variations in slide size and translational velocities.

As illustrated in Figure 10(a) to 10(c), optical fibers (98, 100) are installed on the moving part of translation stage (106) as beacons while stationary stage (104) remains stationary relative to translation stage (106). Alternatively, stage (106) may be held stationary while stage (104) moves. First beacon (98) is the brightest, second beacon (100) is dimmer, and so on. Linear CCD array (102) may also be installed on the stationary (104), or moving (106), part of the stage. The separation between two adjacent beacons (98, 100) is slightly smaller than the length of linear CCD array (102). At the start of the translation, linear CCD array (102) will detect a single bright spot at the one end of its pixel array. The position of this spot indicates the relative position of translation stage (106), as depicted in Figure 10(a). As this spot moves to the other end of linear CCD (102) during the translation of stage (106) and before first beacon (98) is out of the range of linear CCD (102), second beacon (100) has moved in to continue the position

monitoring, as depicted successively in Figures 10(b) and 10(c). Any number of beacons may be used if necessary so that the position of translation stage (106) throughout the entire range can be monitored in an absolute manner.

As an example of beacon separation distances relative to a CCD length, the precise position of the spot along the pixel array can be calculated to at least about 1/50 of pixel pitch using a centroid algorithm. Considering, e.g., a typical low cost CCD array (102) with a conventional 2048 pixels and a 24 μm pitch, the effective length of linear CCD array (102) is approximately 49 mm. By installing two fiber beacons at, e.g., about 40 mm of separation, position sensing device (96) may be capable of monitoring translation stage (106) position over about a 80 mm range at a resolution of about 0.48 μm, which is more than sufficient for a microarray scanner.

Position sensing device (96) may be utilized for an embodiment of a one dimensional microarray (108), as depicted in Figure 11. Here, a one dimensional microarray (108) as described in copending U.S. Patent Application Ser. No. 60/244,418 entitled "Gene Thread," inventors Shiping Chen, Yuling Luo, and Anthony Chen, and filed on Oct. 30, 2000, which is herein incorporated by reference in its entirety, may be positioned on translation stage (106). Disposed upon one dimensional microarray (108) may be standard probes (110) containing samples for hybridization. Probes may alternatively be placed in varying configurations depending upon the desired applications. Some examples may include placing probes in a linear manner (112) or diagonally (114). In any case, the position of the probes may be monitored and read using any of the methods and apparatus as described above.

An embodiment of the scanning apparatus of the invention may alternatively allow scanning by having a read head (50) which is held stationary while one dimensional microarray (108) moves below read head (50). This would eliminate the need for an oscillating read head since only the one dimensional probes (110) are moving on stage (106) in a single direction.

The setup for position sensing device (96) may also incorporate a system which identifies beacons (98, 100) through their relative peak intensities. As seen

in Figures 10(a) to 10(c), the CCD array outputs relative signal strengths as a function of position along the array. A given signal strength corresponds to a given beacon. For instance, first beacon (98) is the brightest beacon and thus corresponds to the brightest signal (98') and second beacon (100), which is dimmer than first beacon (98), corresponds to the dimmer signal (100') on the CCD output, and so on. Therefore, outputs from a CCD array may be utilized in tracking beacons by their relative intensities. Alternatively, different beacons may also be identified through the spot size or the number of fibers in the beacon. For example, the first beacon (98) may comprise a single fiber forming a single spot on the CCD. The second beacon (100) may comprise two closely positioned optical fibers forming two adjacent spots, and so on. Note that the two fibers in second beacon (100) may separate from each other by a very small distance relative to the distance between first and second beacons (98, 100); e.g., first and second adjacent beacons (98, 100) may be separated by 40 mm while the two fibers in second beacon (100) may be separated from each other by a small distance such as 0.1 mm.

In addition, where the required scanning range in the X-direction is less than, approximately 30 mm, a single beacon with a CCD array or an analog position sensing device may be used. The analog position sensing device may be, for instance, a continuous photoresistor strip having differential voltage output at its two terminals proportional to the position of a light spot on the strip.

Figure 12 illustrates an embodiment of a scanning apparatus having a rotating position sensing mechanism (116), where the microarray is fabricated on rotating substrate (122) that has a shape similar to a CD-ROM disk as described in the aforementioned copending U.S. Patent Application Ser. No. 60/244,418. In this embodiment, microarray substrate (122) may be rotated continuously by rotating stage (120) while optical read head (118) or multiple heads moves stepwise in a radial direction to scan across the entire microarray surface (122). An advantage of this design is that it allows full benefit of the mature CD-ROM design and manufacturing capability to reduce the system cost. Because the substrate may be rotated continuously at high speed, the reading speed can be

accomplished faster than by conventional scanning mechanisms. This embodiment of the scanning apparatus may incorporate optical or magnetic markers in the substrate (122), as described above, which provide an indication of the position of the read head.

A scanning apparatus may incorporate read heads based on bulk optical lenses similar to what is found in a compact disk (CD) reader in addition to a read head (118) having a fiber optic as described above. This configuration may take full advantage of the existing mature technology in CD-ROM drives.

When microarray scanners are adapted as readers in high throughput screening, the microarray substrate in the system is replaced by an array of micro reaction wells, which are filled with fluids. All types of microarray scanners maybe adapted for this application. In particular, when the disclosed invention is used for this application, the optical fiber guides excitation light energy to wells and collects emission light at the same time. The well array may moves in one direction while the read head moves in the other, usually orthogonal direction to complete scanning action. On the other hand, the read head may provide the scanning motion in both directions while the array is kept stationary. Because the read head is fiber optic in the disclosed invention, it can be moved independent of the light source. This provides added flexibility to enable a compact, economical structure design.

In any of the embodiments of the invention, the scanning structure may be configured to receive light from a dry area or a wet area on a substrate. In one preferred embodiment of the invention, the area has a diameter between about 10 micron and about 500 micron. In another preferred embodiment, the area is a microwell having a diameter between about 100 and about 1000 micron. The areas are described as having a diameter because typically the areas are spots as are formed by known spotting techniques or liquid contained in wells having a circular cross section, although the areas are not confined to circular shapes.

A scanning structure can be configured to receive light of a second wavelength from an area of a specified size a number of ways. For example, the fiber or rod will have a diameter suitable to read light from the area (that is, the

diameter is not so large that it overlaps two or more areas at all times the fiber or rod is reading a signal from a given area). The detector may be timed by known techniques to read signals at given times and/or given locations that correspond to the light conductive portion of the scanning structure intersecting the area to be read. The scanning speed may be altered to accommodate array density. Any combination of these techniques may also be used.

The substrate areas that react to light may be dry or wet. Microarrays of genes or proteins may be read using the scanner, as can microwells containing products of reaction or association (such as those encountered in high-throughput screening of drugs). Such microarrays include those described in WO99/55460, WO99/55461, EP 0 955 084, WO00/53736, WO00/53739, U.S. application no. 09/758,873, filed Jan. 10, 2001, 09/791,998, filed Feb. 22, 2001, 09/791,410, filed Feb. 22, 2001, and 09/791,411, filed Feb. 22, 2001 (the latter four of which are inventions of the present applicant), all of which are incorporated by reference herein in their entirety.

A scanning structure of the invention may be used in conjunction with a substrate configured for high throughput screening as described above. The scanning structure has a light source and a detector. The substrate receives light from the light source, and a detector receives light from the substrate to detect the presence or absence of one or more wavelengths of light. Other scanners as are used to determine hybridization on a microarray containing oligonucleotides may be used in this manner as well. Such microarrays are discussed in the Background section above. These microarrays may contain at least 100, 400, or 1000 microwells per square centimeter for liquid sample processing, for instance.

Although the invention has been described with reference to particular embodiments, the description is only an example of the invention's application and should not be taken as a limitation. Various adaptations and combinations of features of the embodiments disclosed are now readily apparent to those of ordinary skill and are within the scope of the invention. All references discussed or referred to herein are incorporated by reference in their entirety as if fully put forth herein.

WHAT IS CLAIMED IS:

1. A scanning structure for delivering light to and receiving light emitted from a light-excitable area on a substrate, said scanning structure comprising an optical fiber that transmits light of a first wavelength and light of a second wavelength and having a proximal end and a distal end, which optical fiber is configured to receive at least a portion of the light of the second wavelength that is generated when said light-excitable area receives the light of the first wavelength.

2. A scanning structure according to claim 1 and further comprising additional optical fibers that transmit the light of the first wavelength and the light of the second wavelength, each of said additional optical fibers having proximal ends and distal ends and being configured to traverse the light-excitable area of the substrate in sufficient proximity to the substrate that said optical fibers capture at least a portion of the light of the second wavelength that is generated when said light-excitable area receives the light of the first wavelength.

3. A scanning structure according to claim 2 wherein the distal end of said optical fiber and the distal ends of said additional optical fibers are held in a fixed relationship.

4. A scanning structure according to any of claims 1-3 further comprising a detector optically coupled to said proximal end of said optical fiber to receive the light of the second wavelength.

5. A scanning structure according to any of claims 1-4 further comprising a holder for the substrate that is configured to move the substrate as said optical fiber scans the substrate.

6. A scanning structure according to any of claims 1-5 further comprising a holder for said optical fiber that moves between a first position and a second

position, the distance between said first position and said second position being sufficient that said optical fiber traverses the light-excitable area of the substrate.

7. A scanning structure according to any of claims 1-6 and further comprising a light source that emits light of said first wavelength to excite the light-excitable area, which light source is optically coupled to the proximal end of the optical fiber so that the light of the first wavelength exits the distal end of the optical fiber.

8. A scanning structure for delivering light to and receiving light emitted from a light-excitable area on a substrate, said scanning structure comprising

a) an optical fiber having a proximal end and a distal end that transmits light of a first wavelength and light of a second wavelength;

b) a holder for the optical fiber that moves between a first position and a second position, the distance between said first position and said second position being sufficient that the optical fiber traverses the light-excitable area of the substrate, which light-excitable area emits light of the second wavelength when said light-excitable area receives light of the first wavelength,

c) a light source that emits light of said first wavelength to excite the light-excitable area, which light source is optically coupled to the proximal end of the optical fiber so that the light of the first wavelength exits the distal end of the optical fiber,

d) wherein the optical fiber is attached to the holder so that the optical fiber is sufficiently close to the microarray that the optical fiber receives said light of the second wavelength produced by the light-excitable area at the distal end of the optical fiber and transmits the light of the second wavelength to the proximal end of the optical fiber, and

e) wherein the holder is configured to be moved between the first position and the second position when the light source emits the light having the first wavelength.

9. A scanning structure according to any of claims 1-8, wherein the scanning structure further comprises a beam having a distal portion and a proximal portion, said distal portion comprises said holder, the beam is attached to a mount at the proximal end of the beam, and the beam is configured so that the distal portion of the beam moves between the first position and the second position.

10. A scanning structure according to claim 9, wherein the beam is a movable arm of a galvanometer.

11. A scanning structure according to claim 9, wherein the beam moves between the first position and the second position at a resonant frequency of the beam.

12. A scanning structure according to claim 11, wherein the resonant frequency of the beam is actuated piezoelectrically.

13. A scanning structure according to claim 11, wherein the resonant frequency of the beam is actuated magnetically.

14. A scanning structure according to claim 9, wherein the proximal end of the beam is attached to the mount such that the proximal end of the beam is not movable.

15. A scanning structure according to any of claims 8-14 wherein the holder is configured to move horizontally between the first position and the second position, and wherein the scanning structure further comprises a magnet configured to repel the holder in a vertical direction when the holder reaches at least one of said first position and said second position.

16. A scanning structure according to any of claims 7-15, wherein the light source comprises a laser which emits the light at the first wavelength.

17. A scanning structure according to any of claims 1-16, wherein the scanning structure further comprises an optical filter comprising a first branch channel and a second branch channel, which optical filter is configured to receive the light of the first wavelength from the laser in the first branch channel and which optical filter is configured to transmit the light of the second wavelength from the light-excitable area through the second branch channel.

18. A scanning structure according to any of claims 8-17, wherein the scanning structure further comprises a substrate translation stage that moves the substrate along a direction generally perpendicular to a line defined by the first position and the second position.

19. A scanning structure according to any of claims 8-18, wherein the holder secures the optical fiber at an angle other than perpendicular to the substrate.

20. A scanning structure according to any of claims 8-19, wherein a surface defining the distal end of the optical fiber is substantially parallel with the substrate.

21. A scanning structure according to any of claims 1-20, wherein the optical fiber further comprises a capillary fiber.

22. A scanning structure according to any of claims 1-20, wherein the optical fiber is a double core fiber comprising an inner core having a first refractive index and an outer core exterior to the inner core and having a second refractive index, said first refractive index being greater than the second refractive index.

23. A scanning structure for delivering light to and receiving light emitted from a light-excitable area on a substrate, said scanning structure comprising

a) a plurality of optical fibers each having a corresponding proximal end and a corresponding distal end that transmit light having a plurality of differing preselected wavelengths, each of the wavelengths corresponding to each of the plurality of optical fibers,

b) said plurality of optical fibers comprising at least a first optical fiber that transmits light having a first wavelength and light of a second wavelength,

c) a holder for the plurality of optical fibers that moves between a first position and a second position, the distance between said first position and said second position being sufficient that the plurality of optical fibers traverse the light-excitable area of the substrate, which light-excitable area emits light of at least the second wavelength when said light-excitable area receives light of at least the first wavelength,

d) a plurality of light sources corresponding to each of the plurality of optical fibers that emit light of at least the first wavelength to excite the light-excitable area, which light sources are optically coupled to each of the corresponding proximal ends of the plurality of optical fibers so that the light of at least the first wavelength exits the distal end of at least the first optical fiber,

e) wherein the plurality of optical fibers are attached to the holder so that the plurality of optical fibers are sufficiently close to the microarray that each of the optical fibers receive said light of at least the second wavelength produced by the light-excitable area at the distal ends of the plurality of optical fibers and transmits the light of at least the second wavelength to at least the proximal end of the first optical fiber, and

f) wherein the holder is configured to be moved between the first position and the second position when the plurality of light sources emits the light having at least the first wavelength.

24. A scanning structure according to claim 23, wherein the scanning structure further comprises a beam having a distal portion and a proximal portion, said distal portion comprises said holder, the beam is attached to a mount at the proximal end of the beam, and the beam is configured so that the distal portion of the beam moves between the first position and the second position.

25. A scanning structure according to claim 24, wherein the beam is a movable arm of a galvanometer.

26. A scanning structure according to claim 24, wherein the beam moves between the first position and the second position at a resonant frequency of the beam.

27. A scanning structure according to claim 26, wherein the resonant frequency of the beam is actuated piezoelectrically.

28. A scanning structure according to claim 26, wherein the resonant frequency of the beam is actuated magnetically.

29. A scanning structure according to claim 24, wherein the proximal end of the beam is attached to the mount such that the proximal end of the beam is not movable.

30. A scanning structure according to any of claims 23-29 wherein the holder is configured to move horizontally between the first position and the second position, and wherein the scanning structure further comprises a magnet configured to repel the holder in a vertical direction when the holder reaches at least one of said first position and said second position.

31. A scanning structure according to any of claims 23-30, wherein the plurality of light sources comprises a plurality of lasers which emit the light of at least the first wavelength.

32. A scanning structure according to any of claims 23-31, wherein the scanning structure further comprises a plurality of optical filters, each of said optical filters comprising at least a first branch channel and a second branch channel, which optical filters are configured to receive the light of at least the first wavelength from the plurality of lasers in the first branch channel and which optical filters are configured to transmit the light of at least the second wavelength from the light-excitable area through the second branch channel.

33. A scanning structure according to any of claims 23-32, wherein at least one of the plurality of optical fibers comprises a plurality of capillary fibers.

34. A scanning structure according to any of claims 23-33, wherein at least one of the plurality of optical fibers comprises a double core fiber comprising an inner core having a first refractive index and an outer core exterior to the inner core and having a second refractive index, said first refractive index being greater than the second refractive index.

35. A scanning structure according to any of claims 23-34, wherein the distal ends of the plurality of optical fibers comprise a multiple fiber read head where each of the distal ends have a preselected bundled configuration.

36. A scanning structure according to any of claims 23-34, wherein the distal ends of the plurality of optical fibers comprise a multiple fiber read head where each of the distal ends are linearly adjacent.

37. A scanning structure for delivering light to and receiving light emitted from a light-excitable area on a substrate, said scanning structure comprising

a) a plurality of optical fibers each having a corresponding proximal end and a corresponding distal end that transmit light having a plurality of differing

preselected wavelengths, each of the wavelengths corresponding to each of the plurality of optical fibers,

b) wherein the plurality of optical fibers transmits light of at least a first wavelength and light of at least a second wavelength,

c) a plurality of multiplexers which optically couple each of the corresponding distal ends of the plurality of optical fibers into at least one main optical fiber having a main proximal end and a main distal end,

d) a holder for the main optical fiber that moves between a first position and a second position, the distance between said first position and said second position being sufficient that the main optical fiber traverses the light-excitable area of the substrate, which light-excitable area emits light of at least the second wavelength when said light-excitable area receives light of at least the first wavelength,

e) a plurality of light sources corresponding to each of the plurality of optical fibers that emit light of at least the first wavelength to excite the light-excitable area, which light sources are optically coupled to each of the corresponding proximal ends of the plurality of optical fibers so that the light of at least the first wavelength exits the main distal end via at least one of the multiplexers,

f) wherein the main optical fiber is attached to the holder so that the main optical fiber is sufficiently close to the microarray that the main optical fiber receives said light of at least the second wavelength produced by the light-excitable area at the distal end of the main optical fiber and transmits the light of at least the second wavelength to the proximal ends of the plurality of optical fibers via the plurality of multiplexers, and

g) wherein the holder is configured to be moved between the first position and the second position when the plurality of light sources emits the light having at least the first wavelength.

38. A scanning structure according to claim 37, wherein the scanning structure further comprises a beam having a distal portion and a proximal portion, said

distal portion comprises said holder, the beam is attached to a mount at the proximal end of the beam, and the beam is configured so that the distal portion of the beam moves between the first position and the second position.

39. A scanning structure according to any of claims 37-38 wherein the holder is configured to move horizontally between the first position and the second position, and wherein the scanning structure further comprises a magnet configured to repel the holder in a vertical direction when the holder reaches at least one of said first position and said second position.

40. A scanning structure according to any of claims 37-39, wherein the plurality of light sources comprises a plurality of lasers which emit the light of at least the first wavelength.

41. A scanning structure according to any of claims 37-40, wherein the scanning structure further comprises a plurality of optical filters, each of said optical filters comprising at least a first branch channel and a second branch channel, which optical filters are configured to receive the light of at least the first wavelength from the plurality of lasers in the first branch channel and which optical filters are configured to transmit the light of at least the second wavelength from the light-excitable area through the second branch channel.

42. A scanning structure according to any of claims 37-41, wherein the main optical fibers comprises a capillary fiber.

43. A scanning structure according to any of claims 37-41, wherein the main optical fibers comprises a double core fiber comprising an inner core having a first refractive index and an outer core exterior to the inner core and having a second refractive index, said first refractive index being greater than the second refractive index.

44. A scanning structure according to any of claims 1-43 wherein said optical fiber has a diameter at its distal end that is identical to a diameter along the length of the optical fiber.

45. A scanning structure for delivering light to and receiving light from a surface of an object which emits light at a second wavelength when said surface receives light at a first wavelength, said surface having either (i) a one-dimensional microarray of probes arranged along a first direction, said probes having a width in a second direction, or (ii) a two-dimensional microarray of probes arranged along the first direction and a second direction of the substrate, said scanning structure comprising

a) a light source for illuminating at least a portion of the surface with light at the first wavelength;

b) a light-guiding rod positioned so that a distal end of the light-guiding rod is sufficiently close to said surface that light of said second wavelength emitted at the surface is captured by the light-guiding rod; and

c) a carrier configured to carry the light-guiding rod over the surface from a first position to a second position along said second direction, the distance between said first position and said second position being (i) at least the width of the probes for the one-dimensional microarray or (ii) the distance between probes furthest from one another along said second direction for said two-dimensional microarray.

46. A scanning structure according to claim 45, wherein the light-guiding rod is configured so that a proximal end of the light-guiding rod receives at least a portion of the light of the first wavelength generated by said light source and transmits said light to the distal end of the light-guiding rod.

47. A scanning structure according to claim 45 or claim 46, wherein said light-guiding rod comprises an optical fiber.

48. A scanning structure according to any of claims 45-47, wherein said scanning structure further comprises a second light-guiding rod configured to receive the light of the first wavelength from the light source at a proximal end of the second light-guiding rod and configured so that said light exits a distal end of the second light-guiding rod and near the surface of the substrate.

49. A scanning structure according to claim 48, wherein said second light-guiding rod comprises an optical fiber.

50. A scanning structure according to any of claims 1-49, wherein the substrate is substantially circular and defines a rotational axis perpendicular to the light-excitable area of the substrate.

51. A scanning structure for delivering light to and receiving light from a surface of an object which emits light at a second wavelength when said surface receives light at a first wavelength, said surface having a one-dimensional microarray of probes arranged along a first direction, said probes having a width in a second direction, said scanning structure comprising

a) a light source for illuminating at least a portion of the surface with light at the first wavelength;

b) a light-guiding rod fixedly positioned so that a distal end of the light-guiding rod is sufficiently close to said surface that light of said second wavelength emitted at the surface is captured by the light-guiding rod; and

c) a stage configured to translate the surface from a first position to a second position along said first direction, the distance between said first position and said second position being at least the distance between probes arranged along said first direction.

52. A scanning structure according to claim 51, wherein the light-guiding rod is configured so that a proximal end of the light-guiding rod receives at least a

portion of the light of the first wavelength generated by said light source and transmits said light to the distal end of the light-guiding rod.

53. A scanning structure according to claim 51 or claim 52, wherein said light-guiding rod comprises an optical fiber.

54. A scanning structure according to any of claims 1-53 wherein the scanning structure is configured to receive light of the second wavelength from the light-excitable area on the substrate, and wherein the light-excitable area contains a dry sample in said area having a diameter of between about 10 micron and about 500 micron.

55. A scanning structure according to any of claims 1-53 wherein the scanning structure is configured to receive light of the second wavelength from the light-excitable area on the substrate, and wherein the light-excitable area contains a wet sample in said area having a diameter of between about 10 micron and about 500 micron.

56. A scanning structure according to any of claims 1-53 wherein the scanning structure is configured to receive light of the second wavelength from the light-excitable area on the substrate, and wherein the light-excitable area contains a wet sample in said area having a diameter of between about 100 micron and about 1000 micron.

57. A scanning structure according to any of claims 54-56 wherein said optical fiber or said light-conducting rod has a diameter sufficiently small to receive light of the second wavelength from a first light-excitable area but not an adjacent second light-excitable area.

58. A method for determining the presence of association of a first target molecule to a first probe and a second target molecule to a second probe, the first probe comprising a plurality of first molecules complementary to the first target molecule on a first portion of a surface of a substrate and the second probe

comprising a plurality of second molecules complementary to the second target molecule on a second portion of the surface of the substrate, the probe being a member of a plurality of probes on the substrate in either (i) a one-dimensional microarray of probes arranged along the substrate, wherein said one-dimensional microarray has a plurality of probes arranged along a second direction of the substrate, each probe of said plurality having a width along a first direction on the substrate, or (ii) a two-dimensional microarray of probes arranged along the first direction and the second direction of the substrate, wherein said two-dimensional microarray has a plurality of probes arranged along the first direction and a plurality of probes arranged along the second direction and wherein the target molecule has a component that provides light of a second wavelength when illuminated with light of a first wavelength, said method comprising the acts of

a) illuminating the first portion of the surface of the substrate with light at the first wavelength;

b) detecting the presence of light at the second wavelength by relative movement between a light detector and the first portion of the surface along the first direction to determine whether the first target molecule has associated with the first probe;

c) advancing the substrate in the second direction; and

d) again detecting the presence of light at the second wavelength by illuminating the second portion of the surface of the substrate with the light at the first wavelength and moving the light detector across the second portion of the surface in the first direction to determine whether the second target molecule has associated with the second probe.

59. A method according to claim 58, wherein the light detector comprises a first optical fiber.

60. A method according to claim 59, wherein the act of illuminating comprises shining the light of the first wavelength into a proximal end of the first optical

fiber so that said light of the first wavelength exits a distal end of the first optical fiber to illuminate said first portion and said second portion of the substrate.

61. A method according to claim 59, wherein the act of illuminating comprises shining the light of the first wavelength into a proximal end of a second optical fiber so that said light of the first wavelength exits a distal end of the second optical fiber to illuminate said first portion and said second portion of the substrate.

62. A method of making a scanner that detects the presence of a fluorescent moiety, said method comprising positioning a distal end of an optical fiber in a holder of the scanner such that light of a first wavelength exiting the distal end of the optical fiber excites the fluorescent moiety to emit light of a second wavelength, and positioning said distal end of the optical fiber sufficiently close to the fluorescent moiety that the distal end of the optical fiber receives the light of the second wavelength and transmits the light of the second wavelength to a proximal end of the optical fiber.

63. A method according to claim 62, wherein said method comprises positioning the distal end of the optical fiber so that the distal end is not perpendicular to a surface on which the fluorescent moiety resides.

64. A method for determining the position of a linear stage for use in a scanner, comprising the acts of

a) positioning a first beacon on the stage at a first location, said first beacon having a corresponding first signal;

b) positioning at least a second beacon on the stage at a second location which is at a predetermined distance from the first beacon such that a line from the first beacon to the at least second beacon defines a first direction,

said at least second beacon having a corresponding second signal weaker than the first signal;

c) translating the stage along the first direction;

d) while translating the stage, detecting the first signal and then the second signal with a detector;

e) correlating the detected first signal and the weaker second signal with the corresponding stage location.

65. A method according to claim 64, wherein the detector is a linear CCD.

66. A method according to claim 64 or claim 65, wherein the first beacon and the second beacon comprise optic fibers.

67. A method according to claim 65 or claim 54, wherein the predetermined distance is less than the linear CCD.

68. A method of using a scanning structure for a substrate configured for high throughput screening, wherein the scanning structure has a light source and a detector, said method comprising illuminating the substrate with light from the light source and detecting light received from the substrate with the detector.

69. A method according to claim 68, wherein the substrate contains at least 400 microwells per square centimeter.

70. A method according to claim 68, wherein the substrate contains at least 1000 microwells per square centimeter.

71. A method according to any of claims 68-70, wherein the substrate contains a probe in a liquid.

72. A method according to any of claims 68-71, wherein said light received from the substrate is produced by sample fluorescence.

73. A method according to any of claims 68-71, wherein said light received from the substrate is produced by chemiluminescence.

74. A method according to any of claims 68-71, wherein said light received from the substrate lacks at least one wavelength of light provided by the light source.

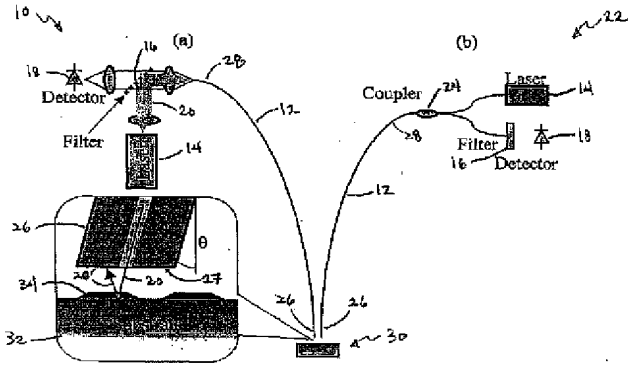


Fig. 1(a) and 1(b)

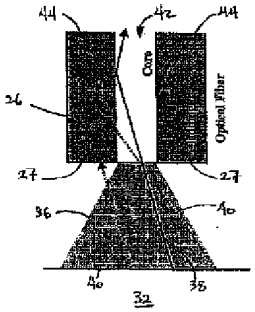


Fig. 2

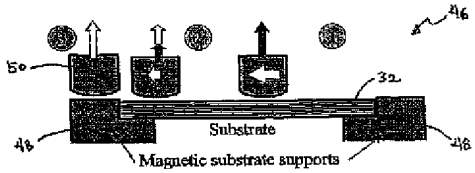

46
50
32
Substrate
48
48
Magnetic substrate supports

Fig. 3

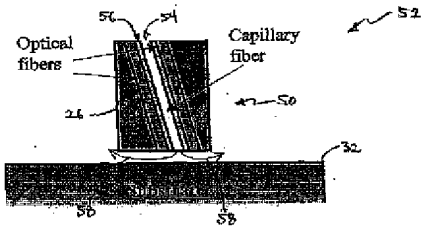

52
56
54
Optical fibers
Capillary fiber
24
50
32
58

Fig. 4

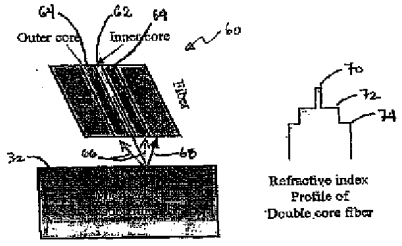

64
62
Outer core
Inner core
60
66
68
32
70
72
74
Refractive index
Profile of
Double core fiber

Fig. 5

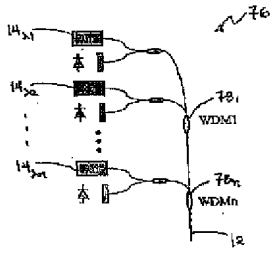


Fig. 6

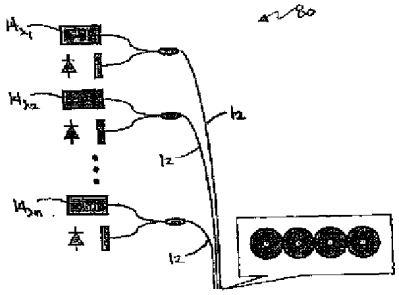


Fig. 7

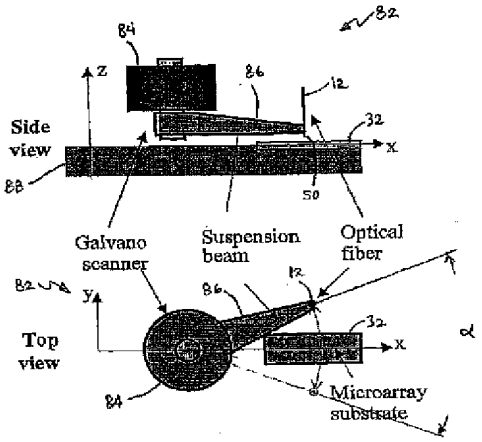

82
84
z
86
12
Side
view
32
x
88
50
Galvano
scanner
Suspension
beam
Optical
fiber
82
y
86
12
Top
view
32
x
α
84
Microarray
substsate

Fig 8

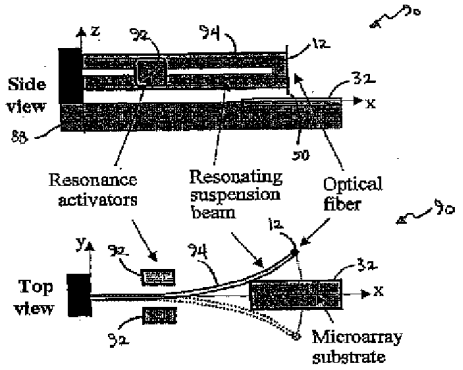
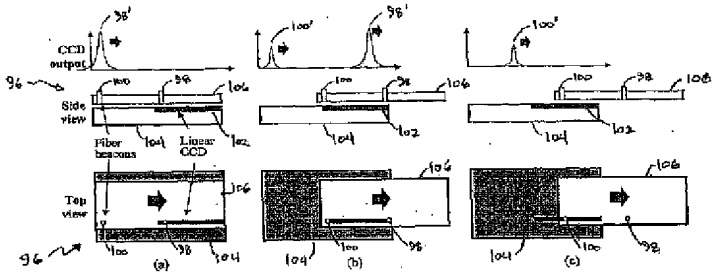

Side view
Top view
Resonance activators
Resonating suspension beam
Optical fiber
Microarray substrate
z
x
y
92
94
12
32
88
50
90

Fig. 9



Figs. 10(a), 10(b), and 10(c)

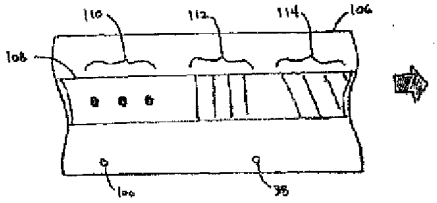


Fig. 11

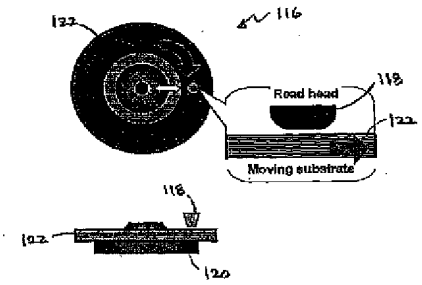


Fig. 12

【国際公開パンフレット（コレクトバージョン）】

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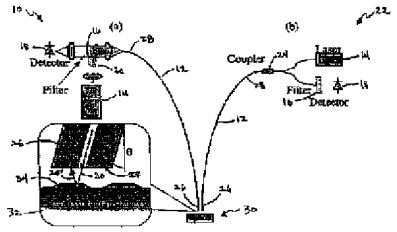
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Published:
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FIBER OPTIC SCANNER



(57) Abstract: The disclosed scanning structure includes an apparatus for light delivery and light receiving from a light-excitable area on a substrate to be measured by the scanning structure. The light delivery and receiving apparatus may include an optical fiber having a proximal end and a distal end which transmits light having a certain wavelength or light with several varying wavelengths to excite the substrate samples. This optical fiber may also simultaneously receive light which may be emitted by fluorescing samples on the substrate. The scanning structure also may further include a holder for the optical fiber that is able to transverse variable distances over the substrate to be measured or examined. Holders may include galvano scanners as well as resonating suspension beams. A light source, e.g., a laser, may be optically coupled to the optic fiber's proximal end. And this light source may be of a certain wavelength, but multiple light sources each having a different wavelength may also be used simultaneously by coupling the light sources into either a single optic fiber through wavelength multiplexers or by placing individual optic fibers carrying differing wavelengths in close proximity to each other. As the light is transmitted down to the substrate through the optic fiber, the fiber is sufficiently close to the substrate microarray so that it can also receive the emitted fluorescing light.

WO 01/69302 A3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/08043

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7　G01N21/64

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	EP 0 266 881 A (ASTROMED LTD) 11 May 1988 (1988-05-11)	1-4,7,8, 23,37, 45-47, 51,58, 64,69,72
A	the whole document	23,37
X	US 5 968 766 A (POWERS LINDA) 19 October 1999 (1999-10-19)	1-5
A	column 5, line 5 - line 15; figure 1	8,23,37, 45,51, 58,64
A	US 4 877 966 A (TOMEI L DAVID ET AL) 31 October 1989 (1989-10-31) column 1 -column 4; figure 1	1,8,23, 37,45, 51,58,64

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[X] Further documents are listed in the continuation of box C.　　[X] Patent family members are listed in annex.

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"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

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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
13 December 2001	20/12/2001

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European Patent Office, P.B. 5818 Patentlaan 2
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Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
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Authorized officer
Hervé, D

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/08043

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 784 157 A (GORFINKEL VERA ET AL) 21 July 1998 (1998-07-21) column 10, line 9 -column 11, line 15; figure 4	1,8,23, 37,45, 51,58,64

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

page 2 of 2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ational Application No
PCT/US 01/08043

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			GB 2196734 A		05-05-1988
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			CA 2237222 A1		29-05-1997
			EP 0862735 A1		09-09-1998
			WO 9719342 A1		29-05-1997

Form PCT/ISA/210 (patent family annex) (July 1992)

(51)Int.Cl.[7]	ＦＩ	テーマコード（参考）
Ｇ０２Ｂ　26/10	Ｇ０２Ｂ　26/10　　１０９	

(81)指定国　　AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EE,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,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZW

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Ｆターム(参考) 2G043 AA01 BA16 EA01 FA06 GA03 GB01 GB19 HA05 HA09 JA02
KA09 LA03 MA01
2G054 AA06 EA03 GA04 GE01
2H045 AE05
2H050 AC13 AC28 AC87

【要約の続き】
られた蛍光をも受光するのに充分に、この基板に近接している。

Espacenet

Bibliographic data: JP2008534960 (A) — 2008-08-28

FLOW CYTOMETER FOR DIFFERENTIATING SMALL PARTICLES IN SUSPENSION

Inventor(s):

Applicant(s):

Classification:	**- international:** ***G01N15/14; G01N21/05; G01N21/49***
	- cooperative: **G01N15/1404**; G01N15/1434
Application number:	JP20080504110 20060316 Global Dossier
Priority number (s):	WO2006US09449 20060316 ; US20050094723 20050330
Also published as:	EP1924837 (A1) US2006221325 (A1) US7113266 (B1) WO2006104699 (A1)

Abstract not available for JP2008534960 (A)
Abstract of corresponding document: US7113266 (B1)

A flow cytometer includes an optical flow cell through which particles to be characterized on the basis of at least their respective side-scatter characteristics are caused to flow seriatim. A plane-polarized laser beam produced by a laser diode is used to irradiate the particles as they pass through a focused elliptical spot having its minor axis oriented parallel to the particle flow path. Initially, the plane of polarization of the laser beam extends perpendicular to the path of particles through the flow cell. A half-wave plate or the like is positioned in the laser beam path to rotate the plane of polarization of the laser beam so that it is aligned with the path of particles before it irradiated particles moving along such path.

(19)日本国特許庁(JP)　(12)公表特許公報(A)　(11)特許出願公表番号
特表2008-534960
(P2008-534960A)
(43)公表日　平成20年8月28日(2008.8.28)

(51)Int.Cl.		F I			テーマコード（参考）
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G01N 21/05	(2006.01)	G01N	21/05		2G059
G01N 21/49	(2006.01)	G01N	21/49	Z	

審査請求 未請求　予備審査請求 未請求　（全 13 頁）

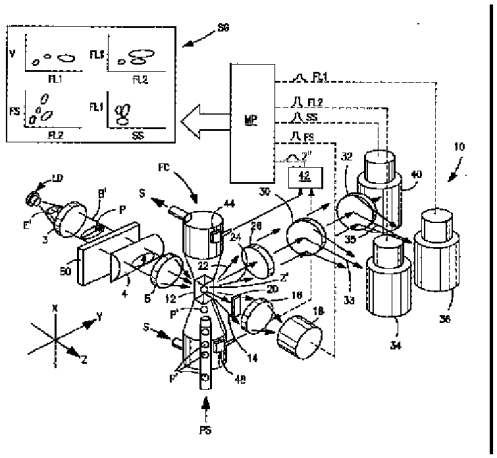
(21)出願番号　特願2008-504110 (P2008-504110)
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最終頁に続く

(54)【発明の名称】懸濁液中の小粒子を区別するためのフローサイトメータ

(57)【要約】
フローサイトメータは、光学フローセルを含み、特性調査されるべき粒子が該光学フローセルを順次流され、該粒子は、少なくともそのそれぞれの側面散乱特性に基づいて、特性調査される。レーザダイオードによって生成される平面偏光レーザビームは、該粒子が、集束楕円スポットを通過する際に、該粒子を照射するために使用され、該集束楕円スポットは、その短軸が、該粒子フロー経路に平行に向けられる。最初に、該レーザビームの該偏光面は、該フローセルを通過する粒子の該経路に対して垂直に延びる。半波長板などが、該レーザビーム経路に配置され、該レーザビームの該偏光面を回転する。その結果として、該レーザビームは、そのような経路に沿って移動する粒子を照射する前に、粒子の該経路と整列される。



【特許請求の範囲】
【請求項1】
（ａ）粒子調査ゾーンを有する光学フローセルであって、特性調査されるべき粒子が実質的に線形な粒子経路に沿って、一度に一個ずつ、該ゾーンを通過し得る、光学フローセルと、
（ｂ）平面偏光レーザビームを生成するためのレーザであって、該レーザは、該レーザビームの偏光面が、該粒子経路に垂直であるように向けられる、レーザと、
（ｃ）該粒子調査ゾーン内に、該粒子経路上に中心を置く楕円スポットとして、該レーザビームを集束するためのビーム成形レンズ系であって、該楕円スポットの短軸は、該粒子経路と平行に配置される、ビーム成形レンズ系と、
（ｄ）該粒子経路と、該レーザビームが該粒子調査ゾーンで粒子を照射する方向とに対して実質的に垂直な方向に、該集束楕円スポットによって照射された粒子によって散乱されると、該レーザビームの一部分を検出するように配置された側面散乱光検出器と、
（ｅ）該レーザと該光学フローセルとの間の該レーザビームの中に配置された偏光回転光学素子であって、該光学素子は、該レーザビームが該粒子調査ゾーンで、粒子を照射する前に、該レーザビームの偏光面を回転する役割を果たす、偏光回転光学素子と
を備える、フローサイトメータ。
【請求項2】
前記偏光回転光学素子は、前記粒子照射レーザビームの偏光面を前記粒子経路に実質的に平行にするために十分な量、該レーザビームの偏光面を回転する役割を果たす、請求項１に記載の装置。
【請求項3】
前記偏光回転光学素子は、前記レーザビームの偏光面を９０°回転するように動作する、請求項２に記載の装置。
【請求項4】
前記偏光回転光学素子は、半波長板を備える、請求項３に記載の装置。
【請求項5】
前記偏光回転光学素子は、一対の四分の一波長板を備える、請求項３に記載の装置。
【請求項6】
（ａ）線形粒子経路を有する光学フローセルであって、該線形粒子経路に沿って特性調査されるべき粒子が順次通過し得る、光学フローセルと、
（ｂ）相互に垂直な長軸と短軸とを有する楕円断面の平面偏光レーザビームを生成するためのレーザダイオードであって、該レーザダイオードは、該楕円断面の該長軸が、該粒子経路に対して垂直に延びるように配置される、レーザダイオードと、
（ｃ）該粒子経路上に互いに垂直な長軸と短軸とを有する楕円スポットに、該レーザビームを集束するためのレンズ系であって、該スポットは、該集束スポットの該短軸が該粒子経路と平行であるように向けられる、レンズ系と、
（ｄ）粒子フローの方向と、該レーザビームが該粒子経路に沿って移動する粒子を照射する方向とに対して実質的に垂直な方向に散乱される、レーザ放射を検出するために配置された側面散乱光検出器と、
（ｅ）該レーザビームの中に配置された光学素子であって、該光学素子は、該レーザビームが、該粒子経路上の粒子を照射する前に、該レーザビームの偏光面を９０°回転する機能を有する、光学素子と
を備える、フローサイトメータ。
【請求項7】
前記光学素子は、半波長板を備える、請求項６に記載の装置。
【請求項8】
前記光学素子は、タンデムに配置された一対の四分の一波長板を備える、請求項６に記載の装置。

【請求項9】
（ａ）粒子調査ゾーンを有する光学フローセルであって、特性調査されるべき粒子が、実質的に線形な粒子経路に沿って、一度に一個ずつ、該ゾーンを通過するようにされ得る、光学フローセルと、
（ｂ）相互に垂直な長軸と短軸とを有する拡大楕円断面の平面偏光レーザビームを生成するレーザであって、該レーザビームは、該拡大楕円断面の該長軸に平行な平面内で平面偏光され、該偏光面が、該線形粒子経路に対して実質的に垂直となるように、該光学フローセルに対して向けられる、レーザと、
（ｃ）該レーザビームの大部分を収集して、コリメートし、コリメートレーザビームを提供するためのコリメートレンズであって、該コリメートレンズは、該拡大楕円断面の一部分を切頭するようにさらに動作し、これによって、該コリメートレンズの両側に現れる偽光源を生成する、コリメートレンズと、
（ｄ）（ｉ）該粒子調査ゾーン内の該線形粒子経路上に中心を置く楕円スポットとして、該コリメートレーザビームを集束するため、および（ｉｉ）該集束楕円スポットの両側上で、該集束楕円スポットの該長軸に沿って、該偽光源からの光を、偽光スポットとして、集光するためのビーム成形レンズ系であって、該ビーム成形レンズ系は、該集束楕円スポットの該短軸が該粒子経路と平行に延びるように、該集束楕円スポットを向けるのに有効であり、これによって、該偽光スポットが、該粒子経路の両側に該粒子経路から間隔を空けて配置される、ビーム成形レンズ系と、
（ｅ）該粒子経路と、該レーザビームが該粒子調査ゾーンで粒子を照射する方向とに対して実質的に垂直な方向に、該集束楕円スポットによって照射される粒子から、散乱される、レーザ放射を検出するために配置された側面散光乱検出器と、
（ｆ）該レーザビームの中に配置された偏光回転光学素子であって、該偏光回転光学素子は、該レーザビームが該粒子調査ゾーンで粒子を照射する前に、該レーザビームの偏光面を９０°回転するように適合されている、偏光回転光学素子と
を備える、フローサイトメータ。
【請求項10】
前記偏光回転光学素子は、前記レーザビームの前記コリメートされる部分の中に配置される、請求項９に記載の装置。
【請求項11】
前記偏光回転光学素子は、半波長板を備える、請求項９に記載の装置。
【請求項12】
前記偏光回転光学素子は、タンデムに配置された一対の四分の一波長板を備える、請求項９に記載の装置。
【発明の詳細な説明】
【技術分野】
【0001】
（発明の背景）
（発明の分野）
本発明は、液体懸濁液中の小粒子、例えば、様々なタイプの血球を区別するために、一般的に使用されるタイプのフローサイトメータにおける改善に関する。より具体的には、本発明は、例えば、レーザダイオードから発光された平面偏光放射を使用し、この平面偏光放射は、光学フローセルを通過する個々の粒子の光学散乱特性を検出するために、このような粒子を照射し、これによって、各粒子を、特定のクラスまたはタイプのメンバとして、特徴づけるタイプのフローサイトメータにおける改善に関する。
【背景技術】
【0002】
（従来技術）
フローサイトメータは、粒子懸濁液中の種々のタイプの個々の小さな粒子を区別するために、一般的に使用され、この区別は、各粒子の光散乱および／または蛍光の特性をベー[illegible]。このような器具は、サンプルからの粒子が一列縦隊で進むようにされた粒

子調査ゾーンを有する光学的に透明なフローセルと、該粒子が該粒子調査ゾーンを通過する際に、このような粒子を一度に一個ずつ照射するためのレーザ光源と、該照射された粒子からの散乱放射と、対象のクラスまたはタイプ内の特定の粒子に事前に付けられた蛍光色素によって発せられる蛍光放射との双方を受けるように、該フローセルの周りに戦略的に置かれている複数の光学検出器とを、一般的に含む。典型的に、光検出器は、光検出器の光感知素子の幾何学的形状によって決定される角度範囲内の前方散乱放射と、照射レーザビームの方向と粒子経路の方向とに、実質的に垂直な方向に散乱される側面散乱放射との双方を検出するように配置される。光検出器のそれぞれの出力は、次いで、公知の方法で処理され、照射された粒子のそれぞれを特定のクラスまたはタイプのメンバとして識別する。通常、フローサイトメータは、各クラスにおける粒子の数または各タイプの粒子の数を示すヒストグラムまたは散布図を提供する。

【0003】

上記のタイプのフローサイトメータにおいて、粒子照射レーザ光源として、レーザダイオードを用いることが、ますます一般的になってきている。このようなソリッドステートデバイスは、サイズおよび経済性を基に考慮すると、より従来型のガスレーザ、例えば、ヘリウムネオンレーザおよびアルゴンレーザよりも好まれることが多い。これらのデバイスは、比較的小さなサイズであるので、器具筐体の中に、容易に配置され、方向付けされ、任意の様々な設計目的を達成し得る。レーザダイオードは、多くの点で、有利であると考慮され得る一方で、レーザダイオードは、不利な点がない分けではない。例えば、比較的低パワーのデバイスであることに加え、レーザダイオードは、典型的に、実用のためにコリメートされなくてはならない非コリメート放射を発する。多くのデバイスにおいて、アクティブな半導体素子または「ダイ」から発せられる出力放射は、比較的速く発散する傾向がある。なぜなら、ダイは、通常、形状が長方形であるために、発せられた放射は、相互に垂直な平面で、異なるように発散するからである。したがって、レーザダイオードからの放射出力の形状は、典型的には、拡大楕円の形状であり、一般に、比較的大きな発散角度、例えば、３０°で一つの平面に拡がり、その一方で、かなり小さな発散角度、例えば、１０°で垂直な平面に拡がる。この拡大ビーム内のレーザエネルギを捕獲し、コリメートするために、レーザ源に非常に接近して、比較的高い開口数のコリメートレンズを配置することは、一般的である。このコリメートレンズは、より小さな角度でレーザ源から発散するエネルギの全てを容易に収集する一方で、しばしば、より大きな角度で発散するビームの一部分を切頭する。多くのレーザダイオードにおいて、この切頭の結果、回折光および／または反射光からなる一対の異質（ｅｘｔｒａｎｅｏｕｓ）光源または偽光源（ｓｐｕｒｉｏｕｓ　ｌｉｇｈｔ　ｓｏｕｒｃｅ）（あるいは遠視野回折パターン）が、ビーム切頭が発生するコリメートレンズの両側に現れる。これらの偽光源は、通常、コリメートされた主ビームに比べて、比較的弱い強度である一方で、偽光源は、フローサイトメータの性能に対して問題となり得る。例えば、レーザダイオードからの出力ビームを、フローセルを通過して移動する粒子を照射するために適合された楕円スポットに集束するとき、その集束楕円スポットは、一対の比較的弱く、境界が不鮮明な放射のローブ（ｌｏｂｅ）、すなわち「光ローブ」をともなう。これらの光ローブは、コリメートレンズの両側から発する放射の集束偽ビームを表す。これらの光ローブは、集束楕円スポットの両側で、その境界の外側に現れる。これらの光ローブが、粒子経路の中に位置する場合には、これらの光ローブは、フローサイトメータの信号処理を複雑化するように機能する低レベルの光散乱信号および蛍光信号を生じる。具体的には、このような低レベルの信号は、小さな粒子から発するように見えるが、これらの小さな粒子は、粒子サンプルには、実際は、存在しない。

【0004】

Ｏｚａｓａらへ付与された米国特許（特許文献１）においては、上述の光ローブ問題が、フローサイトメータにおけるレーザダイオードの向きを単に調整するだけで、対処される。すなわち、上述の光ローブは、フローセルを通過する粒子経路の外側に、すなわち、粒子経路に垂直である平面にローブを配置するように置かれる。Ｏｚａｓａらは、フロー

サイトメータにおけるレーザダイオードを、拡大楕円の長軸が、（通常、垂直である）フローセルを通過する粒子フローの方向に平行となるように向けることは、従来通りであると指摘している。レーザダイオードをこのように向けると、ビームが集束されて、円筒レンズと集光レンズとの組み合わせを通過するときに、粒子照射楕円となることを可能にする。この粒子照射楕円は、（ａ）その楕円の短軸に平行な平面内に、回折限界があり、（ｂ）フローセル内の公称粒子経路上に、その中心が位置し、その短軸がそのような経路と平行に延びる。なぜなら、集束楕円は、粒子経路に平行な平面内に回折限界があるために、集束ビームのフラックス密度が最大化され、このことによって、器具の光散乱および蛍光検出の感度が強化される。また、粒子経路に平行な平面内に回折限界があるので、集束楕円は、時間ドメイン内に（すなわち、粒子フローの方向に）、比較的接近して一緒に移動する複数の粒子の同時照射（および検出）ができない。しかし、Ｏｚａｓａらが指摘するように、フローサイトメータにおけるレーザダイオードの従来の向きの著しい欠点は、フローサイトメータが、上述の光ロープを、直接、粒子経路に配置するように機能するので、この結果、実際には存在しない小さな粒子を誤って検出し、カウントすることである。この問題に対するＯｚａｓａらの解決策は、上述のように、レーザダイオードをそのサポート筐体に対して、単に９０°回転することである。これは、拡大楕円の長軸が、今では水平であること、すなわち、フローセルを通過する（通常は垂直である）粒子経路に垂直となるようにするためである。これは、ロープ放射を、粒子経路の両側で、その外側にシフトする効果を有する。このように向ける結果、フローセルを通過する粒子で、光ロープによって照射されず、したがって、このような照射の結果として、放射を散乱し得ないし、また蛍光放射も発し得ない。したがって、光検出器のそれぞれの出力におけるこのようなロープ放射の存在を相殺する必要はない。レーザダイオードのこのように向けると、その結果として、集束楕円スポットが９０°回転し、この楕円の長軸が望ましからぬように粒子経路と整列されるが、Ｏｚａｓａらは、ビーム形成光学系に追加レンズを追加することによって、この状況を回避していることに留意されたい。追加レンズは、集束楕円の形状を修復して、レーザダイオードが回転する前に生じた形状に、すなわち、短軸が粒子経路と平行である楕円にするために動作する。

【0005】

Ｏｚａｓａらの特許で教示されるレーザダイオードの向きは、確認された光ロープ問題を解決し得るが、照射粒子によって散乱される放射の検出に影響を与える別の光学問題を生成することが見出された。より具体的には、照射粒子からの側面散乱放射（すなわち、照射ビームの光軸と、フローセルを通過する粒子経路との双方に対して、９０°で散乱する放射）を検出することが望ましいとき、レーザダイオードの示唆された（水平の）向きは、側面散乱信号の信号対ノイズ比（ＳＮＲ）を劇的に減少させる効果を有することが見出された。このＳＮＲの減少の程度は、側面散乱パラメータが粒子の特性調査プロセスの一部として使用され得ないほどである。

【特許文献1】米国特許第６，７１３，０１９号明細書

【発明の開示】

【発明が解決しようとする課題】

【0006】

（発明の概要）

上記の議論の観点から、本発明の目的は、確認された問題、すなわち、レーザダイオードの拡大楕円出力ビームの長軸が、フローセルを通過する粒子経路に対して垂直となるように、レーザダイオードが向けられるときの側面散乱放射を検出する問題に対する解決策を提供することである。

【課題を解決するための手段】

【0007】

本発明の一つの局面に従うと、レーザダイオードからの出力ビームは、そのビームが最も速く発散している平面内で、平面偏光されることが、認識される。さらに、フローサイトメータにおける側面散乱放射の測定は、照射レーザビームの偏光面がフローセルを通過

する粒子経路の方向に平行でなくてはならないことを要求することが、認識される。さらに、上述されたタイプ、すなわち、レーザダイオードの向きが、確認された「光ローブ」問題を排除するために、９０°回転するタイプのフローサイトメータにおいて、粒子照射レーザビームの偏光面は、もはや粒子経路に平行ではなく、それゆえ、側面散乱放射を検出する能力が割り引かれることが、認識される。したがって、このような器具を用いて側面散乱測定を行うために、照射ビームの偏光面と粒子経路との間で必要な関係を新たに確立することが必要である。

【0008】

以上の観点から、本発明に従って構成されたフローサイトメータは、以下のエレメント、すなわち、（ａ）粒子調査ゾーンを有する光学フローセルであって、特性調査されるべき粒子が、実質的に線形な粒子経路に沿って、一度に一個ずつ、該ゾーンを通過し得る、光学フローセルと、（ｂ）平面偏光レーザビームを生成するレーザ源であって、このようなレーザは、該レーザビームの該偏光面が、該粒子経路に垂直となるように向けられる、レーザ源と、（ｃ）該粒子調査ゾーン内で、該粒子経路上に中心を置かれた楕円スポットとして、該レーザビームを集束するためのビーム成形レンズ系であって、このような楕円スポットの短軸は、該粒子経路に平行に配置される、ビーム成形レンズ系と、（ｄ）該粒子経路と、該レーザビームが該粒子調査ゾーンで粒子を照射する方向とに、実質的に垂直な方向に、該集束楕円スポットによって照射された粒子によって散乱される、該レーザビームの一部分を検出するように配置された側面散乱光検出器と、（ｅ）該レーザ源と該光学フローセルとの間の該レーザビーム経路に配置された偏光回転光学素子であって、このような光学素子は、従来技術の系のように、このような経路に垂直に配置されるよりもむしろ、該レーザビームの該偏光面を該粒子経路と実質的に平行に配置されるのに十分な量、回転する役割を果たす、偏光回転光学素子とを備える。好ましくは、上記偏光回転光学素子は、半波長板、または２つの四分の一波長板の組み合わせのいずれかを備え、この板は、上記レーザビームの上記偏光面を９０°回転させるように役立つ。

【0009】

本発明およびその利点は、添付図面を参照しながら、以下の好ましい実施形態の詳細な説明から、より良く理解される。添付図面において、同様の記号は、同様のパーツを意味する。

【発明を実施するための最良の形態】

【0010】

（好ましい実施形態の詳細な説明）

ここで、図面を参照すると、図１は、発散レーザビームＬＢを発するタイプの従来型レーザダイオードＬＤを示し、この発散レーザビームＬＢは、２つの異なる発散角θ１およびθ２に従って、サイズを拡大する楕円断面を有する。図示されるように、これらの角度は、相互に垂直な平面内で測定され、一方の角度、この場合は、角θ１が、他方の角度よりも通常は実質的に大きい。レーザダイオードの特徴であるように、発光レーザビームＬＢは、平面Ｐ内で、平面偏光される。平面Ｐは、ビームの拡大楕円断面の長軸Ａ’に平行である。楕円断面の短軸Ａ”は、当然、長軸Ａ’に対して垂直である。

【0011】

図２において、図１のレーザダイオードＬＤが、上述された以前のタイプのフローサイトメータで具現化されているように示される。解析されるべき粒子、例えば、血球を含む粒子サンプルＰＳは、矢印Ａの方向に、光学的に透明なフローセル６の中に、ノズル７によって導入される。シース液体Ｓは、ノズル７によって提供される粒子ストリームを囲むような方法で、フローセルに供給され、これによって、一般的に垂直に配置されるフローセルの中心軸に、粒子ストリームのフローを流体力学的に制約する役割を果たす。シース流体の流速は、粒子サンプルがノズル７によって導入される速度と組み合わさって、粒子が、フローセルの中心に位置する粒子調査ゾーンＺ’を１度に１個ずつ通過するようにさせる。レーザダイオードによって発された発散レーザビームＬＢは、コリメータレンズ３によって、コリメートされ、その結果コリメートされた主ビームＭＢは、円筒レンズ４お

よび集光レンズ５の組み合わせを通され、主ビームを粒子調査ゾーンでの楕円スポット焦点に導く。上述の理由から、この分野において、レーザダイオードの楕円出力ビームの長軸Ａ’がフローセルを通過する（通常垂直な）粒子経路に平行に延びるように、レーザダイオードを向けることが、従来行われてきた。（この向きにおいて、レーザビームの偏光面Ｐもまた、粒子経路に平行に延びることに留意されたい。）楕円ビームがこの垂直な向きであるにも関わらず、ビームは、集束スポットの短軸が粒子経路に平行に延びる、楕円スポット焦点に導かれる。このことは、上述の技術的な利点を提供する。

【0012】

図２に示されるレーザダイオードの向きで、コリメートレンズ３は、通常、ビームがより速く拡大する平面、すなわち、角θ１が測定される垂直な平面内の発散するレーザビームの一部分を切頭する。コリメートレンズが、発散レーザビームに対して中心に位置すると仮定すると、この切頭は、比較的低強度の一対の偽ビームＳＢ１およびＳＢ２を生成する。この一対の偽ビームは、切頭が生じるコリメートレンズ３の両側３ａおよび３ｂから発するように見える。上述のように、楕円スポットとして、主ビームを集束することに加え、レンズ４および５は、主ビームＭＢの集束ビームスポットＢＳの両側に、これらの偽ビーム（「光ローブ」）をビームスポットＢＳ１およびＢＳ２として、集束するように機能する。図示されるように、これらの偽ビームスポットのそれぞれの強度Ｓ１およびＳ２は、主ビームの強度Ｓ０よりも実質的に低い。それにも関わらず、これらの偽ビームスポットは、フローセルを通過する粒子経路上に直接位置する。したがって、フローセルを通過する各粒子は、３箇所で照射され、そのうちの１箇所（中央箇所）のみが、望ましい。以下で議論される光散乱・蛍光検出器は、次いで、偽ビームからの信号を受けるので、このため、粒子分化プロセスにおいて、これらの信号を無視するためのスキームを必要とする。

【0013】

図３に模式的に示されるフローサイトメータにおいて、図１および図２のレーザダイオードＬＤが、９０°回転していることが、理解される。その結果として、発散レーザビームＬＢが、図面の平面内と、粒子経路の平面内とで、その小さな角θ２で拡大する。レーザダイオードのこの向きにおいて、その拡大楕円出力ビームの長軸Ａ’は、今や水平面内に延び、粒子経路と、図面の平面とに対して垂直である。したがって、レーザビームの部分は、コリメートレンズによって、依然として、切頭されるものの、その結果得られる（図２に示される）偽ビームスポットＢＳ１およびＢＳ２は、粒子経路と整列して位置するのではなく、むしろ、今や粒子経路の両側に位置する。レーザダイオードのこの向きは、レーザビームの偏光面Ｐを水平面内に、粒子経路に対して垂直に向ける効果を有することに留意すべきである。したがって、ビームスポットＢＳ１およびＢＳ２は、フローセルを通過する粒子を照射する位置には、もはや位置しない一方で、ビームの偏光面が、ここでは、問題となる。

【0014】

図４Ａおよび図４Ｂを参照すると、ベクトルＥによって示されるように、垂直なＸ／Ｚ平面内で偏光される平面偏光ビームＢによって照射される小粒子Ｐ’は、図示されるような水平なＹ／Ｚ平面の光を散乱する役割を果たすことが、図４Ａに示される。水平面での散乱光は、前方散乱ＦＳＨ、側面散乱ＳＳＨ、および全ての中間角度での前方散乱ＦＳＨ’を含め、その垂直な偏光を維持し、水平面内での任意の角度で測定されるその強度は、照射粒子の物理的および光学的性質のみによって決定される。しかしながら、垂直なＸ／Ｚ平面に前方に散乱される光の強度は、測定角∂に依存し、角度∂が、前方への散乱光が垂直上向き（または下向き）に導かれ、これによって前方への散乱光が垂直方向での側面散乱光ＳＳＶになる位置である、９０°（水平なＹ／Ｚ平面に対して測定）に近づくにつれて、このような散乱光の強度は、示されるように、ゼロに近づくことに、留意されたい。実際、垂直な偏光面での波振動は、垂直な方向の散乱ビーム強度を相殺するように機能する。同様に、図４Ｂに示されるように、ビーム源が、９０°回転し、その偏光面が、今や水平である、すなわち、Ｙ／Ｚ平面にあるような場合、垂直（Ｘ／Ｚ）平面に散乱する

光の強度は、このビーム源の向きによる影響を受けないが、水平（Ｙ／Ｚ）平面に散乱された光は、測定がなされる角βに依存する。水平面内の側面散乱光ＳＳＨの強度は、角βが９０°に近づくと、ゼロになることに、留意されたい。この情報を上述のフローサイトメータ環境に適用すると、側面散乱信号が、図３に示されるダイオードレーザの向きで検出されない理由が、理解される。そのように向くと、図４の散乱条件が適合し、検出する側面散乱信号が存在しない。

【0015】

以上の議論において、図５は、本発明が役立ち、本発明が具現化されるタイプのフローサイトメータ１０を模式的に示す。図示されるように、本発明のフローサイトメータは、光学フローセルＦＣを含み、このフローセルＦＣを介して、特性調査されるべき種々の粒子Ｐ’を含む粒子サンプルＰＳが、流される。サンプルは、例えば、このサンプル内に、特性調査されるべき粒子が、様々なタイプの血球（例えば、単球、リンパ球、好塩基球など）の形で存在する血液全体のサンプルを備え得る。フローセルは、光学的に透明な筐体１２を備え、筐体１２は狭い中央チャネルを規定し、このチャネルを通過して、粒子が一度に一個ずつ流れ、その間に、レーザダイオードＬＤによって提供される集束レーザビームＢ’によって照射される。図示されるように、レーザダイオードは、その発散楕円出力ビームが、垂直平面内でよりも水平面内でより速く拡大するように向けられる。これによって、上述の偽ビームスポットは、図３を参照して上記で議論されたように、フローセルを通過する垂直な粒子の両側で、外側に位置する。粒子調査ゾーンＺを通過し、集束レーザビームによって照射されると、各個々の粒子はビーム光を散乱し、照射された粒子のタイプに従って、蛍光放射を発する。前方散乱光１４は、レンズ１６によって収集され、適切な光検出器１８上に集束され、光検出器１８の出力ＦＳは、マイクロプロセッサＭＰに加えられる。軸方向ビーム光は、適切な光ストップ２０によって吸収される。側面散乱光２２、すなわち、水平（Ｙ／Ｚ）平面内に９０°で散乱されたビーム光は、選択された粒子によって運ばれる蛍光色素によって発せられるマルチカラーの蛍光２４と一緒に、レンズ２６によって収集され、コリメートされ、その結果得られたコリメートビーム２８は、コリメートビーム経路に対して４５°で配置された一対の二色性ミラー３０、３２にパスされるか、あるいは二色性ミラー３０，３２によって反射される。側面散乱光３３は、ミラー３０によって反射され、適切な光検出器３４、典型的には光電子増幅管へと向かう。ミラー３０を通過するか、透過し、ミラー３２によって反射される第一の波長の蛍光３５は、第二の光検出器３６に導かれる。ここでもまた、第二の光検出器は、好ましくは、光電子増幅管である。ミラー３０とミラー３２との双方を通過する第二の波長の蛍光は、第三の光検出器４０に導かれる。ここでもまた、第三の光検出器は、好ましくは、光電子増幅管のタイプのものである。光検出器３４、３６、および４０のそれぞれの出力ＳＳ、ＦＬ１、およびＦＬ２は、処理のために、マイクロプロセッサに供給される。フローセルを通過する各粒子から得られた光散乱信号および蛍光信号に加え、調査される各粒子のコールター体積を表すインピーダンス信号Ｚを検出することは、一般的である。このような信号は、フローセルの調査ゾーンを通過して定電流を通すことと、このゾーン内の粒子の電気インピーダンスによってもたらされる電流の変化をモニタすることとによって、導出される。粒子が大きければ大きいほど、電流の変化が大きい。粒子調査の両側に配置された一対の電極４４、４６の間に接続された定電流回路４２は、そのゾーンを通過するのに必要な電流を提供し、このような電流内の粒子に由来する変化を検出するように動作する。回路４２の出力Ｚ”もまた、マイクロプロセッサに供給される。マイクロプロセッサが、ＦＬ１、ＦＬ２、ＳＳ、ＦＳ、およびＺ”の入力信号を、１つ以上の公知のアルゴリズムに従って、同時に処理すると、マイクロプロセッサは、典型的には、複数の散布図ＳＧの形式で、出力を提供する。散布図ＳＧは、粒子サンプルＰＳの中の粒子の相対数と種々のタイプとを示す。

【0016】

上記で指摘されたように、図３を参照して議論されたように向けられたレーザダイオードには、レーザビームの偏光面Ｐが、粒子フローの方向に対して垂直であることにおいて

、問題がある。図５のフローサイトメータに示されるように、拡大楕円Ｅ’の長軸は、コリメートレンズ３を通過したコリメートビームの長軸がそうであるように、水平面内にある。ビームの偏光面Ｐは、長軸に平行であるので、図４Ａは、水平面内における側面散乱放射が、水平面内の波面振動によって相殺されることを示す。したがって、本発明に従うと、光学素子５０は、レーザビームＢ’の中に、好ましくは、コリメートレンズ３と円筒レンズ４との間のビームのコリメートされる部分の中に配置される。素子５０は、ビームの偏光面を十分な量、回転することによって、レーザビームの偏光面が、フローセルを通過する（垂直な）粒子経路と、今や実質的に平行となるように、レーザビームの偏光面を向ける効果を有する。したがって、レーザダイオードを回転させて、望ましくない偽スポットを排除することのメリットは、側面散乱光を検出することを可能にすることのメリットと組み合わされる。なぜなら、偏光面は、このような側面散乱光を検出するために必要な向きにあるからである。好ましくは、素子５０は、レーザビームの偏光面を９０°回転させるように動作し、従来の半波長板の形式を採る。代替として、素子５０は、タンデムに配置された２つの四分の一波長板の形式であり得る。

【0017】

本発明は、好ましい実施形態を参照して記載されてきたが、本発明の精神から逸脱することなく様々な変更がなされ得ることは、理解される。このような変更は、添付の請求項の範囲内に収まることを意図される。

【図面の簡単な説明】

【0018】

【図1】図１は、従来のレーザダイオードからの発散出力ビームを示す。

【図2】図２は、フローサイトメータの光学フローセル内の所望箇所で、発散レーザビームを集束するための従来技術の光学系の模式図である。

【図3】図３は、フローサイトメータの光学フローセル内の所望箇所で、発散レーザビームを集束するための従来技術の光学系の模式図である。

【図4】図４Ａおよび図４Ｂは、平面偏光光源の偏光面における変化が、光散乱に与える影響を示す。

【図5】図５は、本発明を具現化するフローサイトメータの斜視模式図である。

【図1】

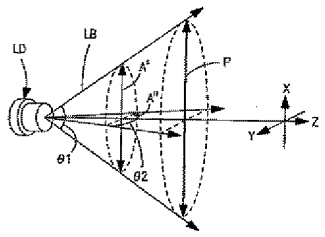


FIG. 1
従来技術

【図2】

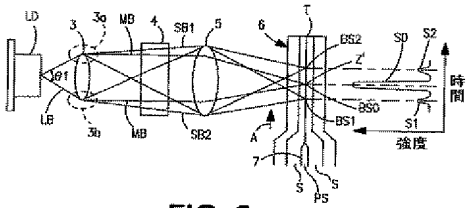


FIG. 2
従来技術

【図3】

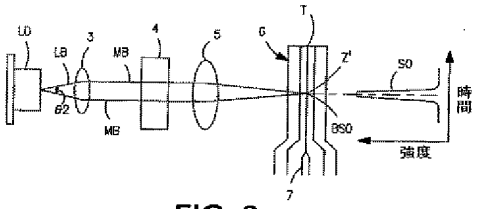


FIG. 3
従来技術

【図4A】

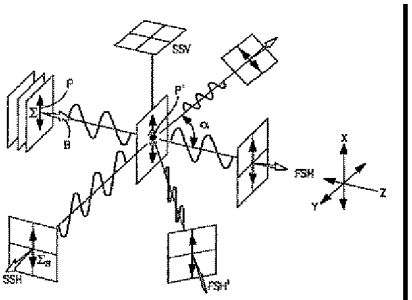


FIG. 4A

【図4B】

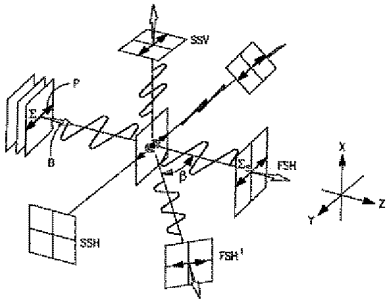


FIG. 4B

【図5】

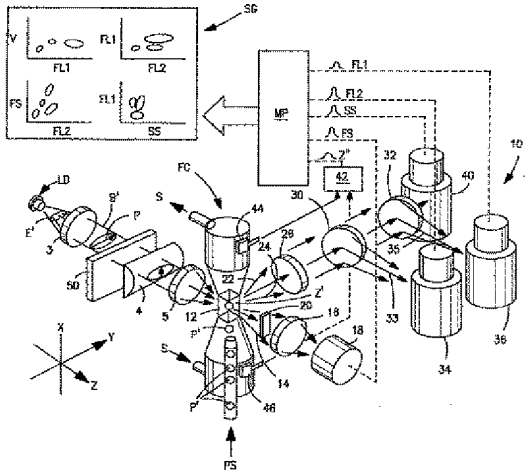


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/09449

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **G01N 21/00**(2006.01)

USPC: 356/73
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)
U.S. : 356/73,336,342,369

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,798,827 A (FRANK et al.) 25 August 1998 (25.08.1998), see entire document.	1-12
A	US 6,228,652 B1 (RODRIGUEZ et al.) 08 May 2001 (08.05.2001), see entire document.	1-12
A	US 6,794,671 B2 (NICOLI et al.) 21 September 2004 (21.09.2004), see entire document.	1-12
A,P	US 2005/0112541 A1 (DURACK et al.) 26 May 2005 (26.05.2005), see entire document.	1-12

☐ 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

"E" earlier application or patent published on or after the international filing date

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"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
21 June 2006 (21.06.2006)

Date of mailing of the international search report
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/09449

Continuation of B. FIELDS SEARCHED Item 3:
USPGPUB, USPAT, USOCR, EPO, JPO, DERWENT, IBM_TDB search notes: (cytomet$3 and (flow adj cell) and laser and spot and (minor adj axis) and ((side adj scatter$3) or side-scatter$3) and (polarisation or polarization or polarized)) and @ad<="20050330"

Form PCT/ISA/210 (extra sheet) (April 2005)

(81)指定国　　AP(BW,GH,GM,KE,LS,MW,MZ,NA,SD,SL,SZ,TZ,UG,ZM,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,BG,CH,CY,CZ,DE,DK,EE,ES,FI,FR,GB,GR,HU,IE,IS,IT,LT,LU,LV,MC,NL,PL,PT,RO,SE,SI,SK,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG),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,KM,KN,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,LY,MA,MD,MG,MK,MN,MW,MX,MZ,NA,NG,NI,NO,NZ,OM,PG,PH,PL,PT,RO,RU,SC,SD,SE,SG,SK,SL,SM,SY,TJ,TM,TN,TR,TT,TZ,UA,UG,US,UZ,VC,VN,YU,ZA,ZM,ZW

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Ｆターム(参考) 2G057 AA02 AA14 AB04 AB08 AC01 AC05 BA05
2G059 AA05 BB06 BB13 DD12 EE02 EE05 EE07 EE11 FF12 GG01
JJ07 JJ11 JJ13 JJ19 JJ20 KK02 KK03

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Bibliographic data: JP2010085194 (A) — 2010-04-15

SAMPLE ANALYZER

Inventor(s): KUSUZAWA HIDEO ± (KUSUZAWA HIDEO)

Applicant(s): SYSMEX CORP ± (SYSMEX CORP)

Classification: - international: ***G01N15/14; G01N21/17; G01N21/64***
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Application number: JP20080253498 20080930 Global Dossier

Priority number (s): JP20080253498 20080930

Also published as: CN101713723 (A) EP2169382 (A2) US2010079762 (A1)

Abstract of JP2010085194 (A)

PROBLEM TO BE SOLVED: To provide a sample analyzer capable of sharply imaging the contour of a particle of erythrocyte, or the like. ;SOLUTION: This sample analyzer 10 is equipped with a flow cell 30 for forming a sample flow, and an imaging system 24 for acquiring a particle image by imaging a particle contained in the sample flow in the flow cell 30. The imaging system 24 has a light source 61 for irradiating near ultraviolet light to the sample flow in the flow cell 30, and a camera 64 for imaging the particle in the sample flow irradiated with the near ultraviolet light from the light source 61. ;COPYRIGHT: (C)2010,JPO&INPIT

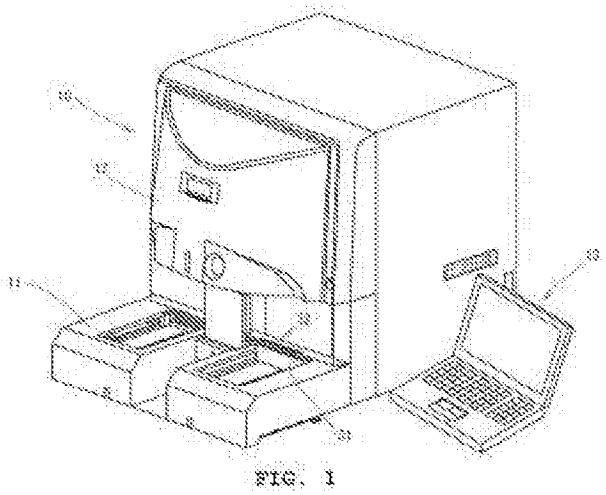


FIG. 1

(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2010-85194
(P2010-85194A)
(43)公開日　平成22年4月15日(2010.4.15)

(51)Int.Cl.		F I			テーマコード（参考）
G01N 15/14	(2006.01)	G01N	15/14	D	2G043
G01N 21/17	(2006.01)	G01N	21/17	A	2G059
G01N 21/64	(2006.01)	G01N	21/64	Z	

審査請求 未請求　請求項の数 10　ＯＬ　（全 14 頁）

(21)出願番号　特願2008-253498 (P2008-253498)
(22)出願日　平成20年9月30日 (2008.9.30)

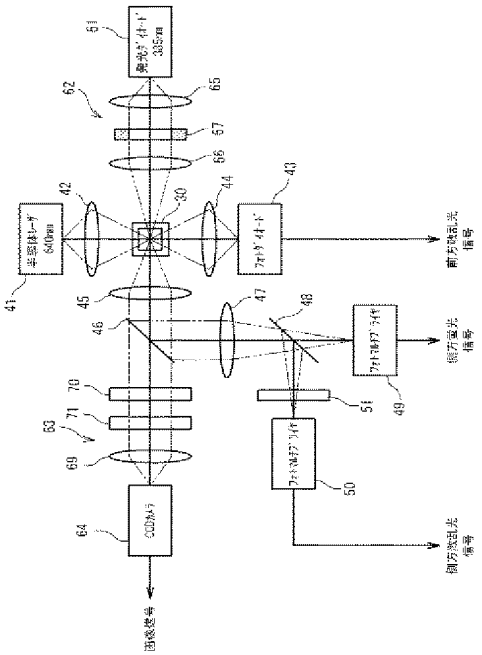
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(72)発明者　楠澤　英夫
兵庫県神戸市中央区脇浜海岸通１丁目５番１号　シスメックス株式会社内

Ｆターム(参考)　2G043 AA04 BA16 DA05 EA01 EA14
FA06 HA09 JA02 KA02 KA03
KA08 KA09 LA02 LA03 NA06

最終頁に続く

(54)【発明の名称】試料分析装置

(57)【要約】
【課題】赤血球等の粒子の輪郭を鮮明に撮像することができる試料分析装置を提供する。
【解決手段】試料分析装置１０は、試料流を形成するためのフローセル３０と、フローセル３０中の試料流に含まれる粒子を撮像して粒子画像を取得するための撮像系２４と、を備える。撮像系２４は、フローセル３０中の試料流に対して近紫外光を照射する光源６１と、光源６１の近紫外光が照射された試料流中の粒子を撮像するカメラ６４と、を有する。
【選択図】　図４



【特許請求の範囲】
【請求項1】
試料流を形成するためのフローセルと、
前記フローセル中の試料流に対して近紫外光を照射する光源と、
前記光源によって近紫外光が照射された試料流に含まれる粒子を撮像する撮像部と、備える試料分析装置。
【請求項2】
前記光源がパルス発光するように構成されている請求項１に記載の試料分析装置。
【請求項3】
前記フローセル中の前記試料流に含まれる粒子を検出するための粒子検出手段と、
前記粒子検出手段によって粒子が検出された場合にその粒子を撮像するように前記撮像部を制御する撮像制御手段と、をさらに備えている請求項１又は２に記載の試料分析装置。
【請求項4】
前記粒子検出手段が、
前記光源によって照射される近紫外光よりも長い波長の光を前記フローセル中の試料流に対して照射する第２の光源と、
この第２の光源の照射光によって励起される粒子からの蛍光を受光する受光部と、を有している請求項３に記載の試料分析装置。
【請求項5】
前記光源、第２の光源および粒子検出手段は、前記光源からの近紫外光の照射によって励起された粒子からの蛍光によって粒子が検出されないように構成されている請求項４に記載の試料分析装置。
【請求項6】
表示部と、
前記撮像部によって撮像された粒子画像を前記表示部に表示させる表示制御手段と、をさらに備えている請求項１～５のいずれか１つに記載の試料分析装置。
【請求項7】
前記表示制御手段は、前記撮像部によって粒子が撮像された場合に、前記表示部にその粒子の粒子画像を表示させるように構成されている請求項６に記載の試料分析装置。
【請求項8】
前記フローセルと前記撮像部との間に配置され、かつ近紫外光を選別して透過させるように構成された光選別部をさらに備える請求項１～７のいずれか１つに記載の試料分析装置。
【請求項9】
前記光源と前記フローセルとの間に配置された照明レンズ系と、前記フローセルと前記撮像部との間に配置された撮像レンズ系とをさらに備え、
前記照明レンズ系の開口数が前記撮像レンズ系の開口数と同等又は前記開口数よりも小さく設定されている請求項１～８のいずれか１つに記載の試料分析装置。
【請求項10】
前記撮像制御手段は、前記粒子検出手段によって変形赤血球が検出された場合に、その変形赤血球を撮像するように前記撮像部を制御する請求項３に記載の試料分析装置。

【発明の詳細な説明】
【技術分野】
【0001】
本発明は、例えば尿や血液などの試料を分析するための試料分析装置に関する。
【背景技術】
【0002】
従来、撮像機能を有するフローサイトメータが知られている。例えば、下記特許文献１には、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が開示されている。

【特許文献1】特開平１０－７３５２８号公報

【発明の開示】

【発明が解決しようとする課題】

【0003】

特許文献１に記載のフローサイトメータは、試料流を照明する光源として近赤外光源が用いられており、この近赤外光源から照明される近赤外光は粒子に対する透過性が高いため、粒子内部の状態を好適に撮像することが可能である。したがって、例えば、試料に含まれる細胞の核部分等を撮像するのに適している。

その反面、近赤外光を用いると、背景像と粒子像とのコントラストが明瞭に現れず、粒子の輪郭がぼやけしまうことがあった。

【0004】

本発明は、上記課題に鑑みてなされたものであり、赤血球等の粒子の輪郭を鮮明に撮像することができる試料分析装置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】

【0005】

本発明は、試料流を形成するためのフローセルと、前記フローセル中の試料流に対して近紫外光を照射する光源と、前記光源によって近紫外光が照射された試料流に含まれる粒子を撮像する撮像部と、を備えることを特徴とする。

以上のように光源の照射光を近紫外光とすると、当該照射光が粒子に照射されたときの散乱効率が向上する。つまり、近紫外光は粒子に対する透過性が低いため、粒子が存在する部分では光が透過せずに暗く映り、粒子の周囲の背景部分は明るく映る。このため、粒子像と背景像とのコントラストが明瞭となり、粒子の輪郭が鮮明に現れた粒子画像を得ることができる。

【0006】

本発明の試料分析装置は、近紫外光源がパルス発光するように構成されていることが好ましい。これにより、フローセルを流れる粒子を瞬間的に照射、撮像することができるため、粒子像が試料流方向に伸びたりすることがなく、実際の粒子形状に近い粒子像を得ることができる。

【0007】

本発明の試料分析装置は、前記フローセル中の前記試料流に含まれる粒子を検出するための粒子検出手段と、前記粒子検出手段によって粒子が検出された場合にその粒子を撮像するように前記撮像部を制御する撮像制御手段と、をさらに備えていることが好ましい。

この場合、粒子が粒子検出手段によって検出されたときだけ、その粒子を撮像部によって撮像することが可能となる。したがって、フローセルを粒子が通過した場合にのみその粒子を撮像するので、試料に含まれる粒子の画像のみを得ることができる。

なお、撮像制御手段は、粒子検出手段によって変形赤血球が検出された場合に、撮像部によってその変形赤血球を撮像するように構成することができる。本発明による試料分析装置は、上記のとおり光源として近紫外光を照射可能な光源を備えているため、試料中に含まれる変形赤血球を自動的に検出し、輪郭が鮮明に撮像された変形赤血球の画像を得ることができる。これにより、臨床的意義の高い変形赤血球の変形の形態等を把握し、あるいはその変形の程度によってその変形赤血球の由来（生体試料に混入した部位）を知るための有用な臨床情報を得ることができる。

【0008】

前記粒子検出手段は、前記光源によって照射される近紫外光よりも長い波長の光を前記フローセル中の試料流に対して照射する第２の光源と、この第２の光源の照射光によって励起される粒子からの蛍光を受光する受光部と、を有していることが好ましい。

一般に、粒子に光を照射することによって励起される蛍光は、照射した光の波長よりも

長くなることが知られている。本発明のように第２の光源として近紫外光よりも波長の長いものを使用すると、第２の光源の照射光により粒子から発せられる蛍光と、近紫外光源によって発せられる蛍光波長帯の差を大きくすることができる。したがって、近紫外光源から発せられた光と、第２の光源によって粒子から発せられた蛍光とが重複する場合であっても、２つの光源からの光をフィルタ等を用いることによって正確に選別することができる。よって、撮像部や受光部に対してそれぞれ適切な波長の光を受光させることが可能となり、鮮明な粒子画像や正確な検出データを得ることができる。

【0009】

さらに、前記近紫外光源、第２の光源および粒子検出手段は、近紫外光源からの近紫外光の照射によって励起された粒子からの蛍光によって粒子が検出されないように構成されていることが好ましい。

このような構成とすることにより、近紫外光源の発光によって粒子検出手段に影響を及ぼすことがなく、粒子を正確に検出することができる。

【0010】

本発明の試料分析装置は、表示部と、前記撮像部によって撮像された粒子画像を前記表示部に表示させる表示制御手段と、をさらに備えていることが好ましい。また、前記表示制御手段は、前記撮像部によって粒子が撮像された場合に前記表示部にその粒子の粒子画像を表示させるように構成されていてもよい。

このような構成を採用することによって、撮像部によって撮像された粒子画像を、表示部を介して目視により確認することができる。

【0011】

本発明の試料分析装置は、前記フローセルと前記撮像部との間に配置され、かつ近紫外光を選別して透過させるように構成された光選別部をさらに備えることが好ましい。これによって、近紫外光源から照射された近紫外光以外の光を遮り、輪郭が鮮明に現れた粒子画像を確実に取得することができる。

【0012】

本発明の試料分析装置は、前記光源と前記フローセルとの間に配置された照明レンズ系と、前記フローセルと前記撮像部との間に配置された撮像レンズ系とを備えていてもよい。この場合、前記照明レンズ系の開口数が前記撮像レンズ系の開口数と同等又は前記開口数よりも小さく設定されていることが好ましい。

このような構成を採用することによって、粒子の輪郭部に生じる回折散乱縞の少ない鮮明な粒子画像を撮像することができる。

【発明の効果】

【0013】

本発明の試料分析装置によれば、輪郭が明瞭な粒子画像を取得することができる。

【発明を実施するための最良の形態】

【0014】

図１は、本発明の実施形態に係る試料分析装置の斜視図、図２は、試料分析装置の全体構成を示すブロック図である。本実施形態においては、試料分析装置１０として、尿中有形成分分析装置を例にとり説明する。

【0015】

〔試料分析装置の全体構成〕

図１に示すように、尿中有形成分分析装置（以下、単に分析装置という）１０は、搬送部１１と、試料の測定等を行う測定部１２と、この測定部１２に接続され、測定結果の分析等を行うシステム制御部１３とを備え、搬送部１１によって移送されたラック３１の試験管３２から試料としての尿を吸引し、この尿から有形成分や細菌等の粒子の情報を検出、分析するように構成されている。

【0016】

図２に示すように、分析装置１０の測定部１２は、試験管３２（図１）に収容された尿から試料を調製するための試料調製部１４と、試料中の有形成分や細菌等を検出し、検出

された粒子の特徴に応じた電気信号をアナログ信号処理回路１６へ出力するための光学検出部（粒子検出手段）１５と、光学検出部１５からの出力の増幅やフィルタ処理等を行うアナログ信号処理回路１６と、アナログ信号処理回路１６の出力をディジタル信号に変換するＡ／Ｄコンバータ１７と、ディジタル信号に対して所定の波形処理を行うディジタル信号処理回路１８と、ディジタル信号処理回路１８と接続されたマイクロコンピュータ２０と、マイクロコンピュータ２０に接続されたＬＡＮアダプタ２１とを備えている。システム制御部１３を構成するパソコン（ＰＣ）１３は、このＬＡＮアダプタ２１を介して測定部１２とＬＡＮ接続されており、このパソコン１３により、測定部１２で取得したデータの解析が行われる。アナログ信号処理回路１６、Ａ／Ｄコンバータ１７、及びディジタル信号処理回路１８は、光学検出部５が出力する電気信号を処理するための信号処理部２２を構成している。

【0017】

さらに、測定部１２は、ディジタル信号処理回路１８に接続された撮像制御手段２３と、この撮像制御手段２３によって試料から粒子（有形成分）を撮像するように制御される撮像系２４とを備えている。撮像制御手段２３は、ＣＰＵやＲＡＭ，ＲＯＭ等のメモリを有する前記マイクロコンピュータ２０の一機能部として構成されている。

【0018】

〔試料調製部の概略構成〕

図３は試料調製部１４及び光学検出部１５の構成を示す概略図である。試験管３２に入った尿（検体）は、吸引管２６を用いて図示しないシリンジポンプにより吸引され、試料調製部１４へ分注される。本実施形態における試料調製部１４は第１試料調製部１４ｕと第２試料調製部１４ｂとで構成されており、これら第１，第２試料調製部１４ｕ，１４ｂのそれぞれに尿の定量されたアリコートが分配される。

【0019】

第１試料調製部１４ｕの尿アリコートは、希釈液２７ｕと染色液（染色試薬）２８ｕが混合されて当該染色液２８ｕに含まれる色素により染色が施される。この染色試料は赤血球、白血球、上皮細胞、円柱等の比較的大きい尿中有形成分を分析するための懸濁液となる。

一方、第２試料調製部１４ｂの尿アリコートは、希釈液２７ｂと染色液（染色試薬）２８ｂが混合されて当該染色液２８ｂに含まれる色素により染色が施される。この染色試料は細菌を分析するための懸濁液となる。

【0020】

以上のようにして調製された２種類の懸濁液（試料）は、先に第１試料調製部１４ｕの懸濁液（第１の試料）が光学検出部１５に導かれ、シースフローセル３０においてシース液に包まれた細い流れを形成し、そこに、光源からの光が照射される。その後同様に、第２試料調製部１４ｂの懸濁液（第２の試料）が光学検出部１５に導かれ、シースフローセル３０において細い流れを形成し、光源からの光が照射される。以上のような各動作は、マイクロコンピュータ２０（制御部）の制御によって図示しない駆動部や電磁弁等を動作させることにより、自動的に行われる。

【0021】

〔光学検出部及び撮像系の構成〕

図４は、光学検出部１５及び撮像系２４の構成を示す模式図である。光学検出部１５は、半導体レーザからなる光源４１、光源４１の光をシースフローセル３０に集光するためのコンデンサレンズ４２、シースフローセル３０を流れる試料中の粒子からの前方散乱光を受光する第１受光部としてのフォトダイオード４３、この前方散乱光をフォトダイオード４３に集光するための集光レンズ４４を備えている。本実施形態の光源４１には、６４０ｎｍ付近にピーク波長を有する赤色光を発光する半導体レーザが用いられている。なお、光源４１として、前記半導体レーザに代えて、ガスレーザや発光ダイオードを用いることもできる。

【0022】

光学検出部１５は、シースフローセル３０を流れる試料中の粒子からの側方蛍光及び側方散乱光を平行光に変換するコリメータレンズ４５、この平行光を反射させる第１ダイクロイックミラー４６、第１ダイクロイックミラー４６によって反射された側方蛍光及び側方散乱光を集光する対物レンズ４７、対物レンズ４７を透過した側方蛍光をさらに透過し、側方散乱光を反射させる第２ダイクロイックミラー４８、第２ダイクロイックミラー４８を透過した側方蛍光を受光する第２受光部としてのフォトマルチプライヤ４９、第２ダイクロイックミラー４８で反射された側方散乱光を受光する第３受光部としてのフォトマルチプライヤ５０を備えている。

【0023】

光学検出部１５のフォトダイオード４３及びフォトマルチプライヤ４９，５０によって受光された光はそれぞれ電気信号に変換され、前方散乱光信号、側方散乱光信号、及び側方蛍光信号として信号処理部２２（図２参照）に入力され、信号処理部２２において所定の処理がなされた後、検出データ（検出信号）としてマイクロコンピュータ２０のメモリに格納される。また、この検出データは、ＬＡＮアダプタ２１を介してシステム制御部１３を構成するパソコンに送信され、パソコン１３において解析される。

【0024】

一方、撮像系２４は、図４に示すように、光学検出部１５の光源４１の光軸に対して上面視で９０度交差した光軸を有する光源６１を備えている。この光源６１は、光学検出部１５の光源４１よりも短い波長の光、具体的には３８５ｎｍ付近にピーク波長を有する光を発光する発光ダイオードが用いられている。また、撮像系２４の光源６１の光軸は、光学検出部１５の光源４１の光軸よりも試料流方向のやや下流側に配置されている。

なお、光源６１は近紫外域にピーク波長を有する光を発光する光源であればよく、例えば２００ｎｍ～４００ｎｍの間にピーク波長を有する光を発光する光源を用いることができる。

【0025】

光源６１はパルス発光するように制御され、好ましくは１００ｎ秒以下の発光時間で発光するように制御される。これにより、シースフローセル３０を流れる粒子を瞬間的に照射して撮像することができる。粒子を瞬間的に照射して撮像することには、次のような利点がある。すなわち、シースフローセル３０に向かって光を照射し続けると、フローセルを高速で流れる粒子が試料流の方向に伸びた像となって撮像されてしまうことがあるが、粒子を瞬間的に照射して撮像することで、実際の粒子の形状に近い、正確な粒子像を撮像することができる。

発光ダイオードをパルス発光させるための駆動回路としては、例えば特開２００５－２６９０５１号公報に記載の回路を用いることができる。

【0026】

さらに、撮像系２４は、光源６１の近紫外光をシースフローセル３０に集光する照明レンズ系６２と、シースフローセル３０を流れる試料中の粒子を撮像する撮像部としてのＣＣＤカメラ６４と、シースフローセル３０に照射された近紫外光をカメラ６４に入射させる撮像レンズ系６３とを備えている。

照明レンズ系６２は、コリメータレンズ６５及び集光レンズ６６を備え、コリメータレンズ６５は、光源６１の近紫外光を平行光に変換し、集光レンズ６６はこの平行光をシースフローセル３０に集光する。また、コリメータレンズ６５と集光レンズ６６との間には、照明レンズ系６２の開口数（ＮＡ）を所定に設定するアイリス（絞り部）６７が設けられている。本実施形態のアイリス６７は、照明レンズ系６２の開口数を０．４に設定するものとされている。

【0027】

撮像レンズ系６３は、コリメータレンズ４５と結像レンズ６９とを備えており、コリメータレンズ４５は、シースフローセル３０を透過した光を平行光に変換する。このコリメータレンズ４５は、光学検出部１５の光源４１による粒子からの側方散乱光及び側方蛍光を平行光に変換するものが兼用されている。

結像レンズ６９は、第１ダイクロイックミラー４６を透過した近紫外光を集光し、カメラ６４の受光面に結像する。

【0028】

また、コリメータレンズ４５と結像レンズ６９との間には、可視光を遮るカットフィルタ７０と、近紫外光のみを透過するバンドパスフィルタ（光選別部）７１とが設けられている。このカットフィルタ７０とバンドパスフィルタ７１とによってカメラ６４には確実に近紫外光が入射するようになっている。

【0029】

撮像レンズ系６３の開口数は、照明レンズ系６２の開口数よりも大きい値に設定されている。具体的に、照明レンズ系６２の開口数はアイリス６７によって０．４に設定されているので、撮像レンズ系６３の開口数はこれよりも大きい値、例えば０．５５に設定される。このように撮像レンズ系６３の開口数を照明レンズ系６２の開口数よりも大きい値に設定することによって、照明レンズ系６２を経てシースフローセル３０に照射された近紫外光は撮像レンズ系６３のコリメータレンズ４５や結像レンズ６９の中心部分のみを透過することになる。このため、各レンズ４５，６９の収差等の影響が小さくなり、ボケや歪み等の少ない良好な粒子画像を得ることが可能となる。さらに、粒子の輪郭部分の回折散乱縞が少なくなり、鮮明な粒子画像を得ることができる。

【0030】

光学検出部１５の光源４１は６４０ｎｍ付近にピーク波長を有する赤色光を照射し、撮像系２４の光源６１は３８５ｎｍ付近にピーク波長を有する光（近紫外光）を照射する。そして、光学検出部１５の光源４１による粒子からの側方蛍光及び側方散乱光は、撮像系２４の光源６１の近紫外光と平行な光となり、これらは互いに重複する。また、光学検出部１５の光源４１による粒子からの側方散乱光は、当該光源４１と同一のピーク波長（約６４０ｎｍ）を有するのに対して、側方蛍光は、これよりも長い波長（例えば約６６０～８００ｎｍ）となる。

【0031】

そのため、本実施形態では、第１ダイクロイックミラー４６として、波長が３７０～４００ｎｍの近紫外光を透過し、それ以外の波長の光を反射するものが用いられており、カメラ６４側には近紫外光以外の光が入射しないように構成されている。また、第２ダイクロイックミラー４８は、波長が６６０～８００ｎｍの光を透過し、それ以外の波長の光を反射するものが用いられており、フォトマルチプライヤ４９には、側方蛍光以外の光が入射しないように構成されている。

【0032】

なお、フォトマルチプライヤ５０には、３７０～４００ｎｍ及び６６０～８００ｎｍの波長を除く波長の光が入射する可能性があり、撮像系２４の光源６１から比較的波長の短い光（例えば、４００～４２０ｎｍ程度）が入射することも考えられるので、フォトマルチプライヤ５０の手前には、光学検出部１５の光源４１による粒子の側方散乱光のみを入射させるべく、約６４０ｎｍ波長付近の光を透過するバンドパスフィルタ５１を設けておくことが望ましい。

【0033】

光学検出部１５は、試料流中の粒子の前方散乱光、側方散乱光、及び側方蛍光を検出し、これらの検出信号は信号処理部２２を介してマイクロコンピュータ２０へ入力される。そして、マイクロコンピュータ２０の一機能部である撮像制御手段２３は、入力された検出信号に応じて粒子画像を撮像するように撮像系２４を制御している。

【0034】

一般に、光学検出部１５によって取得される前方散乱光、側方散乱光、及び側方蛍光は、その信号の強度によって赤血球、白血球、上皮細胞等の粒子の種類や大きさを判別できることが知られている。そして、本実施形態の撮像制御手段２３は、光学検出部１５の検出信号から粒子の種別や大きさを判別し、その粒子が予め定められた所定の粒子であると判断した場合に撮像系２４によって当該粒子を撮像し、所定の粒子以外の粒子であると判

断した場合には、その粒子は撮像しない（光源６１を発光しない）ように構成されている。

【0035】

なお、撮像系２４によって撮像された粒子画像のデータは、マイクロコンピュータ２０のメモリに格納される。また、粒子画像のデータは、ＬＡＮアダプタ２１を介してシステム制御部を構成するパソコン１３に送信され、当該パソコン１３において所定の処理がなされる。

【0036】

ここで、撮像系２４の光源６１、光学検出部１５の光源４１および光学検出部１５は、撮像系２４の光源６１からの近紫外光の照射によって励起された粒子からの蛍光によっては、粒子が検出されないように構成されている。具体的には、次のように構成されている。

光学検出部１５は、光源４１によって照射された粒子からの蛍光に基づいて、その粒子を検出可能な光量に対する閾値が予め設定されている。撮像系２４の光源６１は、光源６１が近紫外光を照射することによって励起された粒子からの蛍光の光量が上記閾値未満となるように、その光量が調整されている。

このような構成とすることにより、光源６１の発光によって光学検出部１５に影響を及ぼすことがなく、正確に粒子を検出することができる。

【0037】

〔システム制御部の構成〕

図５は、システム制御部１３のブロック図である。システム制御部１３は、パーソナルコンピュータ等よりなり、制御部本体７１と、表示部７２と、入力部７３とから主構成されている。制御部本体７１は、ＣＰＵ７１ａと、ＲＯＭ７１ｂと、ＲＡＭ７１ｃと、ハードディスク７１ｄと、読出装置７１ｅと、入出力インターフェース７１ｆと、画像出力インターフェース７１ｇと、通信インターフェース７１ｉから主構成されている。これらの間は、バス７１ｈによって通信可能に接続されている。

【0038】

ＣＰＵ７１ａは、ＲＯＭ７１ｂに記憶されているコンピュータプログラム及びＲＡＭ７１ｃにロードされたコンピュータプログラムを実行することが可能である。ＲＯＭ７１ｂは、マスクＲＯＭ、ＰＲＯＭ、ＥＰＲＯＭ、ＥＥＰＲＯＭ等によって構成されており、ＣＰＵ７１ａに実行されるコンピュータプログラム及びこれに用いるデータ等が格納されている。ＲＡＭ７１ｃは、ＳＲＡＭ又はＤＲＡＭなどによって構成されている。ＲＡＭ７１ｃは、ＲＯＭ７１ｂ及びハードディスク７１ｄに記録されているコンピュータプログラムの読み出しに用いられる。また、これらのコンピュータプログラムを実行するときに、ＣＰＵ７１ａの作業領域として利用される。

【0039】

ハードディスク７１ｄは、オペレーティングシステム及びアプリケーションプログラムなど、ＣＰＵ７１ａに実行させるための種々のコンピュータプログラム及びそのコンピュータプログラムの実行に用いるデータがインストールされている。例えば、ハードディスク７１ｄには、米マイクロソフト社が製造販売するWindows（登録商標）等のグラフィカルユーザーインターフェース環境を提供するオペレーティングシステムがインストールされている。

【0040】

また、ハードディスク７１ｄには、測定部１２への測定オーダ（動作命令）の送信、測定部１２で測定した測定結果の受信及び処理、処理した分析結果の表示等を行う操作プログラムがインストールされている。この操作プログラムは、当該オペレーティングシステム上で動作するものとしている。

【0041】

読出装置７１ｅは、フレキシブルディスクドライブ、ＣＤ－ＲＯＭドライブ、又はＤＶＤ－ＲＯＭドライブなどによって構成されており、可搬型記録媒体に記録されたコンピュ

ータプログラム又はデータを読み出すことができる。入出力インターフェース７１ｆは、例えば、ＵＳＢ、ＩＥＥＥ１３９４、ＲＳ－２３２Ｃなどのシリアルインタフェース、ＳＣＳＩ、ＩＤＥ、ＩＥＥＥ１２８４などのパラレルインタフェース、及びＤ／Ａ変換器、Ａ／Ｄ変換器などからなるアナログインタフェースなどから構成されている。入出力インターフェース７１ｆには、キーボード及びマウスからなる入力部７３やプリンタ等の出力デバイス７４が接続されている。そして、ユーザーが入力部２９を使用することにより、パーソナルコンピュータにデータを入力することが可能である。通信インターフェース７１ｉは測定部１２と接続されており、測定部１２との間でデータ等の送受信を行うことが可能である。

【0042】

画像出力インターフェース７１ｇは、ＬＣＤ又はＣＲＴなどで構成された表示部７２に接続されており、ＣＰＵ７１ａから与えられた画像データに応じた映像信号を表示部２８に出力するようになっている。表示部２８は、入力された映像信号にしたがって、画像（画面）を表示する。

【0043】

システム制御部１３には、測定部１２の信号処理部２２で処理された前方散乱光信号、側方散乱光信号、及び側方蛍光信号がマイクロコンピュータ２０によってＬＡＮアダプタ２１を介して送信される。システム制御部１３では、前方散乱光信号、側方散乱光信号、及び側方蛍光信号に基づいて、試料中の粒子が赤血球、白血球、上皮細胞、円柱、細菌等に分類される。また、システム制御部１３は、尿中の粒子（有形成分）を分析するためのスキャッタグラムを作成し、このスキャッタグラムは表示部７２に表示される。また、システム制御部１３には、撮像系２４により撮像された所定の粒子の画像データがマイクロコンピュータ２０によってＬＡＮアダプタ２１を介して送信され、この画像データはシステム制御部１３の表示部７２に表示される。

【0044】

〔粒子の撮像処理手順〕

以下、撮像系２４によって所定の粒子（本実施形態では変形赤血球）を撮像するための手順について説明する。図６は、当該手順を示すフローチャートである。

図６のステップＳ１では、システム制御部（ＰＣ）１３によって、測定開始の指示が受け付けられたか否かが判断される。この測定開始の指示は、オペレータが入力部７３（図５参照）を構成するキーボードやマウスを操作することによって行われる。システム制御部１３は、測定開始の指示が受け付けられたと判断すると（Ｙｅｓ）、測定部１２のマイクロコンピュータ２０に対して測定開始信号を送信する（ステップＳ２）。

【0045】

測定部１２のマイクロコンピュータ（制御部）２０は、システム制御部１３から送信された測定開始信号が受信されたか否かを判断する（ステップＳ１１）。マイクロコンピュータ２０は、測定開始信号が受信されたと判断すると（Ｙｅｓ）、試料を調製してシースフローセル３０に試料流を形成させるように試料調製部１４を制御する（ステップＳ１２，Ｓ１３）。

【0046】

ついで、ステップＳ１４において、測定部１２のマイクロコンピュータ２０は、光源４１によってシースフローセル３０に光を照射し、フォトダイオード４３及びフォトマルチプライヤ４９，５０によって試料流中の粒子からの前方散乱光、側方散乱光、側方蛍光を受光するように光学検出部１５を制御する。フォトダイオード４３及びフォトマルチプライヤ４９，５０によって光が受光されると、受光された光に基づく電気信号が信号処理部２２によって処理される。信号処理部２２によって処理された電気信号は、検出信号としてマイクロコンピュータ２０に入力され、メモリに格納される。マイクロコンピュータ２０は、撮像制御手段２３によって、当該検出信号が所定の粒子、すなわち変形赤血球の検出信号であるか否かを判断する（ステップＳ１５）。具体的には、マイクロコンピュータ２０は、光学検出部１５によって検出された粒子に基づく検出信号が赤血球に特有の信号

強度であり、かつ正常な赤血球よりも小さい粒子からの信号であると判断した場合に、その検出信号が変形赤血球から得られた信号であると判断する。

そして、マイクロコンピュータ２０は、当該検出信号が変形赤血球の検出信号であると判断した場合（Ｙｅｓ）にステップＳ１６へ処理を進め、変形赤血球の検出信号ではないと判断した場合（Ｎｏ）にステップＳ１７へ処理を進める。

【0047】

ステップＳ１６において、マイクロコンピュータ２０は、粒子画像を撮像するように撮像系２４（光源６１，カメラ６４）を制御する。具体的には、マイクロコンピュータ２０は、撮像系２４の光源６１を発光する制御を実行し、撮像系２４の光源６１が発光したタイミングでカメラ６４によってフローセル中の画像を取り込む制御を実行する。そして、カメラ６４によって撮像された粒子画像のデータはマイクロコンピュータ２０のメモリに格納される。

【0048】

ステップＳ１７において、測定部１２のマイクロコンピュータ２０によってシースフローセル３０内の試料流が終了しているか否かが判断され、マイクロコンピュータ２０は、シースフローセル３０内の試料流が終了していないと判断した場合（Ｎｏ）にはステップＳ１４に処理を戻し、シースフローセル３０内の試料流が終了していると判断した場合（Ｙｅｓ）にはステップＳ１８へ処理を進める。

【0049】

ステップＳ１８において、測定部１２のマイクロコンピュータ２０は、メモリに格納してある光学検出部１５による検出信号のデータや撮像系２４による粒子画像のデータをシステム制御部１３に送信し、処理を終了する。

ステップＳ３において、システム制御部１３の制御部本体７１によって、測定部１２からのデータが受信されたか否かが判断され、システム制御部１３の制御部本体７１は、データが受信されたと判断すると（Ｙｅｓ）、ステップＳ４へ処理を進める。

【0050】

ステップＳ４において、システム制御部１３は、受信したデータの解析を行う。そして、システム制御部１３は、測定部１２から送信されたデータの中に粒子画像のデータが含まれているか否かを判断し（ステップＳ５）、粒子画像データが含まれていると判断した場合（Ｙｅｓ）には、その粒子画像を表示部７２に表示させ（ステップＳ６）、処理を終了する。

【0051】

以上説明したように、本実施形態では、撮像系２４の光源６１として、近紫外光源が用いられている。近紫外光は、可視光や近赤外光に比べて波長が短いので、粒子に対する透過性が低く、散乱効率が高い。したがって、近紫外光が照射された粒子の部分は近紫外光が強く散乱してカメラ６４には暗く撮像され、粒子の周囲では近紫外光が散乱しないので明るく撮像される。この作用によって粒子とその背景とのコントラストが明瞭で、粒子の輪郭が鮮明に現れた画像を得ることができる。

【0052】

また、例えば光源として近赤外光を用いた場合には、光学分解能を上げるために高開口数のレンズを使用する必要があり、焦点深度が浅くなるため、焦点深度内に粒子が収まるようにフローセルを狭窄化しなければならず、単位時間あたりに処理できるサンプル量が少なくなってしまう。

この点、本実施形態では、近赤外光より波長の短い近紫外光を用いたことにより、高開口数のレンズを使用したとしても十分な焦点深度をとることができ、フローセルを狭める必要がない。

【0053】

一般に、血尿、特に糸球体性の血尿に含まれる赤血球には変形が見られ、その変形の態様もこぶ状、ねじれ状等多様である。そのため、試料としての尿に含まれる変形赤血球を判別するには、当該赤血球の輪郭を正確に捉えることが要求される。この点、本実施形態

のように撮像系２４の光源６１として近紫外光源を用いれば、撮像された粒子が変形赤血球であるか否かという判断や、変形赤血球の形態の判断を正確に行うことが可能となり、粒子画像を腎臓疾患等の診断に有効に役立てることができる。

特に、変形赤血球のうち、脱ヘモグロビン化された赤血球（ゴースト赤血球）は、その粒子像のコントラストが現れにくい。そこで、このようなゴースト赤血球を撮像する場合に、光源として近紫外光を用いた本実施形態の試料分析装置を用いることで、粒子の輪郭が明確なゴースト赤血球の粒子画像を得ることができる。

【0054】

なお、光学検出部１５の光源４１には赤色光源が用いられているが、この光源４１の光が照射された粒子によって励起される側方蛍光は、当該光源４１の光よりも波長が長くなる。このため、本実施形態では、撮像系２４の光源６１として光学検出部１５の光源４１よりも波長の短い近紫外光源を用いることによって、撮像系２４の光源６１の近紫外光と、光学検出部１５の光源４１による粒子の側方蛍光との波長の差が大きくなるように構成してある。これにより、図４に示すように、撮像系２４の光源６１の近紫外光と、粒子の側方蛍光とは互いに平行な重複する光となるが、これらの波長の差が大きいために、第１ダイクロイックミラー４６を用いて適切に選別することができる。

【0055】

本発明は上記実施の形態に限定されるものではなく適宜設計変更可能である。

例えば、本発明の試料分析装置は、尿中有形成分分析装置に限らず、血液分析装置等の他の試料分析装置にも適用することができる。また、撮像系によって撮像する粒子は赤血球に限定されるものではなく、白血球や上皮細胞等、近紫外光源を用いて撮像することが可能なあらゆる粒子を対象とすることができる。撮像系の光源は、発光ダイオードに限らず半導体レーザを用いることも可能である。

【図面の簡単な説明】

【0056】

【図1】本発明の実施形態に係る尿中有形成分分析装置（試料分析装置）を示す斜視図である。

【図2】尿中有形成分分析装置の全体構成を示すブロック図である。

【図3】試料調製部の概略構成図である。

【図4】光学検出部及び撮像系の構成を示す模式図である。

【図5】システム制御部のブロック図である。

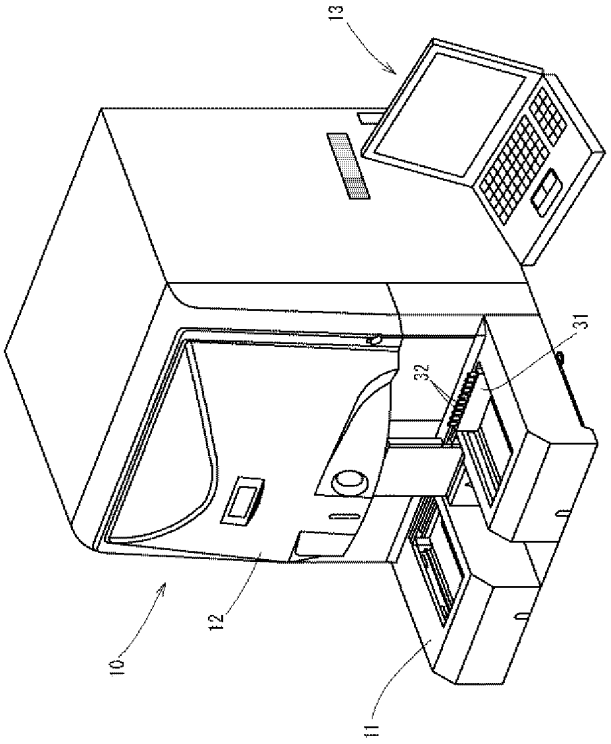
【図6】粒子画像の撮像処理手順を示すフローチャートである。

【符号の説明】

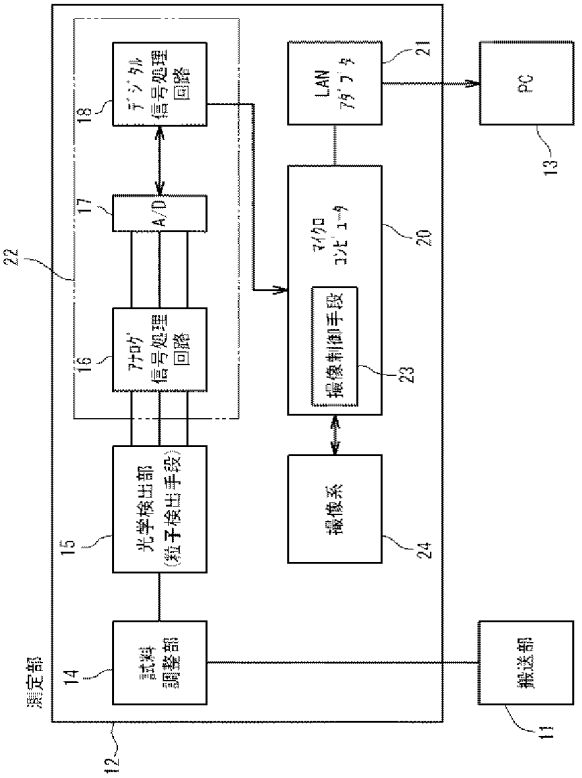
【0057】

１０　試料分析装置
１２　測定部
１３　システム制御部
１５　光学検出部
２０　マイクロコンピュータ
２３　撮像制御手段
２４　撮像系
３０　シースフローセル
４１　光源（第２の光源）
４９　フォトマルチプライヤ（受光部）
６１　光源
６２　照明レンズ系
６３　撮像レンズ系
６４　ＣＣＤカメラ（撮像部）

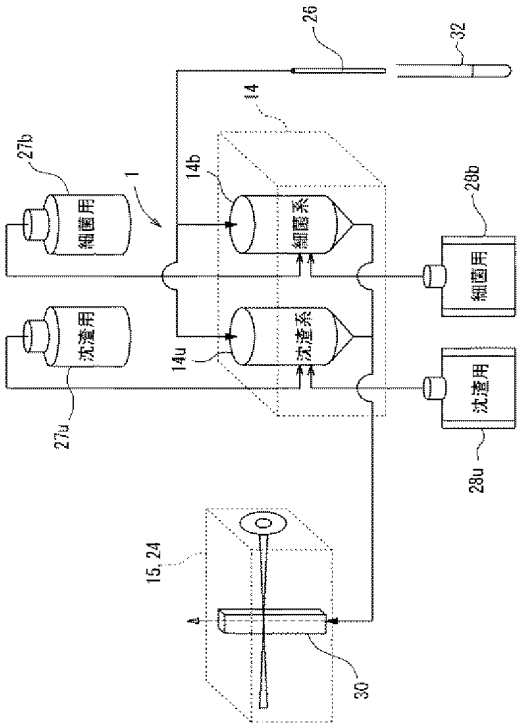
【図1】


13
31
32
10
12
11

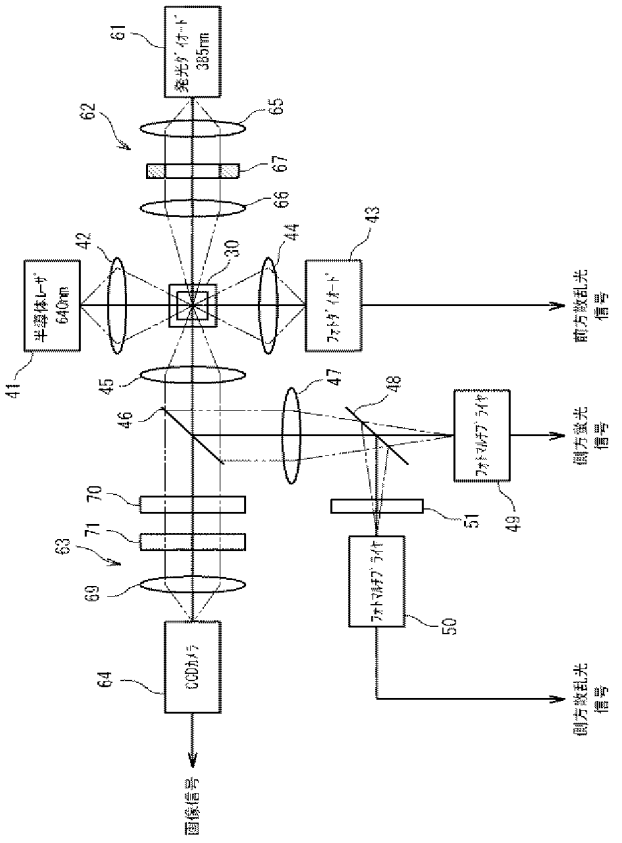
【図2】


測定部
試料調整部
光学検出部(粒子検出手段)
信号処理回路
A/D
デジタル信号処理回路
撮像制御手段
撮像系
LAN
PC
搬送部
14
15
16
17
18
20
21
22
23
24
11
12
13

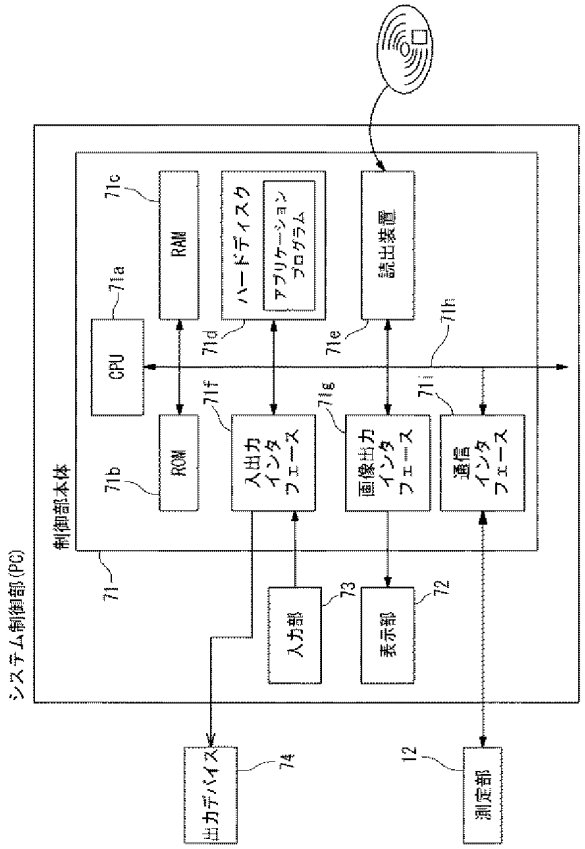
【図3】


細菌用
洗滌用
細胞系
洗滌系
細菌用
洗滌用
26
32
14
14b
14u
27b
27u
28b
28u
1
15, 24
30
A

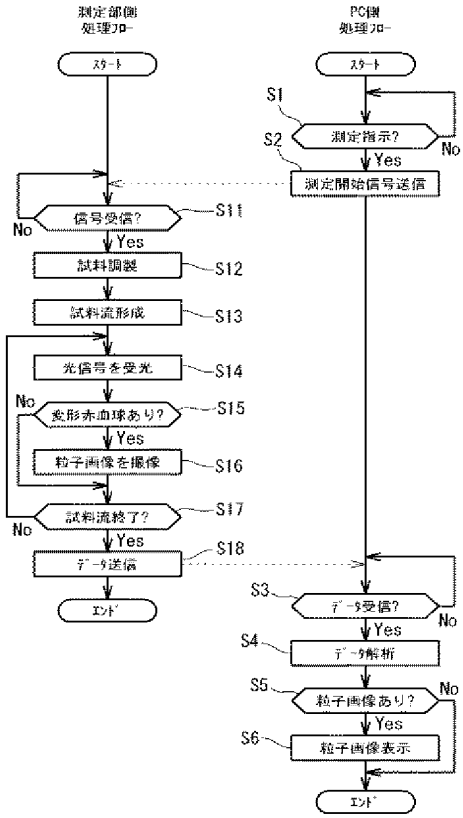
【図4】


発光ダイオード 385nm
半導体レーザ 640nm
フォトダイオード
フォトマルチプライヤ
フォトマルチプライヤ
CCDカメラ
前方散乱光信号
側方蛍光信号
側方散乱光信号
画像信号
41
42
43
44
45
46
47
48
49
50
51
61
62
63
64
65
66
67
69
70
71

【図5】


システム制御部(PC)
制御部本体
71
71a
CPU
71b
ROM
71c
RAM
71d
ハードディスク
アプリケーション
プログラム
71e
読出装置
71h
71f
入出力
インタ
フェース
71g
画像出力
インタ
フェース
71i
通信
インタ
フェース
73
入力部
72
表示部
74
出力デバイス
12
測定部

【図6】


測定部側
処理フロー
PC側
処理フロー
スタート
スタート
S1
S2
測定指示?
No
Yes
測定開始信号送信
No
信号受信?
S11
Yes
試料調製
S12
試料流形成
S13
光信号を受光
S14
No
変形赤血球あり?
S15
Yes
粒子画像を撮像
S16
試料流終了?
S17
No
Yes
データ送信
S18
エンド
S3
データ受信?
No
Yes
S4
データ解析
S5
粒子画像あり?
No
Yes
S6
粒子画像表示
エンド

Ｆターム(参考)　2G059　AA05　BB13　BB14　CC16　DD12　EE01　EE02　EE07　EE11　FF01
　　FF03　FF12　GG01　GG02　GG03　GG08　HH02　HH03　HH06　JJ02
　　JJ07　JJ11　KK02　KK03　KK04　MM10　PP04

Espacenet

Bibliographic data: JPH03197641 (A) — 1991-08-29

LEAD FRAME MATERIAL

Inventor(s): ETO MASATOSHI; YUKI NORIO ± (ETO MASATOSHI, ; YUKI NORIO)

Applicant(s): NIPPON MINING CO ± (NIPPON MINING CO LTD)

Classification: **- international:** ***C22C19/05; C22C38/00; C22C38/10; C22C38/58; H01L23/48;*** (IPC1-7): C22C19/05; C22C38/00; C22C38/10; C22C38/58; H01L23/48

- cooperative:

Application number: JP19890337768 19891226

Priority number (s): JP19890337768 19891226

Abstract of JPH03197641 (A)

PURPOSE:To obtain a high strength lead frame material excellent in etching characteristic and sealing characteristic by specifying a composition consisting of C, Si, P, S, O, N, Co, Ni, elements for improving strength and thermal expansion coefficient, such as Cu, and Fe. CONSTITUTION:A lead frame material having high strength and hardness and excellent in etching characteristic, sealing characteristic, and formability can be obtained by providing a composition which consists of, by weight, <=0.015%, preferably <=0.005%, of C, 0.001-0.15%, preferably 0.001-0.05%, of Si, <=0.01%, preferably <=0.003%, of P, <=0.005% S, <=0.010% O, <=0.005% N, 5-30% Co, 15-55% Ni, further 0.01-5.0% of one or more elements among Cu, Mn, Cr, Mo, W, V, Nb, Ta, Ti, Zr, Hf, B, Be, Mg, and Ca, and the balance Fe with inevitable impurities and also providing a structure in which crystalline grain size at the time of the final annealing is regulated, preferably, to <=50mum.

⑲日本国特許庁（JP）　⑪特許出願公開

⑫公開特許公報（A）　平3－197641

⑤1Int.Cl.[5]	識別記号	庁内整理番号
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19/05	J	8928－4K
38/10		
38/58		
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⑬公開　平成3年(1991)8月29日

審査請求　未請求　請求項の数 5　（全6頁）

⑤4発明の名称　リードフレーム材

⑳特　願　平1－337768
㉒出　願　平1(1989)12月26日

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⑦1出願人　日本鉱業株式会社　東京都港区虎ノ門2丁目10番1号
⑦4代理人　弁理士　今井　毅

明　細　書

1．発明の名称

リードフレーム材

2．特許請求の範囲

(1)　重量割合にて

C：0.015％以下，　Si：0.001～0.15％，

P：0.01％以下，　S：0.005％以下，

O：0.010％以下，　N：0.005％以下，

Co：5～30％，　Ni：15～55％

であって、更にCu，Mn，Cr，Mo，W，V，Nb，Ta，Ti，Zr，Hf，B，Be，Mg及びCaの1種以上をも合計で0.01～5.0％含むと共に 残部がFe及び不可避的不純物から成ることを特徴とする、エッチング加工性及び封着性に優れた高強度リードフレーム材。

(2)　C含有量が0.005重量％以下である、請求項1に記載のリードフレーム材。

(3)　Si含有量が0.001～0.05重量％である、請求項1又は2に記載のリードフレーム材。

(4)　P含有量が0.003重量％以下である、請求項1乃至3の何れかに記載のリードフレーム材。

(5)　最終焼鈍時の結晶粒径が50㎛以下である、請求項1乃至4の何れかに記載のリードフレーム材。

3．発明の詳細な説明

〈産業上の利用分野〉

この発明は、エッチング加工性，封着性並びに成形加工性に優れ、かつ強度の高いリードフレーム材に関するものである。

〈背景技術とその課題〉

一般に、半導体機器類においてはリード材の特性もその性能やコストに大きな影響を及ぼすことが知られているが、このような半導体機器のリード材としては、従来から、熱膨張係数が低く、かつ半導体素子やセラミックスと比較的良好な接着性，封着性を示すFe－Ni系合金が好んで使用されてきた。

ところが、最近、一段と加速の度合を増してきたＬＳＩの高集積化傾向は使用するリードフレームの多ピン化を更に推進する結果をもたらしているが、リードフレームの多ピン化には素材厚の薄い方が有利であるため、薄板化に対応できるように従来にも増して強度及び硬度の高いリードフレーム材が要求されるようになった。

また、リードフレームが多ピン化されると必然的にピン間隔が狭くなり、ピン自体の幅も小さくなるが、それに対処するには精度の一層高いエッチング加工が必要となる。そのため、多ピン用に供されるリードフレーム材では、高強度や高硬度の他に、形成されるピン幅やピン間隔の制御性につながる"エッチング加工性"が優れていることも重要な要求特性となっていた。

ところで、Fe－Ni系合金製リードフレーム材のエッチング加工工程は、一般に、脱脂したリードフレーム材の両面にフォトレジストを塗布しパターンを焼き付けて現像した後、塩化第２鉄を主成分とするエッチング液でエッチング加工し、その後前記レジストを除去する工程から構成されているのが普通である。そして、この際のエッチング性を決める要因としては"レジストの密着性"や"エッチング速度"等が挙げられるが、これらの中でも素材のエッチング速度が最も重要な因子となっており、エッチング速度が速くなるにつれてリードフレーム材に形成されるピン幅，ピン間隔の制御性が容易化することから、該エッチング速度によってエッチング加工性の評価が概ね決定されてしまうと言っても過言ではなかった。

従って、半導体機器の集積度が上昇するに伴い、リードフレーム材には強度特性に加えて"より速いエッチング速度(即ち良好なエッチング加工性)"特性も求められてきた訳であるが、未だ十分に満足できるエッチング加工性，強度及び硬度、更には封着性並びに成形加工性等を兼備した材料が見出されていないのが現状であった。

このようなことから、本発明の目的は、強度及び硬度が高く、しかも 優れたエッチング加工性，封着性並びに成形加工性をも併せ持つところの、集積度の高い半導体機器への適用を意図した場合でも十分な性能を発揮し得るリードフレーム材を工業的に安定して提供し得る手段を確立することに置かれた。

（課題を解決するための手段）

本発明者等は、上記目的を達成すべく、特にFe－Ni－Co系合金リードフレーム材が有する比較的高い強度や低い熱膨張係数等に着目して、これらの特性を更に向上させると共に、そのエッチング加工性や成形加工性を顕著に改善することの可能性を探りながら研究を重ねた結果、次のような新しい知見を得ることができた。即ち、

(a) Ni及びCoを主要成分としたFe合金において、そのＣ，Si及びＰの含有量を、更にはＮ含有量をも特定の低い値に制限した場合には、該合金のエッチング速度が顕著に改善されるようになる，

(b) しかも、上記合金に幾つかの選ばれた特定の元素の１種又は２種以上を所定の割合で含有させた場合には、リードフレーム材としての所要特性に格別な悪影響を及ぼすことなく材料の強度を効果的に向上することができる上、Ni及びCo含有量の注意深い調整の下での上記特定元素の添加は、その熱膨張係数をモールドレジンや半導体チップのそれに近付けるのに極めて有効な手段となる，

(c) また、上記合金材料においてもその結晶粒径が強度及び成形加工性に少なからぬ影響を及ぼすが、該結晶粒径を特定値以下に抑える手立てを講じることによってリードフレームの多ピン化にとって好ましい"材料強度の更なる向上"が期待できる上、成形加工性も改善される，

(d) 従って、Fe－Ni－Coを基本成分とした合金におけるNi，Co，Ｃ，Si及びＰ等の含有量を総合的に調整すると同時に、これに特定合金元素の添加を行うか、或いは更に結晶粒径調整をも実施すると、強度，熱膨張係数，封着性，成形加工性等の特性に優れ、しかも非常に良好なエッチング加工性をも備えたリードフレーム材の実現が可能となる。

本発明は、上記知見事項等を基にして完成されたものであり、

「リードフレーム材を、

C：0.015％以下（以降、成分含有割合は重量％とする），

Si：0.001～0.15％，　P：0.01％以下，

S：0.005％以下，　O：0.010％以下，

N：0.005％以下，　Co：5～30％，

Ni：15～55％

であって、更にCu，Mn，Cr，Mo，W，V，Nb，Ta，Ti，Zr，Hf，B，Be，Mg及びCaの1種以上をも合計で0.01～5.0％含むと共に 残部がFe及び不可避的不純物から成る成分組成に構成することにより、優れたエッチング加工性，封着性及び成形加工性と、高い強度，硬度とを兼備せしめた点」

に特徴を有している。

なお、上記本発明に係るリードフレーム材において、

a）C含有量を0.005％以下とする，

b）Si含有量を0.001～0.05％に調整する，

c）P含有量を0.003％以下とする，

なる条件を単独で、或いは組み合わせて採用すれば、得られるエッチング加工性改善効果は特に顕著となり、また

d）最終焼鈍時（最終焼鈍終了時）の結晶粒径を50㎛以下に調整する，

との手立てによって強度や成形性が一層向上して安定化することから、必要に応じてこれらの1つ又は幾つかを適用することにより多ピンリードフレームの製造にも十分な対応が可能である。

次に、本発明において、リードフレーム材の成分組成を前記の如くに限定した理由を各成分の作用と共に詳述する。

（作用）

Ni

Niはリードフレーム材の熱膨張係数を決定するのに重要な成分であり、封着時や封着後におけるパッケージとの熱膨張差を小さくして優れた封着性，耐湿信頼性を確保するためには、Ni含有量を15～55％に調整する必要がある。また、Niにはリードフレーム材の強度及び硬度を向上させる作用もあるが、Ni含有量が15％未満では所望強度，硬度の確保が困難となる。従って、Ni含有量は15～55％と定めた。

Co

Coもリードフレーム材の熱膨張係数を決定するのに重要な成分であると同時に、材料の強度及び硬度を向上させるのに有効な成分でもある。しかし、Co含有量が5％未満であると熱膨張係数が所望値を超えて大きくなるばかりでなく、リードフレーム材の所望強度，硬度の確保も困難となる。一方、30％を超えてCoを含有させても熱膨張係数が所望値を超えて大きくなってしまう。従って、Co含有量は5～30％と定めた。

C

リードフレーム材中のC含有量が 0.015％を超えると鉄炭化物の生成が起こり、これがリードフレーム材のエッチング加工性を害する。従って、C含有量の上限を 0.015％と定めたが、固溶Cもエッチング加工性に悪影響を与えることからC含有量は低いほど良く、出来れば 0.005％以下にまで抑制するのが望ましい。

Si

Siは脱酸材として必要な元素であるが、一方でリードフレーム材のエッチング加工性に大きな影響を及ぼす元素でもある。即ち、Si含有量が増加するとエッチング速度が遅くなってエッチング加工性を悪化する。このため、良好なエッチング加工性を確保するにはSi含有量を0.15％以下に調整する必要がある。特に、多ピンタイプのリードフレーム材の場合には一段と良好なエッチング加工性が要求されることから、Si含有量は好ましくは0.05％以下にまで低減するのが良い。ただ、Si含有量を0.001％未満の領域にまで低減すると 脱酸効果が認められなくなってしまう。従って、Si含有量は0.001～0.15％と定めたが、上述したように 出来れば0.001～0.05％に調整するのが好ましい。

P

Pも、Siと同様、過剰に含有させるとリードフレーム材のエッチング加工性に害を与える元素である。そして、上記エッチング加工性への悪影響

はP含有量が0.01%を超えるとより顕著化することから、P含有量は0.01%以下と定めた。しかし、P含有量を0.003%以下にまで低減すると エッチング加工性改善効果が一層顕著となるため、望ましくは0.003%以下に調整するのが良い。

S

S含有量が0.005%を超えると リードフレーム材中に硫化物系介在物が多くなり、エッチング加工時の欠陥となってピン折れ等を引き起こすようになる。従って、S含有量は 0.005%以下と限定した。

O

O含有量が0.010%を超えると リードフレーム材中に酸化物系介在物が多くなり、やはりエッチング加工時の穿孔欠陥となることから、O含有量を 0.010%と限定した。

N

N含有量が0.005%を超えても リードフレーム材のエッチング加工性が悪化することから、N含有量の上限を0.005%と定めた。

Cu, Mn, Cr, Mo, W, V, Nb, Ta, Ti, Zr, Hf, B, Be, Mg及びCa

これらの元素は何れもリードフレーム材の強度や熱膨張係数を上昇させる作用を有しているため、材料強度の向上、並びに熱膨張係数を上げてレジンモールドのそれに近付けることで封着性をより改善する目的で1種又は2種以上が含有せしめられる。しかし、それらの含有量が合計で0.01%未満であると前記作用による所望の効果が得られず、一方、合計の含有量が5.0%を超えた場合には 材料が硬くなり過ぎて成形加工性の悪化を招く他、適正な熱膨張係数の確保も困難となることから、上記成分の含有量を 合計量で0.01～5.0%と定めた。

なお、先にも説明したように、最終焼鈍を施した際の結晶粒径を50㎛以下に制御することは強度と成形加工性の効果的な改善につながることから、好ましくは最終焼鈍時の結晶粒径を50㎛以下に調整する手立てを講じるのが良い。ここで、結晶粒度の制御は、焼鈍前の加工度，焼鈍温度，焼鈍時間等の調整によって可能であることは言うまでもない。

続いて、本発明の効果を実施例により更に具体的に説明する。

（実施例）

まず、真空溶解により調整した材料を鋳造し、熱間圧延及び酸洗の後に冷間圧延と焼鈍を繰り返すと共に、最終焼鈍後に加工度：40%の冷間圧延を施し、更に500℃で1時間の歪取り焼鈍を施して第1表に示す如き成分組成の冷延板(板厚:0.15㎜)を製造した。

次に、これらの冷延板を脱脂後、レジスト膜を塗布し所定パターンを焼き付けて現像してから、塩化第2鉄溶液を用いたエッチング加工により何れも同一条件の下で128ピンのリードフレームを作成した。

そして、得られたリードフレームにつき、「エッチング性」の評価として“エッチング加工後のアウターリードピン幅とそのバラツキ”を、「機械的特性」の評価として“引張強さ”，“ビッカー

第 1 表の1

材料種別		化学成分 (重量%) C	Si	P	S	O	N	Ni	Co	他の添加元素	Fe及び不純物	結晶粒径 (μm)	エッチング性 ピン幅平均値 (μm)	ピン幅標準偏差 (μm)	機械的性質 硬さ〔Hv〕	加工性 (曲げ性)	封着性
本発明材	1	0.001	0.03	0.003	0.002	0.004	0.002	29.3	16.8	Cu : 4.36	残	35	403	2	255	○	○
	2	0.003	0.03	0.002	0.003	0.002	0.003	29.5	17.1	Nb : 3.21	残	20	401	3	249	○	○
	3	0.004	0.02	0.001	0.004	0.004	0.002	28.3	17.4	Mn : 4.29	残	19	407	3	240	○	○
	4	0.005	0.02	0.003	0.001	0.005	0.004	29.3	17.4	Cr : 3.15	残	22	405	3	252	○	○
	5	0.001	0.04	0.002	0.002	0.002	0.002	28.4	17.0	Mo : 4.74	残	33	404	4	245	○	○
	6	0.003	0.05	0.002	0.004	0.006	0.002	29.8	17.8	W : 3.21	残	34	405	5	239	○	○
	7	0.002	0.04	0.001	0.002	0.007	0.004	28.5	16.9	V : 2.65	残	20	407	8	250	○	○
	8	0.003	0.03	0.003	0.005	0.005	0.003	29.9	17.5	Nb : 3.46	残	15	405	6	262	○	○
	9	0.004	0.02	0.002	0.003	0.004	0.005	29.7	16.8	Ta : 3.30	残	26	406	4	260	○	○
	10	0.003	0.02	0.002	0.002	0.007	0.002	28.2	17.2	Ti : 2.74	残	29	409	4	255	○	○
	11	0.004	0.03	0.003	0.003	0.005	0.003	29.1	17.4	Zr : 1.45	残	24	407	6	246	○	○
	12	0.003	0.01	0.001	0.001	0.009	0.002	28.6	16.7	Hf : 3.29	残	26	408	7	248	○	○
	13	0.001	0.01	0.002	0.002	0.004	0.001	29.6	16.9	B : 0.95	残	24	404	4	255	○	○
	14	0.004	0.04	0.001	0.002	0.002	0.003	29.5	17.0	Be : 0.44	残	22	403	2	254	○	○
	15	0.003	0.03	0.001	0.001	0.004	0.002	30.8	17.2	Mg : 0.68	残	30	406	7	242	○	○
	16	0.002	0.03	0.003	0.001	0.004	0.004	27.2	16.8	Ca : 0.45	残	31	407	8	239	○	○
	17	0.003	0.05	0.002	0.002	0.002	0.001	27.8	16.7	Ti : 0.87, Mo : 3.32	残	20	408	7	259	○	○
	18	0.002	0.04	0.003	0.004	0.002	0.001	29.6	17.0	Zr : 0.38, Nb : 1.39	残	22	406	6	242	○	○

(注) 「加工性」及び「封着性」の評価結果は、○…良好, ×…不良, でそれぞれ表示した。

第 1 表の2

材料種別		化学成分 (重量%) C	Si	P	S	O	N	Ni	Co	他の添加元素	Fe及び不純物	結晶粒径 (μm)	エッチング性 ピン幅平均値 (μm)	ピン幅標準偏差 (μm)	機械的性質 硬さ〔Hv〕	加工性 (曲げ性)	封着性
本発明材	19	0.013	0.03	0.002	0.002	0.009	0.004	30.4	16.8	Ti : 3.13	残	18	410	9	261	○	○
	20	0.003	0.14	0.002	0.003	0.007	0.003	30.3	16.7	Zr : 2.18	残	20	413	10	256	○	○
	21	0.008	0.04	0.001	0.003	0.003	0.005	28.8	17.4	Mo : 4.48	残	34	411	12	247	○	○
	22	0.006	0.02	0.009	0.004	0.004	0.003	29.2	17.2	Nb : 3.40	残	31	413	9	242	○	○
	23	0.010	0.10	0.004	0.002	0.003	0.001	29.6	17.4	Nb : 4.31	残	35	414	7	235	○	○
	24	0.002	0.03	0.009	0.003	0.004	0.003	30.0	17.9	Cr : 4.28	残	42	415	10	239	○	○
	25	0.004	0.12	0.003	0.004	0.002	0.002	29.2	17.2	Zr : 0.38, Nb : 1.42	残	20	412	10	243	○	○
	26	0.003	0.03	0.002	0.003	0.002	0.003	29.5	17.1	Nb : 3.21	残	52	403	3	231	○	○
比較材	27	0.005	0.05	0.001	0.004	0.003	0.003	28.5	16.7	* —	残	20	405	8	204	○	×
	28	*0.022	0.04	0.001	0.002	0.004	0.002	29.5	16.8	Ti : 2.84	残	22	437	21	243	○	○
	29	0.005	*0.27	0.002	0.002	0.002	0.002	30.2	17.0	Zr : 1.50	残	19	428	18	245	○	○
	30	0.004	0.04	*0.014	0.003	0.004	0.003	30.5	17.4	Nb : 3.51	残	28	424	14	235	○	○
	31	0.003	0.10	0.005	*0.012	0.005	0.005	29.3	17.1	Mo : 4.65	残	33	408	8	244	×	○
	32	0.006	0.04	0.009	0.005	0.002	0.003	30.5	17.4	* Ti : 3.42, Cr : 4.55	残	29	438	20	264	×	○
	33	0.004	0.05	0.005	0.002	0.003	0.002	28.7	16.9	* W : 4.30, Cr : 3.32	残	39	425	17	275	×	○
	34	0.002	0.02	0.004	0.002	*0.011	0.003	30.5	16.7	Nb : 3.36	残	105	407	6	218	○	○
	35	0.004	0.03	0.003	0.004	0.004	0.005	29.7	17.0	* Nb : 0.003	残	30	404	6	205	○	○

(注1) *印は、本発明で規定する条件から外れていることを示している。

(注2) 「加工性」及び「封着性」の評価結果は、○…良好, ×…不良, でそれぞれ表示した。

ス硬さ"及び"加工性(曲げ性)"を、また「封着性」の評価として 樹脂封着後に熱サイクル(80℃×60min×1回)を付与した際のクラック発生の有無を それぞれ調査した。なお、前記加工性(曲げ性)の評価は、90度繰り返し曲げ試験（曲げ半径：0.15㎜）に従って実施した。

これらの調査結果を、最終焼鈍時の結晶粒径と共に第1表に併記した。

第1表に示される結果からも明らかなように、本発明材では何れも十分な強度と硬度を有し、かつ優れたエッチング性，成形加工性並びに封着性を示すのに対して、成分組成が本発明で規定する条件を満たしていない比較材では上記リードフレーム材に要求される特性の何れかが劣っていることが分かる。

即ち、本発明材1～26は、比較材28～30に比べてピン幅が狭く、エッチング速度が速いことが確認できる。また、エッチングによって形成されたピン幅の標準偏差(S.D.)も小さく、優れた寸法精度のリードフレーム製品が得られることも分かる。

比較材35は、逆に強度改善成分の含有量が少ないために十分な強度(硬さ)を示さない。

〈効果の総括〉

以上に説明した如く、この発明によれば、優れたエッチング加工性，封着性及び成形加工性と、高い強度及び硬度とを兼備したリードフレーム材の提供が可能となり、半導体機器の更なる高集積化を容易化できるなど、産業上極めて有用な効果がもたらされる。

出願人　日本鉱業株式会社
代理人　弁理士　今井　毅

そして、本発明材の中でも、特に本発明材1～18はC，Si，Pの含有量が共により好ましい範囲にコントロールされているため、本発明材19～25と比較して一段と優れたエッチング性を示していることも確認できる。

また、本発明材8と比較材34との比較からも、リードフレーム材の結晶粒径が50㎛を大きく上回るようになると十分な強度(硬さ)を確保出来ないことが分かる。

一方、比較材28～30は、各々Si，P，Cの含有量が高いためにエッチングによって形成されたピン幅が広く、バラツキも大きい。

比較材27は、強度改善並びに熱膨張係数上昇成分が添加されていないために十分な強度が得られず、熱膨張係数も小さ過ぎて封着性にも劣る。

比較材31は、S含有量が高いために曲げ加工試験でピン折れが発生した。

比較材32及び33は、強度改善成分の含有量が多過ぎるため曲げ加工性が悪く、エッチング加工性にも劣っている。

Espacenet

Bibliographic data: JPH05119035 (A) — 1993-05-14

IMAGING FLOW SIGHT METER

Inventor(s): OGINO SHINICHI ± (OGINO SHINICHI)

Applicant(s): TOA MEDICAL ELECTRONICS ± (TOA MEDICAL ELECTRONICS CO LTD)

Classification: - international: ***G01N15/14; G01N21/64; G01N33/48; G01N33/49;*** (IPC1-7): G01N15/14; G01N21/64; G01N33/48; G01N33/49
- cooperative: **G01N15/1434; G01N15/1463;** G01N15/1436; G01N15/1459; G01N2015/144; G01N2015/1488; G01N2021/177; G01N21/6456

Application number: JP19910277560 19911024

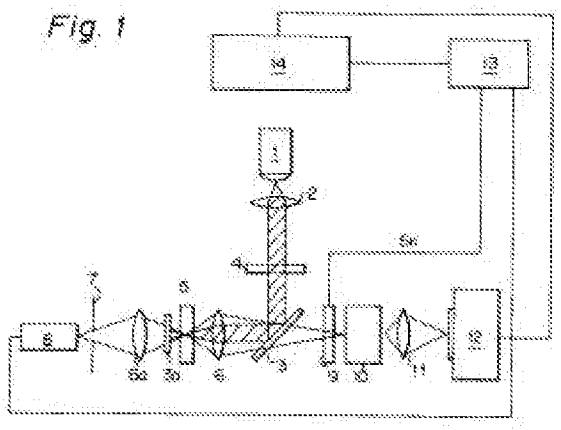
Priority number (s): JP19910277560 19911024

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Abstract of JPH05119035 (A)

PURPOSE:To increase a fluorescent intensity emitted from cells and to enable all the time image taking of a fluorescent image flame by providing a wave length selection means that reflects exciting beam to an opposite side to an irradiation side of flow cell irradiation light and makes fluorescence permeate through it, or a reflecting means that reflects the exciting beam. CONSTITUTION:An image flow sight meter takes image of a fluorescent image flame of flowing particles in a flow cell 5. As a light source, a light source 1 for fluorescence excitation is used.; Dichroic mirrors 3 and 3a which are a wave length selection means to reflect exciting beam permeating from the flow cell 5, and to make the fluorescent from the

Fig. 1



cells permeate, a photomulitiplier tube 8 to detect the fluorescence from the cells, and a judging circuit 13 for cell passing, which judges whether the detected particles are suitable for image taking of fluorescent image flame or not, and then generates the predetermined signal Sn.

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特開平5－119035

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	33/49	Ａ	7055－2Ｊ		
// Ｇ０１Ｎ	21/64	Ｚ	9115－2Ｊ		

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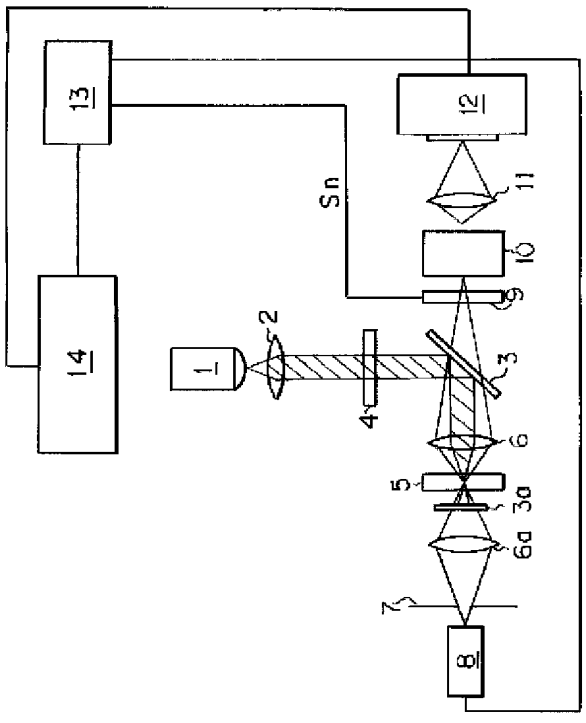
(74)代理人　弁理士　湯浅　恭三　（外５名）

(54)【発明の名称】　イメージングフローサイトメータ

(57)【要約】

【目的】　フローセルの照射光が照射される側とは反対側に励起光を反射し蛍光は透過させる波長選択手段あるいは、励起光を反射させる反射手段を設けることで、励起光の強度を向上することによって、細胞から発せられる蛍光強度を増し蛍光画像の撮像を常に可能とする。

【構成】　フローセル５を流れる粒子の蛍光画像を撮像するイメージングフローサイトメータにおいて、光源として蛍光励起用光源１を使用し、フローセル５から透過した励起光を反射し細胞からの蛍光を透過させる波長選択手段であるダイクロイックミラー３、３ａと、細胞からの蛍光を検出する光電子増倍管８と、該検出信号に基づき被検出粒子が蛍光画像撮像に適したものであるか否かを判定し所定の制御信号ｓｎを発する細胞通過判定回路１３を備えたものである。



【特許請求の範囲】
【請求項１】　細胞の被検出粒子成分を含む試料液を扁平な流れにする流路が形成されたフローセルと、該フローセルの試料液に光を照射する光源と、試料液中の被検出粒子の静止画像を撮像する撮像手段と、該撮像手段からの画像データに基づき所望のデータ処理を行う画像処理手段とを含み、該フローセル中を流れる粒子の蛍光画像を撮像し分析するイメージングフローサイトメータにおいて、該光源からの照射光は蛍光励起用の光であり試料液の扁平流に対し正面から照射され、該撮像手段は正面あるいは背面からの細胞の蛍光画像を撮像するものであり、該フローセルから透過した励起光を反射させ細胞から発せられた蛍光を透過させる波長選択手段と、該撮像手段とは別に該フローセル内の細胞から発せられた蛍光を検出する検出手段と、該検出手段の検出信号に基づき被検出粒子を検出し該被検出粒子が該撮像手段の蛍光画像撮像に適したものであるか否かを判定し所定の制御信号を発する判定制御手段とを備えたことを特徴とするイメージングフローサイトメータ。
【請求項２】　請求項１の波長選択手段は平面を有することを特徴とするイメージングフローサイトメータ。
【請求項３】　請求項１の波長選択手段は凹面を有することを特徴とするイメージングフローサイトメータ。
【請求項４】　請求項１の波長選択手段は前記フローセル背面に密着して設けられたことを特徴とするイメージングフローサイトメータ。
【請求項５】　請求項１の波長選択手段は前記フローセル背面上に形成された蒸着膜であることを特徴とするイメージングフローサイトメータ。
【請求項６】　細胞の被検出粒子成分を含む試料液を扁平な流れにする流路が形成されたフローセルと、該フローセルの試料液扁平流領域に光を照射する光源と、試料液中の被検出粒子の静止画像を撮像する撮像手段と、該撮像手段からの画像データに基づき所望のデータ処理を行う画像処理手段とを含み、該フローセル中を流れる粒子の蛍光画像を撮像し分析するイメージングフローサイトメータにおいて、該光源からの照射光は蛍光励起用の光であり試料液扁平流に対し一側面から照射され、該撮像手段は正面あるいは背面からの細胞粒子の蛍光画像を撮像するものであり、該フローセルから透過した励起光を反射させる反射手段と、該撮像手段とは別に該フローセル内の細胞から発せられる蛍光を検出する検出手段と、該検出手段の検出信号に基づき被検出粒子を検出し、該被検出粒子が該撮像手段の蛍光画像撮像に適したものであるか否かを判定し所定の制御信号を発する判定制御手段とを備えたことを特徴とするイメージングフローサイトメータ。
【請求項７】　請求項６の反射手段は平面を有することを特徴とするイメージングフローサイトメータ。
【請求項８】　請求項６の反射手段は凹面を有することを特徴とするイメージングフローサイトメータ。
【請求項９】　請求項６の反射手段はフローセル面に密着して設けられたことを特徴とするイメージングフローサイトメータ。
【請求項１０】　請求項６の反射手段はフローセル背面上に形成された蒸着膜であることを特徴とするイメージングフローサイトメータ。
【請求項１１】　請求項６のフローセルの一側面に照射励起光を通過させる開口部を設けた凹面を有する反射手段をさらに備えたことを特徴とするイメージングフローサイトメータ。
【発明の詳細な説明】
【０００１】
【産業上の利用分野】本発明は細胞の蛍光像を撮像するイメージングフローサイトメータに関するものである。
【０００２】
【従来の技術】細胞から発せられる蛍光または蛍光染色を施した細胞から発せられる蛍光を測定するフローサイトメータにおいて、励起用光源としてレーザ光源が使用されることが多い。これは、レーザを使用することによって測定領域を小さく絞り込むことが可能となるため測定領域での単位面積当たりの照射強度を強くすることができ、細胞から得られる蛍光の強度を強くすることができるからである。
【０００３】しかし、レーザを使用した場合、励起波長が特定の波長に制限されるため蛍光染色液が制限されるという問題があった。また、光源の大きさが大きく、価格が高いという問題もあった。これらの問題を解決するために励起用光源としてＸｅランプ等を使用し、励起光選択フィルターとして干渉フィルターを使用することによって励起光の波長を選択するという方法が用いられていた。しかし、レーザのように照射領域を小さく絞ることができないため、高い蛍光強度が得られないという問題があった。励起光強度の問題は、特願平３－３３１５１，３３１８９，３３１３７，３３１３８号のように蛍光像を撮像する装置において顕著に示されている。そして、細胞の蛍光像を得るためより強い蛍光強度とするため以下の方法が取られていた。
【０００４】１．励起光源として発光強度の高いものを使用する。
【０００５】２．励起光を極力小さな領域に絞り込む。
【０００６】３．光電子増倍率素子であるイメージインテンシファイアにより弱い蛍光を増幅する。
【０００７】しかしながら、細胞から発せられる蛍光強度が弱い場合に、イメージインテンシファイアによって過度の増幅をすると光電ノイズによりＳ／Ｎ比が下がるため画質が劣化するという欠点があった。このため、実質的な最大増幅率を制限されてしまい、細胞の蛍光像の撮像に必要な蛍光の強度が不足するという問題点があった。

【０００８】
【発明が解決しようとする課題】解決しようとする問題点は、励起用光源の光強度が弱い場合に細胞の蛍光像の撮像に必要な蛍光の強度が不足するという点である。
【０００９】
【課題を解決するたもの手段】本発明は、イメージングフローサイトメータにおいてフローセルの照射光が照射される側とは反対側に励起光を反射し蛍光は透過させる波長選択手段あるいは、励起光を反射させる反射手段を設け、励起光の強度を向上させるものである。
【００１０】
【実施例】図１は、本発明装置の第一の実施例の構成図である。本装置は、試料液を扁平な流れにする流路が形成されたフローセル５中に細胞を流し特定の細胞に対してその細胞像を撮像するものである。特に、本発明では、フローセル５を流れる細胞を常時監視し蛍光を発する細胞の蛍光像のみを選択的に撮像するものである。なお、図１、図７の実施例は落射型と呼ばれる構成となっている。蛍光励起用光源１は、連続発光型のＸｅランプを使用し、励起光を発するものであり、撮像用光源として作用する。コリメータレンズ２は、蛍光励起用光源１から発する光を平行にすることを目的とする。励起光選択フィルター４は、フローセル５を流れる細胞を通過する光の波長を選択するものである。具体的には、対象とする細胞あるいは、蛍光染色液に対して最適な波長を選択して、光を透過するものである。 ダイクロイックミラー３は、励起光選択フィルター４を透過した励起光を所定の方向に反射することによって対物レンズ６に入射させるものである。なお、ダイクロイックミラー３は、蛍光は透過する特性を有する。また、ダイクロイックミラー３ａはフローセル５内の細胞から発せられた蛍光を選択透過し、励起光を反射するものである。ダイクロイックミラー３、３ａの波長透過率の特性例を図２に示す。対物レンズ６ａは、細胞から発せられた蛍光を集光、収束して、検出手段である光電子増倍管８に導くものである。光電子増倍管８と対物レンズ６ａとの間に配され、光電子増倍管８の前方にあるスリット７は、フローセル５内の撮像領域において細胞から発せられる蛍光の検出領域を制限するものである。スリット７の光透過部ａは、図３に示すように一辺は、ビデオカメラ１２の視野領域の幅150μｍに一致しており、他辺は、フローセル５に対応して約20μｍであることが望ましい。このため、対物レンズ６ａとして１０倍のレンズを使用する場合には0.2×1.5㎜，４０倍を使用する場合には0.8×6㎜とすれば良い。従って、スリット７の光透過部ａは、20×150μｍであり、蛍光像撮像部ｂは、150×150μｍの大きさとなっている。細胞通過判定回路１３は光電子増倍管８から出力された信号に所定の処理を行い目的とする細胞であるか否かを判断し、目的の細胞である場合には、電子シャッター９を動作させるシャッター動作トリガー信号ｓｎを発する機能を有する回路である。細胞通過判定回路１３の信号処理の例を図８、図９により説明する。細胞から発せられた蛍光信号ｋｓのパルス幅が所定のパルス幅ｐｗを有する信号である場合、もしくは信号強度が所定のスレショホルドレベルｓ１以上の値である場合、もしくはその両方の条件を満たす場合のいずれかにより目的とする細胞であるか否かを判断する。電子シャッター９は、細胞から発せられた蛍光のうち対物レンズ６によって受光された光をイメージインテンシファイア１０に入射させるか否かを選択するものであり、細胞通過判定回路１３によって、その動作が制御される。また、電子シャッター９の開放時間はサンプルとして使用される細胞の流速によって決定される。例えば、細胞の流速が１ｍ／ｓｅｃの場合、およそ１μｓとしなければならない。これ以上の時間開放すると、イメージインテンシファイア１０に結像する蛍光像が流れてしまい静止像が得られなくなってしまう。イメージインテンシファイア１０は、光電子増倍素子であり、その光電面に入射した光を10^3から10^6倍に増幅して蛍光面側に出力する。また、電子シャッター９の代わりに電子シャッター９の機能を内蔵したイメージインテンシファイア１０とすることもできる。結像レンズ１１は、イメージインテンシファイア１０の蛍光面より出力された像を集光、収束し、ビデオカメラ１２のＣＣＤ素子に結像するものである。
【００１１】以下、装置の動作に従って説明する。蛍光励起用光源１から出力された励起光は、コリメータレンズ２を通過後、ダイクロイックミラー３によって反射される。その反射光は、対物レンズ６で集光されて常時、フローセル５に照射されている。フローセル５内の細胞から発せられる蛍光のみがダイクロイックミラー３ａを通過してスリット７を透過後、光電子増倍管８に入射する。光電子増倍管８からの検出信号は細胞通過判定回路１３によって目的とする細胞か否かが判定される。そして、目的とする細胞であれば、細胞通過判定回路１３から出力されるシャッター動作トリガー信号ｓｎにより電子シャッター９が開放される。なお、ダイクロイックミラー３ａによって反射された励起光成分の光は、再びフローセル５内の細胞に照射される。この時、フローセル５とダイクロイックミラー３ａの間隔は十分に短いので細胞に直接照射される励起光と反射されてきた励起光の時間差は殆ど無く、同時に照射されたのと同じ状態となるため、細胞には約２倍の照度の励起光が照射されたのと等価な励起光強度が得られることとなる。細胞から発せられる蛍光は、すべての方向に対して等方的であるので、フローセル５からの蛍光は、光電子増倍管８が蛍光信号を検出するのと同時に対物レンズ６を介して電子シャッター９にも入射する。この際、細胞通過判定回路１３によって目的とする細胞であると判定されている場合には、電子シャッター９が開放されており、細胞の蛍光

像がイメージインテンシファイア１０の光電面に結像する。イメージインテンシファイア１０によって蛍光像が増幅され出力面である蛍光面に出力され、この増幅された像が結像レンズ１１によりビデオカメラ１２のみがＣＣＤ素子に結像され、細胞の蛍光像が得られることとなる。　図４は、本発明のフローセル５付近の図を示す。

【００１２】励起光は、対物レンズ６で集光されて図の右側からフローセル５に照射される。また、フローセル５の左側のダイクロイックミラー３ａにより、励起光は反射され再び細胞に照射される。一方、細胞から発せられた蛍光はダイクロイックミラー３ａを通過し、光電子増倍管８に入射する。なお、ダイクロイックミラー３ａは、図５に示すような凹面形状のものを使用することも可能である。この場合、ダイクロイックミラー３ａからの反射光の焦点位置をフローセル５内の測定領域に合わせておけば凹面鏡の周辺部に入射する励起光についても測定領域に集光できるため励起光の照度をさらに向上することができる。また、図６に示すように、フローセル５の背面に波長選択膜を蒸着することも可能である。

【００１３】図７に、本発明の第二の実施例を示す。

【００１４】本実施例は、細胞監視系と撮像系を同一光路内に配置することことによって構成を簡略化したものである。なお、この場合にはダイクロイックミラー３と電子シャッター９との間にハーフミラー１６を挿入する必要がある。これは、ハーフミラー１６により反射した蛍光を光電子増倍管８で検出し、ハーフミラー１６を透過した蛍光を撮像手段１２で撮像するためである。図１０に本発明の第三の実施例を示す。

【００１５】本実施例は、細胞監視系、撮像系と励起光照射系を直角に配置することによってダイクロイックミラー３、３ａを省いたものである。この場合、細胞から発する蛍光として、側方からの蛍光を測定するため波長選択手段としてのダイクロイックミラー３、３ａの代わりに反射手段１７を蛍光励起用光源１とは反対側のフローセル５の側方に配置する必要がある。反射手段１７としては、例えば平面鏡または、図５と同様に焦点位置をフローセル５内の測定領域の位置に合わせた凹面鏡を使用する。反射手段１７の配置は図４、図５、図６の実施例のダイクロイックミラー３ａと同様に考えれば良い。

【００１６】さらに、図１１に示すように励起光の集光レンズ１５とフローセル５と間に図１２に示すような中心に円形開口を有する凹面鏡１７を設けることによって凹面部の反射のため励起光の照度をさらに向上させることも可能である。

【００１７】

【発明の効果】以上説明したように本発明のイメージングフローサイトメータは、試料液扁平流の背後に設けられた波長選択手段あるいは反射手段の反射によって、細胞から発せられる励起光を細胞全体に照射することができ、かつ試料液の細胞に反射による励起光が照射されるため、試料液の片側から照射した場合の約２倍の励起光照度が得られるという効果がある。

【図面の簡単な説明】

【図１】イメージングフローサイトメータの第一の実施例の構成図である。

【図２】波長選択手段であるダイクロイックミラーの波長透過率の特性図である。

【図３】フローセルにおける撮像エリアとスリットの関係を示す図である。

【図４】フローセル付近の構成図である。

【図５】ダイクロイックミラーを凹面とした場合のフローセル付近の構成図である。

【図６】フローセルの背面に波長選択手段である蒸着膜を設けた場合のフローセル付近の構成図である。

【図７】イメージングフローサイトメータの第二の実施例の構成図である。

【図８】細胞通過判定回路の信号処理の説明図である。

【図９】細胞通過判定回路の信号処理の説明図である。

【図１０】イメージングフローサイトメータの第三の実施例の構成図である。

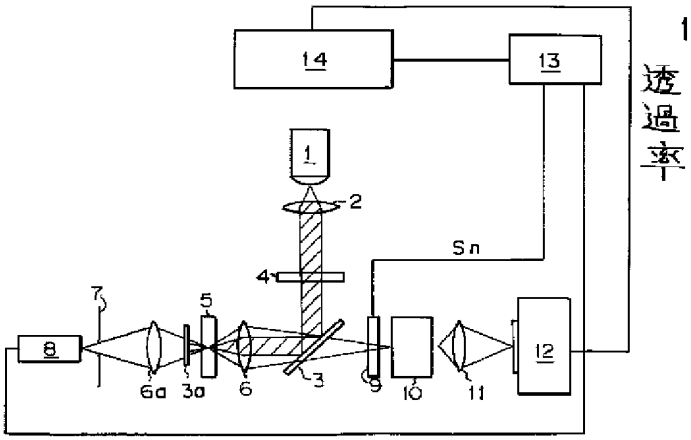
【図１１】第三の実施例において凹面鏡を設けた場合のフローセル付近の構成図である。

【図１２】凹面鏡の円形開口をしめす平面図である。

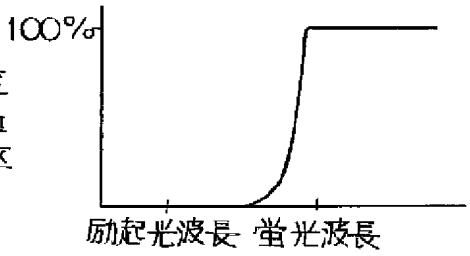
【符号の説明】

１　蛍光励起用光源
２　コリメータレンズ
３、３ａ　ダイクロイックミラー
４　励起光選択フィルター
５　フローセル
６、６ａ　対物レンズ
７　スリット
８　光電子増倍管
９　電子シャッター
１０　イメージインテンシファイア
１１　結像レンズ
１２　ビデオカメラ
１３　細胞通過判定回路
１４　画像処理装置
１５　集光レンズ
１６　ハーフミラー
１７、１７ａ　反射鏡

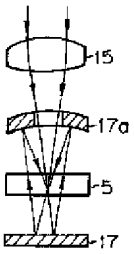
【図１】



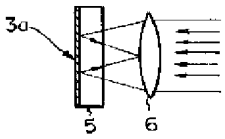
【図２】



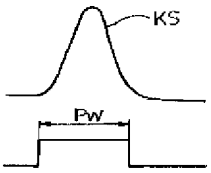
【図１１】



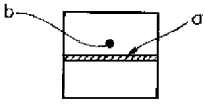
【図６】



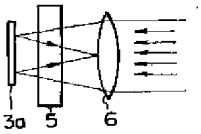
【図８】



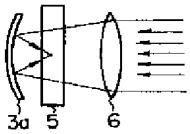
【図３】



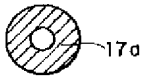
【図４】



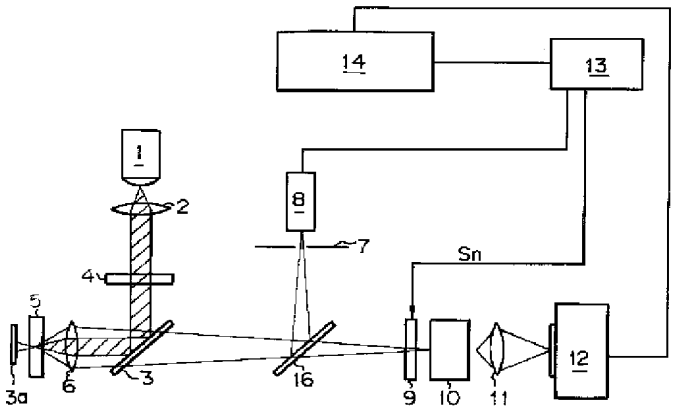
【図５】



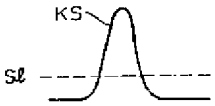
【図１２】



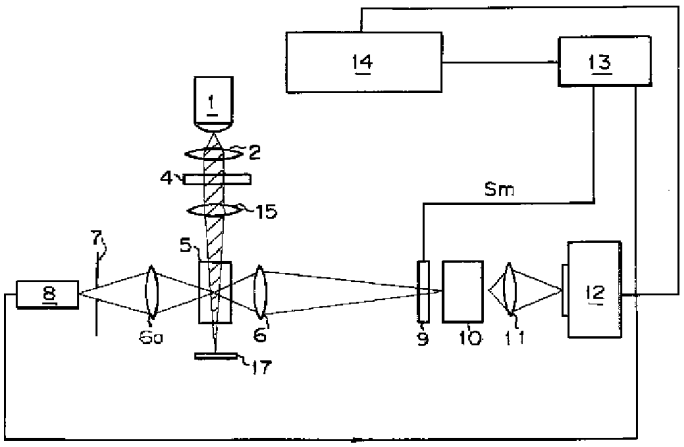
【図７】



【図９】



【図１０】



【手続補正書】
【提出日】平成４年３月１３日
【手続補正１】
【補正対象書類名】明細書
【補正対象項目名】０００９
【補正方法】変更
【補正内容】
【０００９】
【課題を解決するための手段】本発明は、イメージングフローサイトメータにおいてフローセルの照射光が照射される側とは反対側に励起光を反射し蛍光は透過させる波長選択手段あるいは、励起光を反射させる反射手段を設け、励起光の強度を向上させることによって、細胞から発せられる蛍光強度を向上させるものである。
【手続補正２】
【補正対象書類名】明細書
【補正対象項目名】００１６
【補正方法】変更
【補正内容】
【００１６】さらに、図１１に示すように励起光の集光レンズ１５とフローセル５との間に図１２に示すような中心に円形開口を有する凹面鏡１７を設けることによって凹面部の反射させ励起光の照度をさらに向上させることも可能である。
【手続補正３】
【補正対象書類名】明細書
【補正対象項目名】００１７
【補正方法】変更
【補正内容】
【００１７】
【発明の効果】以上説明したように本発明のイメージングフローサイトメータは、試料液偏平流の背後に設けられた波長選択手段あるいは反射手段による励起光の反射によって、励起光を細胞全体に照射することができ、かつ試料液中の細胞に反射による励起光が照射されるため、試料液の片側から照射した場合の約２倍の励起光照度が得られる。その結果、細胞から発せられる蛍光の強度が向上するという効果がある。

Espacenet

Bibliographic data: JPS55144226 (A) — 1980-11-11

IMAGE PROJECTOR

Inventor(s): KOUNOSU OSAMU ± (KONOSU OSAMU)

Applicant(s): MATSUSHITA ELECTRONICS CORP ± (MATSUSHITA ELECTRONICS CORP)

Classification: **- international:** ***G02B13/16; G02B13/18; G02B17/08; G02B27/18; G03B21/00; G03B21/10; G03B21/28; H04N5/74;*** (IPC1-7): G03B21/10; G03B21/28; H04N5/74

- cooperative:

Application number: JP19790053125 19790427

Priority number (s): JP19790053125 19790427

Also published as: JPS6249602 (B2)

Abstract of JPS55144226 (A)

PURPOSE:To eliminate the need for changing the curvature of the light emitting surface of a braun tube gainst the change of screen by providing an aberration correction lens used for the device of projecting images on a screen by way of a concave mirror, movably in the main beam axis direction. CONSTITUTION:A concave mirror 9 and fluorescent screen 10 are fixed to a holding and fixing means 11. An aberration correction lens 8 is designed in the position suitable for the intermediate curvature of two kinds of screens 1, 2 is fixed to a moving means 12 and is movable on the main beam axis Z-Z'. If the position of the aberration correction lens 8 is determined at, e.g., A, with respect to the screen 1 of a front projection type television, the sharp images having no deviations are obtainable in the screen 1.; For the screen 2 of back projection type, only required is to determine the position of the aberration correction lens at, e.g., B. At this time, the radius of curvature of the fluorescent screen 10 may be constant and therefore there is no need for changing the kinds of the braun tube.

⑲ 日本国特許庁（JP）　　⑪ 特許出願公開

⑫ 公開特許公報（A）　　昭55—144226

⑤1Int. Cl.³	識別記号	庁内整理番号	⑬公開　昭和55年(1980)11月11日
G 03 B 21/10		6401—2H	
21/28		6401—2H	発明の数　1
// H 04 N 5/74		6246—5C	審査請求　未請求

（全 4 頁）

⑤4画像投写装置

⑦1出　願　人　松下電子工業株式会社
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⑦2発　明　者　鴻巣理
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⑦4代　理　人　弁理士　中尾敏男　外1名

⑳特　願　昭54—53125
㉒出　願　昭54(1979)4月27日

明　細　書

1、発明の名称

画像投写装置

2、特許請求の範囲

画像を凹面鏡および収差補正レンズを介してスクリーン上に投写する装置において、上記収差補正レンズを主光線軸方向に移動可能としたことを特徴とする画像投写装置。

3、発明の詳細な説明

本発明は、画像投写装置に関するものである。

投写型テレビジョンの画像投写装置として従来より第1図に示すものが知られている。

すなわち、発光面3からの映像は、凹面鏡5で反射され収差補正レンズ6を通過して反射スクリーン1または2に写し出される。

このような投写型テレビジョン装置において、投写スクリーンは、前面投写型の場合は、凹面の反射体から成る球面反射スクリーン1が用いられ、また裏面投写型の場合は光の透過体から成る平面透過型スクリーン2が用いられる。

前者の投写型テレビジョン装置においては、視聴者は、スクリーン1に対して凹面鏡5の側に位置し、後者の投写型テレビジョンにおいては視聴者は、スクリーン2に対して凹面鏡5と反対側に位置する。

以上の例のように、投写型テレビジョン受像装置のタイプが異なると、スクリーン1，2の曲率が変化するため、同スクリーン上にずれのない鮮明な画像を得るためには、螢光面3の曲率を変化させる必要があった。

しかし、複数種類のスクリーン曲率に対してブラウン管4の発光面3の曲率を変化させることはブラウン管4全体の設計を変更させることになり、工場での生産性向上およびコストの面から好ましくなかった。

本発明は上記問題点を解決するためになされたもので、スクリーン1，2の変更があっても発光面3の曲率の補正を必要としない画像投写装置を提供するものである。

以下、図面にもとづいてこの発明の構成を説明

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する。第１図は投写型テレビジョン受像装置の側面図であり、１，２はそれぞれ前面投写型テレビジョンのスクリーン、裏面投写型テレビジョンのスクリーン、３はブラウン管の螢光面、４はブラウン管、５は凹面球、６，７は収差補正レンズをそれぞれ示すことは前述の説明の通りである。

図中の記号 Rs はスクリーン１の曲率半径、Ro は収差補正レンズ６の曲率半径、R_1 は収差補正レンズ６と螢光面３との距離、R_2 は螢光面３の曲率半径、Ｌはスクリーン１またはスクリーン２と収差補正レンズ６との距離、$X\ell$ は補正レンズ６が７へ移動したときの移動距離、To は補正レンズの中央厚み、Do は凹面球５の全長をそれぞれあらわす。

この発明者はスクリーン１，２の変更があっても発光面３の曲率の補正を必要としない画像投写装置を実現しょうと研究の結果、主光線軸 Z－Z′ 上の位置を適切にえらぶことによってスクリーン面曲率がある範囲で変化しても、螢光面３の曲率を固定したままでスクリーン上にズレのない鮮明な画像を得ることに成功した。

即ち、第１図において前面投写型テレビジョンのスクリーン１に対しては収差補正レンズ６の形状と位置が設計され、裏面投写型の２に対して収差補正レンズ７の形状と位置が設計される。上記設計においては、ブラウン管４を変更せず螢光面３の曲率を一定にしてある。発明者の研究によると上記２種類の収差補正レンズ曲面の形状差は極めてわずかであり、実用上同一のものにすることが可能であることが判明（第１表に示す）した。

第１表に、スクリーン曲率半径の変化による各定数の最適値を一実施例として示す。

（以　下　余　白）

第１表　スクリーン曲率半径の変化による各定数の最適値の差

項　　目	記　号	前面投写型テレビの各定数	裏面投写型テレビの各定数
スクリーン曲率半径	Rs/Ro	6.667	∞
凹面鏡直径	Do/Ro	0.827	0.827
CRT 面位置	R_1/Ro	0.47	0.47
CRT面曲率半径	R_2/Ro	0.48	0.48
補正レンズ中央厚み	To/Ro	0.0213	0.0213
補正レンズ位置	$X\ell/Ro$	0	0.0133
補正レンズ屈折率	n	1.5163	1.5163
投写距離	L/Ro	4.298	4.298
収差補正レンズ曲面の係数	a_1	-3.81660×10	-3.83507×10
	a_2	2.32335×10^2	2.34307×10^2
	a_3	3.42567×10^2	3.19424×10^2
	a_4	1.03328×10^3	2.82048×10^3
	a_5	-9.48900×10^3	-5.19199×10^4
	a_6	1.72538×10^5	7.30862×10^5
	a_7	-1.45198×10^6	-5.48461×10^6
	a_8	6.31129×10^6	2.15508×10^7
	a_9	-1.10177×10^7	-3.45189×10^7

第１表で収差補正レンズ曲面の係数について注目し、まずそれらが何をあらわすかを考案し、次に上記２種類のテレビの収差補正レンズの形状を比較する。

収差補正レンズ６の中央からの距離 r におけるレンズの厚みは次式であらわされる。

$$T(r) = To + \sum_{N=1}^{9} a_N \left(\frac{r}{Ro}\right)^{2N} \quad \cdots\cdots (A)$$

ただし、To は収差補正レンズ６の中央での厚み、Ro は凹面球５の曲率半径である。上式（A）において、$\frac{r}{Ro} \leq 1$ より、$a_N\left(\frac{r}{Ro}\right)^{2N}$ の値は、

Ｎ（１～９）が大きいほど、小さくなり、収差補正レンズの形状決定に寄与する度合いは小さくなる。すなわち、２つのレンズの形状を比較する場合、収差補正レンズ曲面の係数 a_1，a_2 …… a_9 のうち、a_1，a_2，a_3 のみ比較すればほぼ十分である。

第１表より、スクリーン曲率半径が6.667の前面投写型のスクリーン１とスクリーン曲率半径

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が無限大の裏面投写型のスクリーン２とのそれぞれの収差補正レンズ曲面の係数a_1，a_2 …… a_9の数値を比較すると、収差補正レンズ曲面の形状決定の大きな要件となる。低次の係数a_1，a_2，a_5の値は、上記両方のスクリーンの場合で極めて近似していることがわかる。高次の係数a_4，a_5，…… a_9の値は上記両者のスクリーンで、大きく異なっているが、これらの高次係数の値は低次係数よりも収差補正レンズ曲面の形状決定に及ぼす影響はきわめて小さい。

以上のことより、曲率半径の異なる２種類のスクリーン１，２に対して収差補正レンズ６の曲率半径をほぼ一定に保ち、かつ螢光面４の曲率半径を一定にしたままで、上記２種類のスクリーン１，２にそれぞれずれのない鮮明な画像を得ることが可能であることが確かめられた。その際、２種類の投写型テレビジョンで調整すべきパラメータは収差補正レンズ６の移動距離 X_ℓ のみである。

第２図は、本発明の収差補正レンズが移動する画像投写装置であり、８は収差補正レンズ、９は凹面球、１０はブラウン管の螢光面、１１は保持固定部、１２は収差補正レンズと一体の移動部をそれぞれ示す。凹面鏡９、螢光面１０は保持固定具１１に固定されている。収差補正レンズ８は第１図の２種類のスクリーン１，２の中間の曲率に対して適当な位置で設計してあり、移動具１２に固定され、主光線軸 Z−Z′ 上で移動することができる。前面投写型テレビジョンのスクリーン１に対しては収差補正レンズ８の位置をたとえばAに定めれば、スクリーン１にズレのない鮮明な画像が得られる。また、裏面投写型のスクリーン２に対しては収差補正レンズの位置をたとえばBに定めればよい。その際、螢光面１０の曲率半径は一定でよいため、ブラウン管の種類を変化させる必要はない。

この発明は投写型テレビ用の画像投写装置は、上記のような構成をもつものであるから、曲率半径の異なる複数のスクリーンに対して光学的に適合することが可能であり、ブラウン管は異なった型のテレビジョンに対して共通して使用できる。

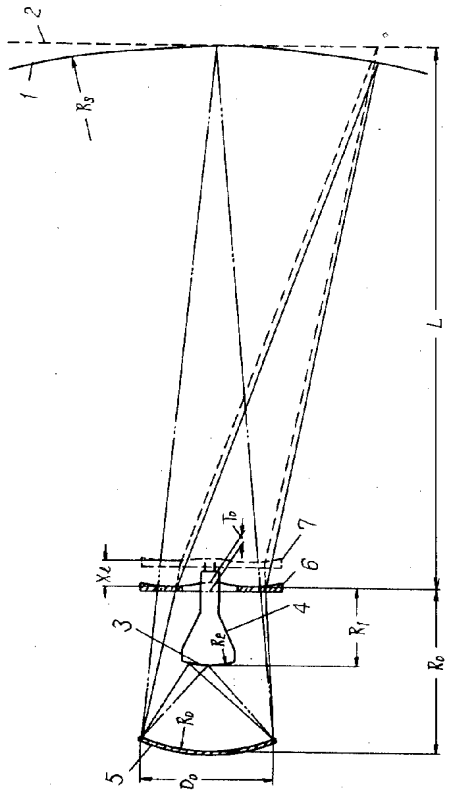
そのためこの発明は生産性向上およびコストの低減に貢献する発明と言える。

4、図面の簡単な説明

第１図は投写型テレビジョン受像装置の一例を示す側面図、第２図は本発明の一実施例における投写型テレビジョンの画像投写装置の側面図である。

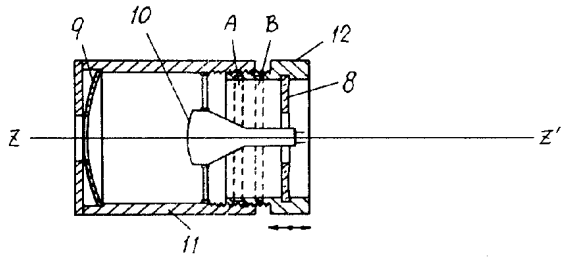
８……収差補正レンズ、９……凹面鏡、１０……螢光面、１１……保持固定具、１２……収差補正レンズと一体の移動部。

代理人の氏名　弁理士　中　尾　敏　男　ほか１名



第１図

第 2 図



Espacenet

Bibliographic data: JPS61184427 (A) — 1986-08-18

APPARATUS FOR MEASURING LASER WAVELENGTH FOR COHERENT ANTI-STOKES RAMAN SPECTRAL DIFFRACTION

Inventor(s): TOCHIGI KENJI ± (TOCHIGI KENJI)

Applicant(s): HITACHI LTD ± (HITACHI LTD)

Classification: **- international:** ***G01J3/44; G01J9/00; G01N21/65;*** (IPC1-7): G01J3/44
- cooperative: **G01J3/44**

Application number: JP19850024415 19850213

Priority number(s): JP19850024415 19850213

Also published as: JPH0588408 (B2)

Abstract of JPS61184427 (A)

PURPOSE:To dispense with temp. control in measurement, by using the atomic spectrum of neon or argon and the molecular spectrum of an iodine molecule receiving no effect of circumferential temp. as the measuring standards of a wavelength. CONSTITUTION:The wavelength of pulse laser beam from wavelength variable pulse laser 4 is continuously changed to record the change in the intensity of coherent anti-stokes Raman beam 11 on a recorder 21 as a spectrum and, at the same time, the absorption spectra based on optical galvanic effect of pulse laser beams by neon and iodine are recorded on the recorder 27.; Therefore, by using the known absorption wavelengths of neon and iodine recorded on the recorder 27 simultaneously with the coherent anti-stokes Raman beam 11 as standards, the wavelength of the wavelength variable pulse laser 4 generating the coherent anti-stokes Raman beam 11 can be measured with high accuracy.

⑲日本国特許庁（JP）　⑪特許出願公開

⑫公開特許公報（A）　昭61－184427

⑤1Int.Cl.⁴　識別記号　庁内整理番号　⑬公開　昭和61年(1986)8月18日
G 01 J 3/44　7172－2G

審査請求　未請求　発明の数 1　（全5頁）

⑤4発明の名称　コヒーレント反ストークスラマン分光用レーザ波長測定装置

⑳特　願　昭60－24415
㉒出　願　昭60(1985)2月13日

⑦2発明者　栃木　憲治　横浜市戸塚区吉田町292番地　株式会社日立製作所生産技術研究所内
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⑦4代理人　弁理士　中村　純之助

明　細　書

1. 発明の名称

コヒーレント反ストークスラマン分光用レーザ波長測定装置

2. 特許請求の範囲

発振波長固定のパルスレーザと、該パルスレーザに同期して上記発振波長固定パルスレーザの発振波長より長波長のレーザを発振する波長可変パルスレーザと、上記発振波長固定パルスレーザ光および波長可変パルスレーザ光を試料部に集光させる光学系と、上記試料のレーザ集光部から発生する固定波長パルスレーザ光より短波長のコヒーレント反ストークスラマン光を集光する光学系と、上記コヒーレント反ストークスラマン光を検出、記録する記録部とからなるコヒーレント反ストークスラマン分光用レーザ波長測定装置において、上記試料透過後の波長可変パルスレーザ光をネオンあるいはアルゴン封入およびよう素封入の各中空陰極放電管に入射し、直流放電することにより得られるネオンあるいはアルゴンの原子吸収線波長とよう素分子の分子吸収線波長とが、上記波長可変パルスレーザ光の波長に一致した場合に、上記中空陰極放電管内にそれぞれ生じる電圧変化量を検出する手段と、上記電圧変化量をコヒーレント反ストークスラマンスペクトルと並列に上記記録部に記録する手段とを備えたことを特徴とするコヒーレント反ストークスラマン分光用レーザ波長測定装置。

3. 発明の詳細な説明

〔発明の利用分野〕

本発明はコヒーレント反ストークスラマン分光用波長可変パルスレーザの波長測定に係り、特に高分解能測定時のレーザ波長位置の高精度測定に好適なコヒーレント反ストークスラマン分光用レーザ波長測定装置に関するものである。

〔発明の背景〕

従来、レーザの発振線幅が回折格子分光光度計の分解能である約0.5 cm^{-1}（20 GHz）以下の場合は、発振波長の測定法としてAIAA-83-1478(1983

年）における Stufflebeam，Verdieck および Hall による“CARS DIAGNOSTICS OF HIGH PRESSURE AND TEMPERATURE GASES”と題する文献に記載されているように Fabry-Perot エタロンを用いた干渉法を使用していた。この方法においては、波長が変化することによって Fabry-Perot エタロンを透過する光強度が、干渉により周期的に変化することを利用するものである。しかし上記方法では、透過光のピーク強度の位置の間隔は正確に求められても波長の位置の絶対値測定ができないため、波長が既知である Kr ランプの発光線などで波長位置の校正を行わなければならないという欠点がある。また Fabry-Perot エタロンの温度変化により干渉周期変化が起るため、このエタロンの温度コントロールが必要であった。さらに Fabry-Perot エタロンを透過した光の強度で測定しなければならないため、測定精度がよい光検出器が必要であり、また照明光など周囲からの妨害光の影響も除去しなければならないという欠点があった。

〔発明の目的〕

本発明は上記の欠点がなく、高精度、高感度で発振線幅が1～2 GHz程度の非常に狭帯域なレーザ光波長を絶対測定できるコヒーレント反ストークスラマン分光用レーザ波長測定装置を得ることを目的とする。

〔発明の概要〕

ネオン、アルゴン等の原子あるいはよう素のような分子を封入した中空陰極放電管を直流放電し、この放電部にレーザ光を入射すると、レーザ波長がネオン、アルゴン、よう素などの吸収波長に一致した場合は、放電条件が変化しこれに基づいて放電電圧が変化する光ガルバノ効果が知られている。この場合にパルスレーザ光を入射すれば電圧変化もパルス状になり、コンデンサを介して変化電位差だけを取出すことができる。ネオン、アルゴンなどの希ガス原子の吸収スペクトルは7桁程度の精度で絶対波長が既に知られており、紫外波長から近赤外波長領域にわたって、波長間隔0.1～5 nm程度で吸収がある。同様にしてよう素分子も紫外波長から赤外波長にわたって波長間隔0.04 cm^{-1} 以下で非常に多数の吸収が高精度に測定されている。したがってこれらの原子、分子の光ガルバノ効果を組合わせて用い、コヒーレント反ストークスラマン分光用の波長可変パルスレーザ光をネオンまたはアルゴンと、よう素の中空陰極放電管に入射させて光ガルバノ効果を測定することにより、波長可変パルスレーザ光の発振波長の絶対測定が可能になる。この場合、吸収波長間隔が長いネオンあるいはアルゴンの光ガルバノ効果測定と、吸収波長間隔が短かいよう素の光ガルバノ効果測定とを併用することにより、ラマン線幅が0.5 cm^{-1} 程度の気体分子のコヒーレント反ストークスラマン分光測定の波長可変パルスレーザの発振波長測定、ひいてはラマンシフト値の高精度測定が可能になる。すなわち、本発明によるコヒーレント反ストークスラマン分光用レーザ波長測定装置は、発振波長固定のパルスレーザと、該パルスレーザに同期して上記発振波長固定パルスレーザの発振波長より長波長のレーザを発振する波長可変パルスレーザと、上記発振波長固定パルスレーザ光および波長可変パルスレーザ光を試料部に集光させる光学系と、上記試料のレーザ集光部から発生する固定波長パルスレーザ光より短波長のコヒーレント反ストークスラマン光を集光する光学系と、上記コヒーレント反ストークスラマン光を検出、記録する記録部とからなるコヒーレント反ストークスラマン分光用レーザ波長測定装置において、上記試料透過後の波長可変パルスレーザ光をネオンあるいはアルゴン封入およびよう素封入の各中空陰極放電管に入射し、直流放電することにより得られるネオンあるいはアルゴンの原子吸収線波長とよう素分子の分子吸収線波長とが、上記波長可変パルスレーザ光の波長に一致した場合に、上記中空陰極放電管内にそれぞれ生じる電圧変化量を検出する手段と、上記電圧変化量をコヒーレント反ストークスラマンスペクトルと並列に上記記録部に記録する手段とを備えたものである。

〔発明の実施例〕

つぎに本発明の実施例を図面とともに説明する。

第1図は本発明によるコヒーレント反ストークスラマン分光用レーザ波長測定装置の一実施例を示すブロック図、第2図は光ガルバノ効果による吸収スペクトルの一例を示す図で、(a)はネオンの場合、(b)はよう素分子の場合をそれぞれ示す。第1図において、発振波長固定のパルスレーザ1から発振されたレーザ光2は、一部をハーフミラー3で分割して波長可変パルスレーザ4の励起光源に用いている。上記波長可変パルスレーザ4から発振されたレーザ光5はダイクロイックミラー6で上記波長固定パルスレーザ光2と同一光軸に重ねられ、集光レンズ7によって試料8中に焦点を結ぶ。焦点部から発生したコヒーレント反ストークスラマン光11と上記レーザ光2およびレーザ光5は、レンズ9によってそれぞれ平行光束に戻される。コヒーレント反ストークスラマン光11はダイクロイックミラー10によって光路を90°曲げられ、光学フィルタ12によりさらにレーザ光2とレーザ光5とを除かれたのち、光検出器13で検出される。また、レーザ光2とレーザ光5とはダイクロイックミラー10を透過後にダイクロイックミラー17で分離され、該ダイクロイックミラー17を透過したパルスレーザ光2は光検出器14で検出されトリガ信号15を発生する。上記光検出器13で検出されたコヒーレント反ストークスラマン光11は、上記トリガ信号15に同期してゲート検出器16で検出され記録計27に記録される。またダイクロイックミラー17で上記パルスレーザ光2と分離されたパルスレーザ光5はさらにハーフミラー18で2分割され、それぞれネオンを充填した中空陰極放電管19およびよう素を充填した中空陰極放電管20に入射する。バラスト抵抗21、21′と直流電源22、22′とで直流放電する上記中空陰極放電管19、20におけるパルスレーザ光5の吸収に伴う光ガルバノ効果によって生じるパルス状変動電位差は、それぞれコンデンサ23および24を経て取出され、上記ゲート信号15に同調してゲート検出器25および26により検出され記録計27に記録される。

上記のようにして波長可変パルスレーザ4からのパルスレーザ光5の波長を連続的に変化させることにより、コヒーレント反ストークスラマン光11の強度変化が上記記録計27にスペクトルとして記録され、これと同時にネオンおよびよう素によるパルスレーザ光5の光ガルバノ効果に基づく吸収スペクトルが、上記記録計27に記録される。第2図(a)はネオンを封入した中空陰極放電管19にパルスレーザ光5を入射して、発振波長を連続的に変えた場合に得られる光ガルバノ効果に基づくネオンの吸収スペクトルの一例を示す図で、第2図(b)はよう素を封入した中空陰極放電管20によって上記同様に測定される光ガルバノ効果に基づくよう素分子の吸収スペクトルの一例を示す図である。よう素分子の吸収スペクトルは紫外波長から可視波長の全域にわたって、第2図(b)と同様に多数の吸収線が光ガルバノ効果に基づいて測定される。したがって記録計27にコヒーレント反ストークスラマン光11と同時に記録されたネオンおよびよう素の既知吸収波長を標準とすることにより、コヒーレント反ストークスラマン光11を発生する波長可変パルスレーザ4の波長を高精度に測定することができる。

〔発明の効果〕

上記のように本発明によるコヒーレント反ストークスラマン分光用レーザ波長測定装置は、発振波長固定のパルスレーザと、該パルスレーザに同期して上記発振波長固定パルスレーザの発振波長より長波長のレーザを発振する波長可変パルスレーザと、上記発振波長固定パルスレーザ光および波長可変パルスレーザ光を試料部に集光させる光学系と、上記試料のレーザ集光部から発生する固定波長パルスレーザ光より短波長のコヒーレント反ストークスラマン光を集光する光学系と、上記コヒーレント反ストークスラマン光を検出、記録する記録部とからなるコヒーレント反ストークスラマン分光用レーザ波長測定装置において、上記試料透過後の波長可変パルスレーザ光をネオンあるいはアルゴン封入およびよう素封入の各中空陰極放電管に入射し、直流放電することにより得られるネオンあるいはアルゴンの原子吸収線波長とよう素分子の分子吸収線波長とが、上記波長可変

パルスレーザ光の波長に一致した場合、上記中空陰極放電管内にそれぞれ生じる電圧変化量を検出する手段と、上記電圧変化量をコヒーレント反ストークスラマンスペクトルと並列に上記記録部に記録する手段とを備えたことによって、周囲温度の影響を受けないネオンあるいはアルゴンの原子スペクトルおよびよう素分子の分子スペクトルを波長測定の基準として用いるため、測定に際して温度のコントロールが一切不要であり、またホトマルやダイオード等の妨害光の影響を受けやすい光検出器を用いないため、照明光等の設置環境の条件も少なく、安定して高精度に、コヒーレント反ストークスラマン分光用波長可変パルスレーザの波長測定を行うことができる。

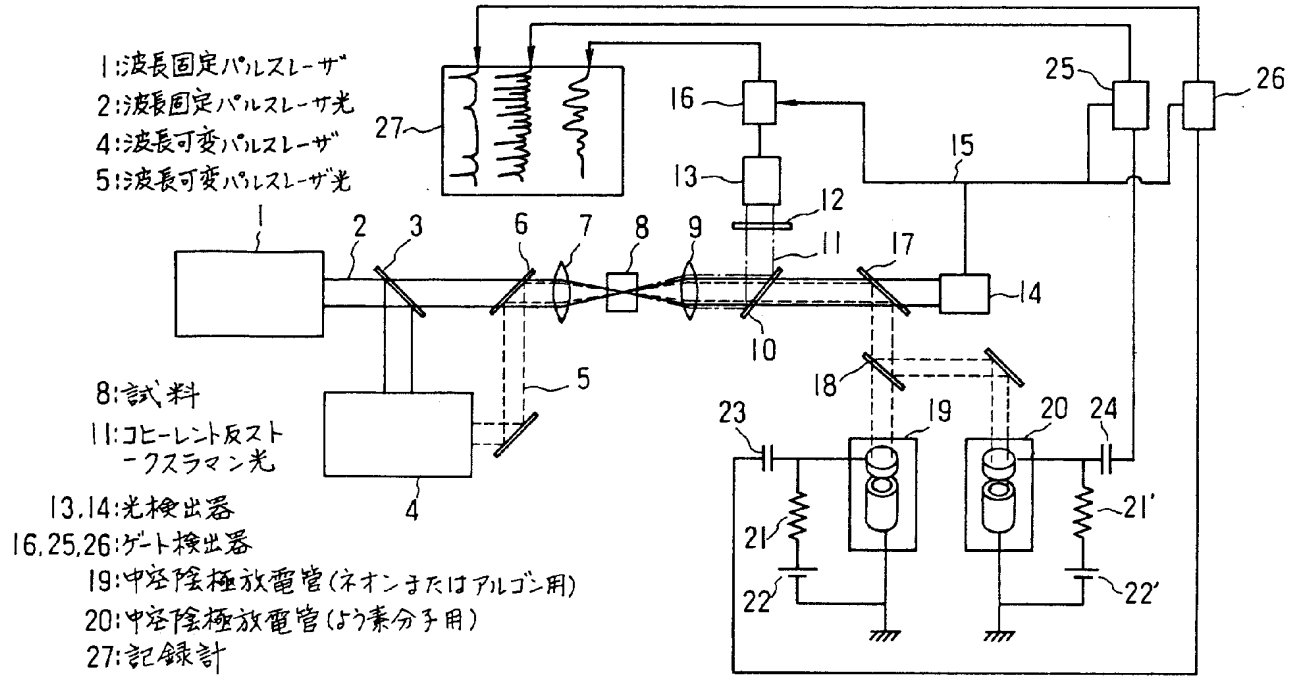
4. 図面の簡単な説明

第1図は本発明によるコヒーレント反ストークスラマン分光用レーザ波長測定装置の一実施例を示すブロック図、第2図は光ガルバノ効果による吸収スペクトルの一例を示す図で、(a)はネオンの場合、(b)はよう素分子の場合を示すものである。

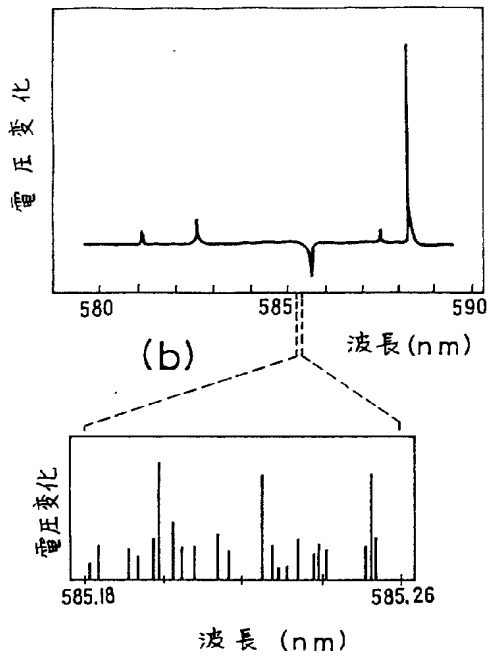
1…波長固定パルスレーザ、2…波長固定パルスレーザ光、4…波長可変パルスレーザ、5…波長可変パルスレーザ光、8…試料、11…コヒーレント反ストークスラマン光、13、14…光検出器、16、25、26…ゲート検出器、19…中空陰極放電管（ネオンまたはアルゴン用）、20…中空陰極放電管（よう素分子用）、27…記録計。

代理人弁理士　中　村　純之助

第1図



第２図



手続補正書（方式）

昭和６０年　６月　５日

特許庁長官　志賀　学　殿

１．事件の表示　　昭和６０年特許願第２４４１５号

２．発明の名称　　コヒーレント反ストークスラマン分光用レーザ波長測定装置

３．補正をする者

事件との関係　　特許出願人

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５．補正命令の日付　　昭和６０年　５月２８日

６．補正の対象　　明細書の発明の詳細な説明の欄

７．補正の内容　　添付別紙のとおり

特許庁 60.6.5

補正の内容

１．明細書第２頁下から１行目～第３頁上から３行目の「ＡＩＡＡ－83－1478（1983）……ＧＡＳＥＳ”」を

エーアイエーエー（ＡＩＡＡ）－83－1478（1983）におけるスタッフルビーム，ベルディエック（Stuffelbeam，Verdieck）およびホール（Hall）による“カーズ　ダイアグノスティックス　オブ　ハイ　プレッシャー　アンド　テンパラチャー　ガスイズ”（CARS DIAGNOSTICS OF HIGH PRESSURE AND TEMPERATURE GASES”」

と補正する。

２．明細書第３頁上から第４行目「Ｆabry－Perot」を「ファブリィーペロット（Ｆabry－Perot」と補正する。

３．明細書第３頁上から第６行目「Ｆabry－Perot」を「ファブリィーペロット（Ｆabry－Perot」と補正する。

４．明細書第３頁下から第８行目「Ｆabry－Perot」を「ファブリィーペロット（Ｆabry－Perot」と補正する。

５．明細書第３頁下から第６行目「Ｆabry－Perot」を「ファブリィーペロット（Ｆabry－Perot」と補正する。

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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[Continued on next page]

(54) Title: FLOW CYTOMETER

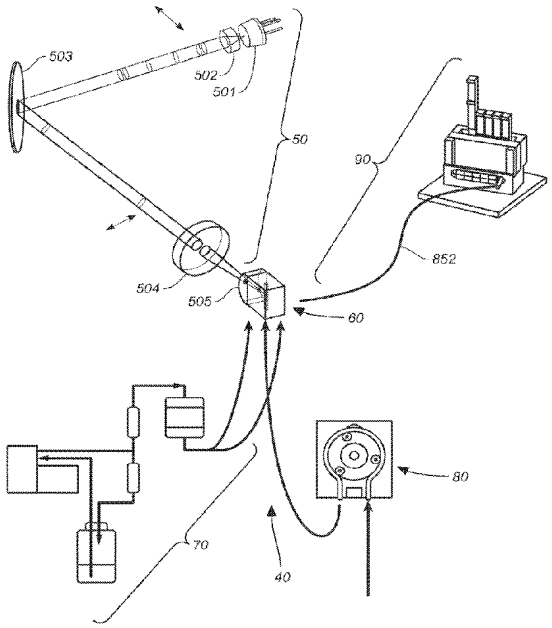


FIG. 1

(57) Abstract: The disclosed flow cytometer (40) includes; 1. a laser diode ("LD") based optical subsystem (50) for impinging a beam of light upon particles passing through a viewing zone; 2. a composite microscope objective (60) for gathering and imaging light scattered from or fluoresced by particles passing through the viewing zone; 3. a fluidic subsystem (70) for supplying a liquid sheath flow to the viewing zone; 4. a peristaltic pump (80) for injecting into the liquid sheath flow a liquid sample flow carrying particles that passes together with the liquid sheath flow through the viewing zone; 5. a multimode optical fiber (852) that receives scattered and fluoresced light from the viewing zone that the composite microscope objective (60) gathers and images; and 6. a wavelength division multiplexer (90) for optically separating into color bands light received via the optical fiber (852).

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TM), European (AL, 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, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

FLOW CYTOMETER

Technical Field

The present disclosures relate 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 Art

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.

An entire flow cytometer system includes the following main components.

1. A flow cell through which a liquid stream, usually called a sheath flow, liquid or fluid, 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 subsystems 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.

Sample Illumination

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 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.

Viewing Zone
Microscope Objective

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 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.

Sheath Liquid Supply

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 air-tight 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 differential between the inlet and outlet of the flow cell.

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.

Peristaltid Pump
Sample Liquid Supply

Peristaltic pumps are volumetric pumps in which a set of linearly or circularly moving rollers progressively compresses 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.

Mmulticolor Fluorescence Detection

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 detectors 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 $1/20^{th}$ 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 no. 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.

Disclosure

The present disclosure provides an improved flow cytometer together with various improved components included therein.

It is an object of the present disclosure to provide 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.

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

An object of the present disclosure ia 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 spatitally separated excitation laser beams or in droplet sorters.

A object of the present disclosure is simple design for a peristaltic pumb can provide a pulseless liquid flow is highly desirable.

An object of the present disclosure to provide a peristaltic pump with minimal pulsation.

A object of the present disclosure to provide a peristaltic pump that is simple to manufacture and operate.

an object of the present disclosure to provide a device capable of collimating a light beam from an extended light

source over an extended distance without significanly expanding the said beam diameter. It is also an object of the present disclosure to provide a WDM system using the said device to separate the said light beam into multiple colored bands. Further, it is an object of the present disclosure to provide such a WDM system that is compatible with low noise semiconductor detectors. In addition, due to the diversity of fluorescent probes, it is an object of the present disclosure to provide such a WDM system that is reconfigurable.

Disclosed herein is a flow cytometer that includes:

1. a 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 into the liquid sheath flow a liquid sample flow carrying particles that passes 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 and images; and
6. a wavelength division multiplexer for optically separating into color bands light received via the optical fiber .

The LD based optical subsystem in accordance with the present disclosure for illuminating particles passing through the flow cytometer's viewing zone generally includes:

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 transposes 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.

The composite microscope objective in accordance with the present disclosure generally includes:

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 is 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 is made of an optically transparent material, such as glass or optical quality plastics, of piano-convex shape with a highly-reflective coating on the convex side for internal reflection. The piano-side of the mirror is either gel-coupled or bonded to one side surface of the cuvette. The plano-aspheric compensation plate is made of a transparent material, such as glass or optical quality plastics, with the piano side gel-coupled or bonded to the opposite side of the cuvette. The piano-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 is in a jet stream with both the concave

mirror and the compensation plate being free standing from the viewing zone, and the mirror is preferably a front surface concave mirror.

The fluidic system in accordance with the present disclosure generally includes 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 fludic 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.

The peristaltic pump in accordance with the present disclosure generally includes 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 tub that the rollers compress against the track. In one embodiment of the present disclosure, the track of the peristaltic pump's housing has 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 is used to drive the pump. When a roller reaches the exit section, the motor's rotation programmatically speeds up to compensate for the tube's expansion.

The wavelength division multiplexer ("WDM") in accordance with the present disclosure generally includes at least two optical elements. The first optical element collimate 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 in the present disclosure to further extend the collimated optical path. The cascaded unit-magnification image relay architecture of the present disclosure provides a greatly extend 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 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. Similar to its counterpart in optical communication, 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 is folded into a zig-zag configuration. Preferably, to facilitate the flow cytometer's reliable reconfiguration each dichroic filter is 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.

These and other features, objects and advantages will be understood or apparent to those of ordinary skill in the art from the following detailed description of the preferred embodiment as illustrated in the various drawing figures.

Brief Description of Drawings

FIG. 1 is a diagram schematically illustrating a preferred 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 via an optical fiber light 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, prior art 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 that improves 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.

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.

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. 6 is a perspective view of an alternative embodiment of a LD based optical illumination subsystem in accordance with

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 depicting in greater detail the jet stream of liquid passes through the viewing zone.

FIG. 7 is a perspective view of an alternative embodiment LD based optical illumination subsystem in accordance with 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 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. 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 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 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 yet another alternative embodiment a composite microscope objective in accordance with the present disclosure adapted for use in the flow cytometer system depicted in FIG. 1, the alternative embodiment composite microscope objective being adapted for a viewing zone that is located inside the jet stream depicted in FIGs. 6 and 6A.

FIG. 13 is a perspective view of yet another alternative embodiment a composite microscope objective in accordance with 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 subsystem in accordance with the present disclosure for supplying stable liquid sheath flow to a flow cytometers 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 alternative embodiment 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 sheath liquid pump the and the flow cell (FIG. 16B).

FIG. 17 is a perspective view of a 3-roller peristaltic pump in accordance with 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 provided by the peristaltic pump.

FIGs. 21 is 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.

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

FIGs. 23 is a simplified plan view of a 6-roller peristaltic pump in accordance with 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 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 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 resect 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 zig-zag configuration in accordance with the present disclosure.

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 ports WDM using a combination of zig-zag and branched configurations in accordance with the present disclosure.

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

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

FIGs. 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 the present disclosure depicting installing into the WDM the replaceable dichroic filter assembly depicted in FIG. 29C and its removal therefrom.

Best Mode for Carrying Out the Disclosure

Flow Cytometer

FIG. 1 depicts a flow cytometer in accordance with the present disclosure identified by the general reference number 40. The flow cytometer 40 includes:

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

Optical Subsystem 50

The optical subsystem 50 includes 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 impinges 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, reduces 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 is preferably 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 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 passes 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 minute 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 diminished 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 cylindrical lens 505, depicted in FIGs. 5A and 5B as a cylindrical plano-convex lens, is placed along the beam of light after the spherical beam focusing lens 504 and is preferably juxtaposed with the composite microscope objective 60. As shown in FIGs. 5A and 5B, the cylindrical lens 505 focuses 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 establishes 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 is 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. 6 depicts yet another alternative diode laser based optical subsystem in accordance with 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 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

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 images a viewing zone that is located inside a prismatically-shaped glass cuvette 603 within a small flow channel 604, that preferably has a rectangular cross-sectional shape, through which passes the particle carrying combined liquid sample and sheath flows. A piano-concave back-surface mirror 601 included in the composite

microscope objective 60 is made of an optically transparent material preferably having 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 includes 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 also includes a plano-ashperic corrector plate 602 that is also made of an optically transparent material preferably having 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 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 has negative optical power while inside the neutral zone the corrector plate 602 has 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.

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 emission from three (3) spatially separated locations in the flow channel 604 near the center of the cuvette 603:

1. initially propagate toward back-surface mirror 601 to be internally reflected by the back-surface mirror 601;
2. then pass first 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 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 in viewing zone within the flow channel 604 that are separated 150 micron from each other. The diameters of all images depicted in FIGs. 9B1 through 9B3 are 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 suffers 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 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' also includes 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 also serves 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 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 is 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 addition of the 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 emission from cells or other microscopic particles carried in the jet stream 519 emitted by the nozzle 518. The composite microscope objective 60'" consists of 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 piano-aspheric corrector plate 612 is 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. Preferably, both surfaces of the corrector plate 612 are 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 the present disclosure. It is understood that the front surface mirror 610 and the corrector plate 612 are 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 is reflected by the concave surface 611 of the front surface mirror 610. The aberration due to reflection from the concave surface 611 is 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 includes two (2) optical elements, one a piano-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 is fixed to a front surface 615 of a transparent, usually glass slide 616. The slide 616 is optically coupled, preferably 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:

1. initially propagates through the slide 616 and the back surface mirror 617;
2. is internally reflected by the back surface mirror 617 back through the slide 616;
3. then through the corrector plate 618; and
4. finally forms an image at an image plane that is located beyond the corrector plate 618.

Fluidic Subsystem 70

FIG. 15 depicts a fluidic subsystem 70 in accordance with 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, or a peristaltic pump, or a piston pump or any types of continuous fluid pump. An outlet of the liquid pump 701 connects to an inlet of a T-coupling 703 that receives sheath liquid from the liquid pump 701. The T-coupling 703 has two (2) outlets a first one of which connects 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. 1, 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 is then

$$R_p = \frac{rR}{r+R} \tag{1}$$

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 are temperature insensitive. Thus, the configuration of the fluidic subsystem 70 depicted in FIG.

15 also provides a simple mechanism for achieving a temperature insensitive sheath liquid flow to the flow channel 604.

As depicted in FIG. 15, a second outlet of the T-coupling 703 connects to the to the flow channel 604 that extends through the cuvette 603 preferably first via a small reservoir capsule 704 and then via a filter cartridge 705. As depicted in FIG. 16, a piece of tubing 704' approximately 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 acts 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 FIG. 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 is not adjustable. As should be apparent to those ordinary skilled in the art, flow restrictors such a fixed restrictor or adjustable valves 711, 711' and 712, 712' 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

One embodiment of an peristaltic pump 80 in accordance with the present disclosure appears in FIG. 17. The pump includes a housing 809 with arcuate curved track 808, three

rollers 810, 811 and 812 attached to a rotor 816rotatable within the housing 809, and a compressible tube 807 sandwiched between the compressible tube 807 of the housing 809 and the 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. 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 experiences no compression;
2. a pump inlet section between point 801 and point 802 where the 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 is fully closed by the roller;
4. a recess section between point 803 and point 804 in which the 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 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 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 from inlet point 801 to outlet point 806, the inner gap of the :

1. progressively decreases from fully open at point 801, to fully closed at point 802 and remain closed until point 803;
2. then progressively expands back to fully open at point 813;
3. then progressively decreases to fully closed at point 804, and remain closed until the roller reaches point 805; and
4. finally progressively expands back to fully open at point 806.

The size of the gap inside the 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 866 are 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 completely determines 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 underneath the roller 810 starts to progressively expand and a gap starts to grow. Meanwhile, the roller 811 arrives at the compression part of the recess section and starts to progressively compress the . 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 substantially fills the volume created by the expansion underneath roller 810 in the exit section between point 5 and point 6. During this period, the compressible is partially open underneath both rollers 810 and 811 and completely closed underneath roller 12. Consequently, the pumping action in mainly delivered by roller 12. In particular, since by design the total volume of liquid in the

section of the between point 13 and point 6 remains substantially constant during this period, the flow rate of the peristaltic pump 80 in the state shown in FIG. 18C remains 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 therefore remains substantially constant throughout the entire process.

The mechanism of the pulseless peristaltic pump 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 hatched area 818 shown in FIG. 19. Clearly, V depends on the angular position, θ, of the roller 20, as well as δ, the amount of tube compression exerted by all other downstream rollers.

$$V = V(\theta, \delta_1, \delta_2, \ldots) \quad (1)$$

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

$$-\mathrm{F} = \frac{dV}{dt} = \frac{\partial V}{\partial \theta} R + \sum_i \frac{\partial V}{\partial \delta_i} \frac{d\delta_i}{dt} \quad (2)$$

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. (2) 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 , and L 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 tube by the roller, follows Eqn. (3):

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

where D is the inner diameter of the compressible tube and G 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 compressible tube 807 of the housing 809. 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. (2) vanishes. FIG. 20B corresponds to the state of the pump shown in FIG. 18C. The is closed off by roller 12' 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.(2) vanishes as well. As a result, the flow rate of the peristaltic pump 80 remains substantially constant regardless of roller positions.

The shape of the compressible tube 807 satisfying the above requirement can be readily derived from Eqn. (3). Referring to FIG. 18C, if the gaps of the arcuate compressible tube 807 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

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

then the total fluid volume in the two sections remains substantially constant, as shown in FIG. 21. In the peristaltic pump 80, the shape of the pump housing 809 is 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 very little pulsation, although it is understood that the symmetry is not required to realize a pulseless peristaltic pump in accordance with the

present disclosure. For example, as long as the gaps of the arcuate compressible tube 807 in the section between point 13 and point 3, $G_{13,3}$, and in the section between point 2 and point 1, $G_{2,1}$, follow Eqn. (5),

$$(D - G_{13,3})^3 + (D - G_{2,1})^3 = D^3 \quad (5)$$

a peristaltic pump in accordance with 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 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' includes a compressible tube 807' having two (2) recesses 820 and 821, and four (4) rollers 822, 823, 824 and 825. 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 822 and 823 near the two recesses 820 and 821.

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" includes six (6) rollers and an arcuate compressible tube 807" 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 826, 827 and 829 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. (2) is much simplified:

$$-\mathrm{F} = R\left(\frac{\partial V_c}{\partial \theta} + \frac{\partial V_c}{\partial \delta}\frac{d\delta}{d\theta}\right) \qquad (6)$$

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, is 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, remains constant, as indicated by the solid line at the top of FIG. 24C. Note the terms inside the parentheses of Eqn. (6) are 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. (3). 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

FIG. 25 shows the optical ray trace for an exemplary 6 ports 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, captures the light from source 901, and projects a magnified image of the object near a final focusing lens 905. The size of the image near 905 is kept approximately the same as the effective size of the collimating optical element 902. Consequently, beam of light propagating between lens 902 and the lens 905 is 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 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, is inserted into the optical path approximately midway between the collimating optical element 902 and the lens 905. The dichroic filter 903 passes 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 impinges upon a second optical element 907, preferably a concave mirror. The concave mirror 907 has 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 has 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. A second dichroic filter 909 is then inserted approximately midway between the relay imaging concave mirror 790 and the second image near focusing lens 908. The second dichroic filter 909 passes another band of color in the beam of light received by the WDM 90 at location 901 and reflects the remainder of the impinging beam of light for further processing.

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 1. As shown in FIG. 25, due to the present disclosure's 1:1 imaging relay architecture, the spots of light produced by focusing lenses 906, 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 the present disclosure. It is also apparent to those skilled in the art that although the WDM 90 preferably uses achromatic doublets as the first collimating optical element, singlet lens can also be used since the images created before the focusing lenses 906, 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 as relay element to extend the path of the collimated beam of light. A clear advantage 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. 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 Mt exclusively for

fluorescence detection such as in the main stream flow cytometers manufactured by Becton-Dickinson, Becman 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 is 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 relay imaging mirrors 907 and 910 in FIG. 27 with concave shaped dichroic filters 907' and 910', the WDM depicted in FIG. 28 provides 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 is 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 ensures 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 is then pressed against the surface 929 of the template. A filter holder 928, which loosely fits the dichroic filter 927, includes a reference surface 931 and a filter slot 933. During assembly of the replaceable dichroic filter, the filter slot 933 is partially filled with epoxy adhesive and the reference surface 931 of the filter holder 928 is 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 the E934~ fabricated as depicted in FIGs. 29A and 29B and described above. The assembly process depicted in FIGs. 29A and 29B and described above ensures that front surface 932 of the replaceable dichroic filter assembly 934 will 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.

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 slides against the flat surface of the glass reference block 935 and is kept in contact therewith by a spring loaded screw 936. Consequently, the coated front surface 932 of the replaceable dichroic filter assembly 934 remains optically parallel to the optical flat and accurately located. In the meantime, the indentation of front surface 932 with respect to the reference reference surface 931 protects 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 is 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 rests against a surface of the first optical flat and the coated surface of the dichroic filter rests against the flat surface of the second optical flat. Epoxy bonding then holds 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.

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 invention has been described in terms of the presently preferred embodiment, 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.

The Claims

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:
i. 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 a 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 piano-concave back surface mirror made from an optically transparent material.

13. The flow cytometer (40) of claim 12 wherein the piano-surface of said piano-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 piano-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 piano-aspherical lens.

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

30. The flow cytometer (40) of claim 29 wherein the piano-surface of said piano-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 piano-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 ashperic 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 piano-concave back surface mirror made from an optically transparent material.

78. The composite microscope objective (60) of claim 77 wherein a piano-surface of said piano-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 piano-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 piano-aspherical lens.

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

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

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

123. The method of claim 122 wherein a piano-surface of said piano-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 piano-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 Tee 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 reservior 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 menas 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 a 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).

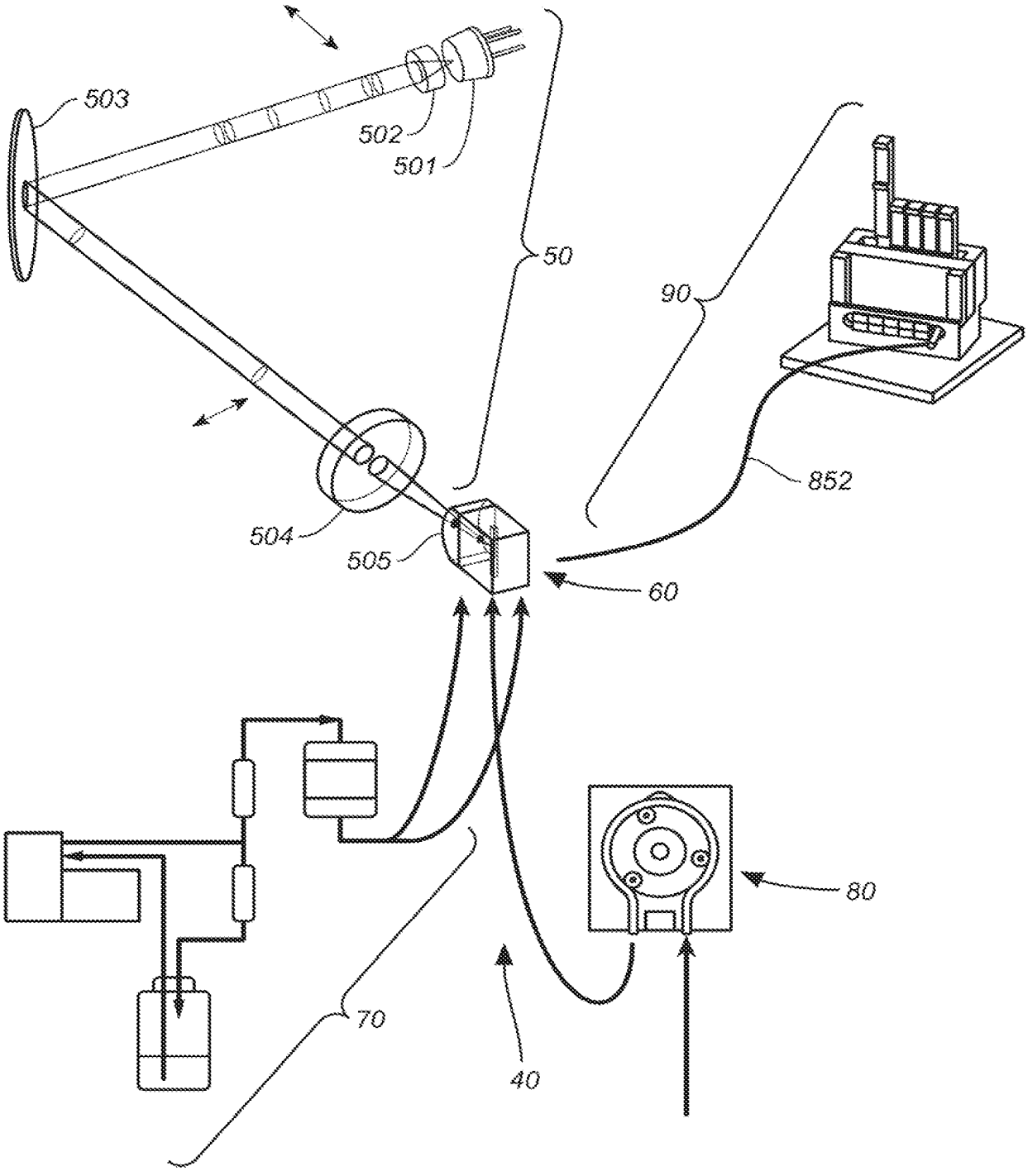


FIG. 1

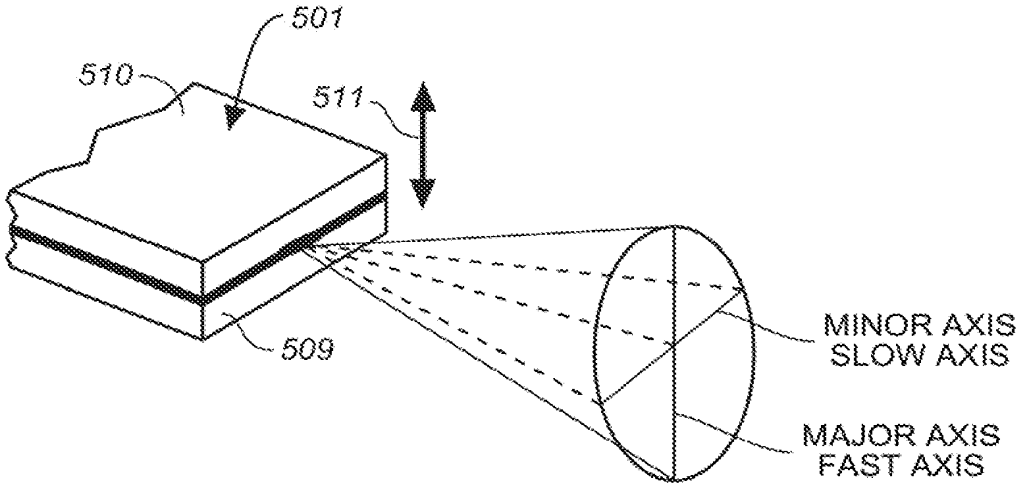


FIG. 2

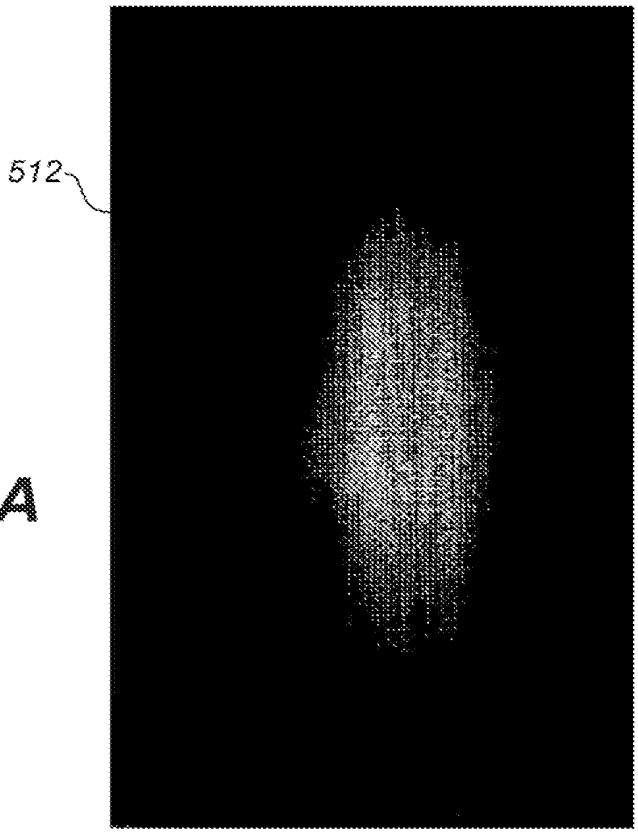


FIG. 2A

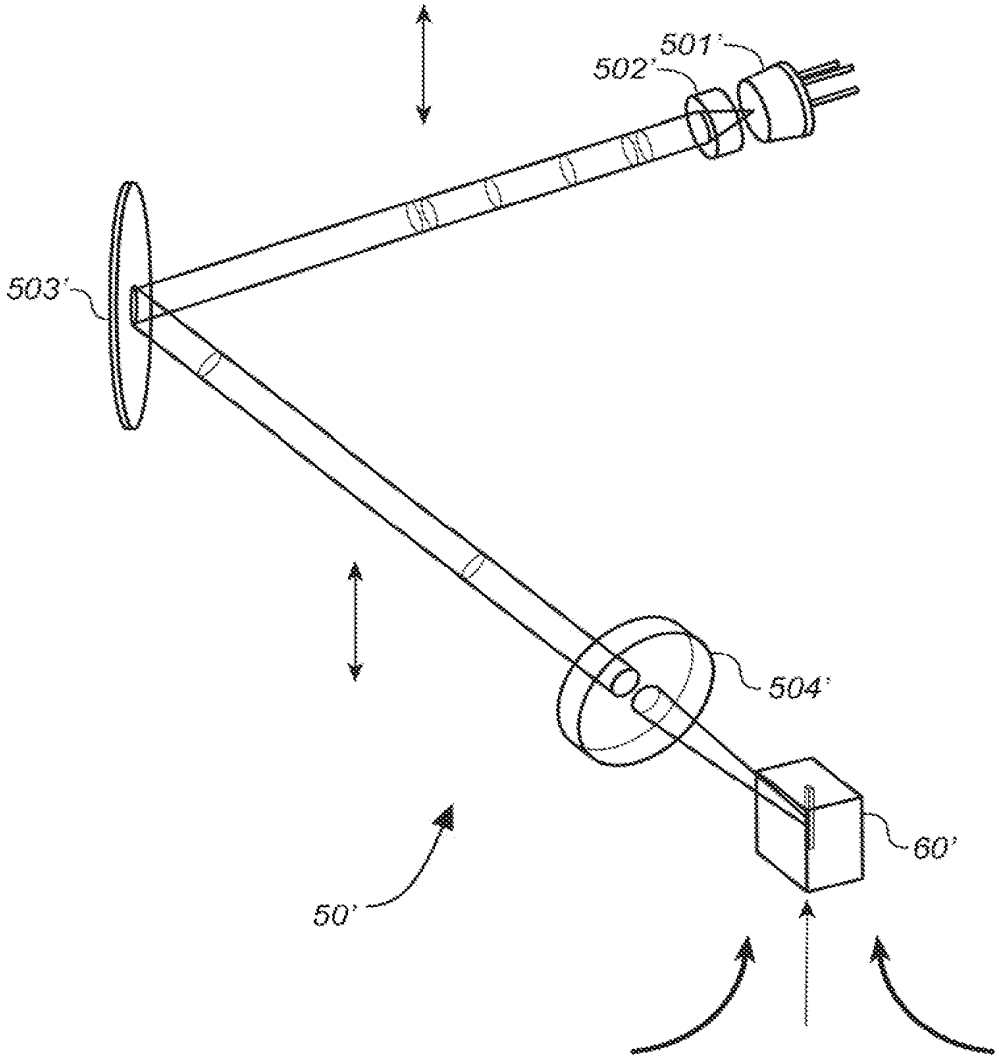


FIG. 3A

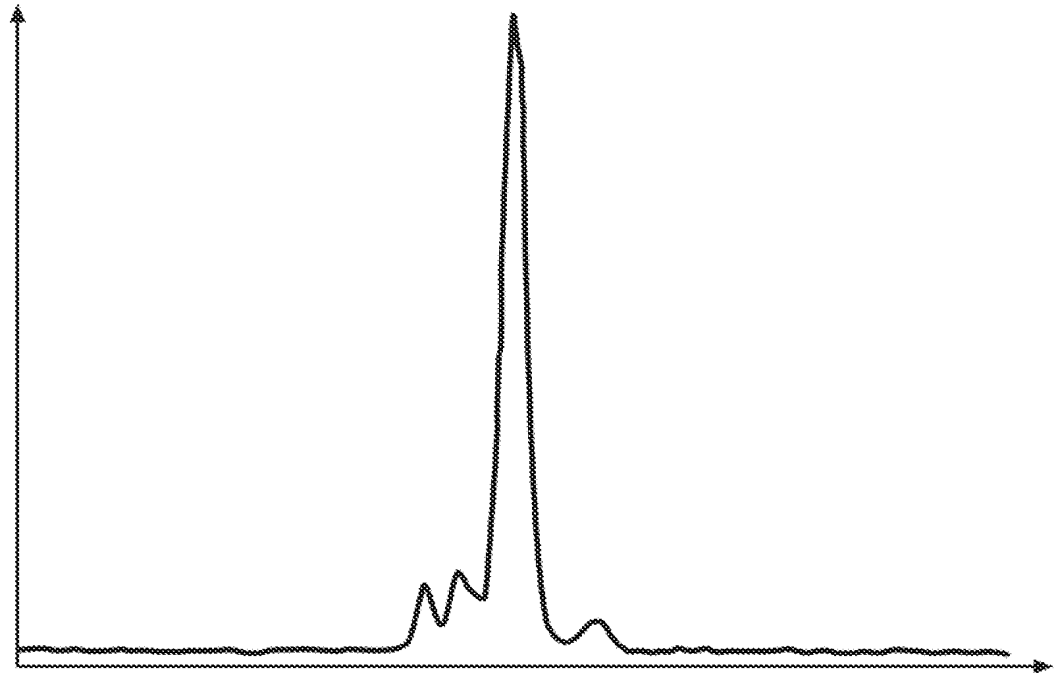


FIG. 3B

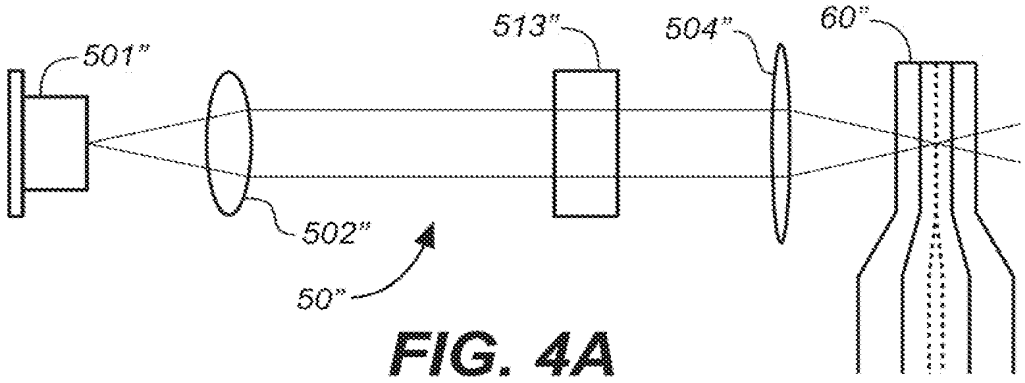


FIG. 4A

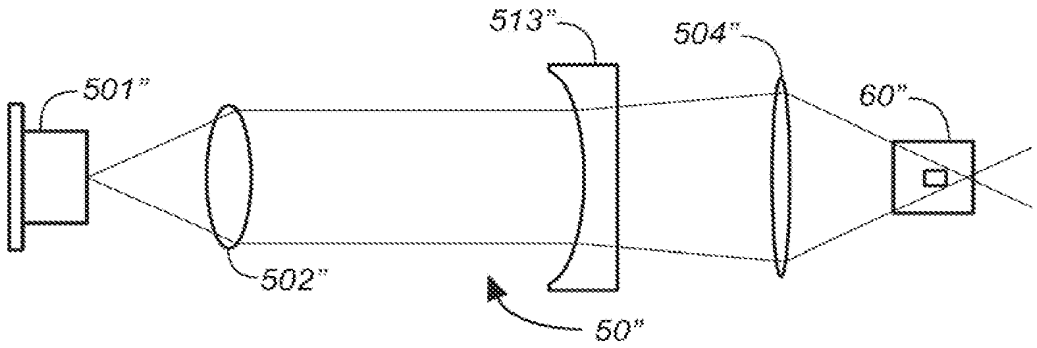


FIG. 4B

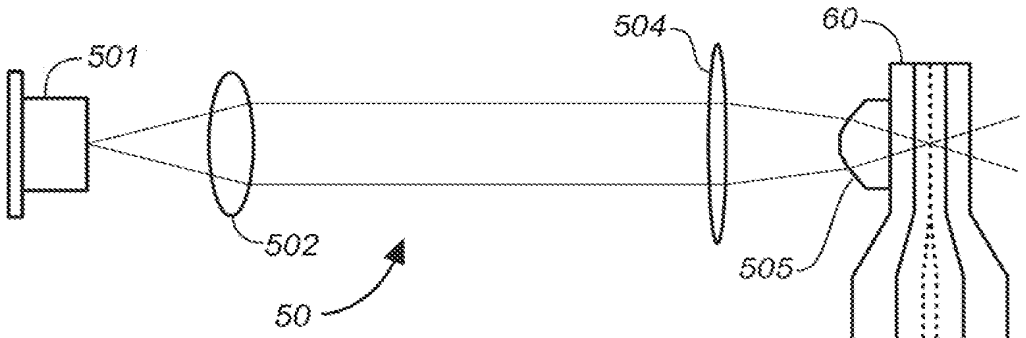


FIG. 5A

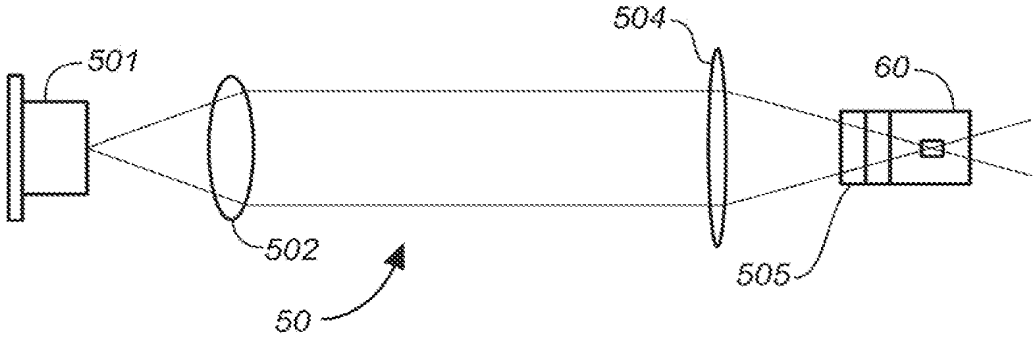


FIG. 5B

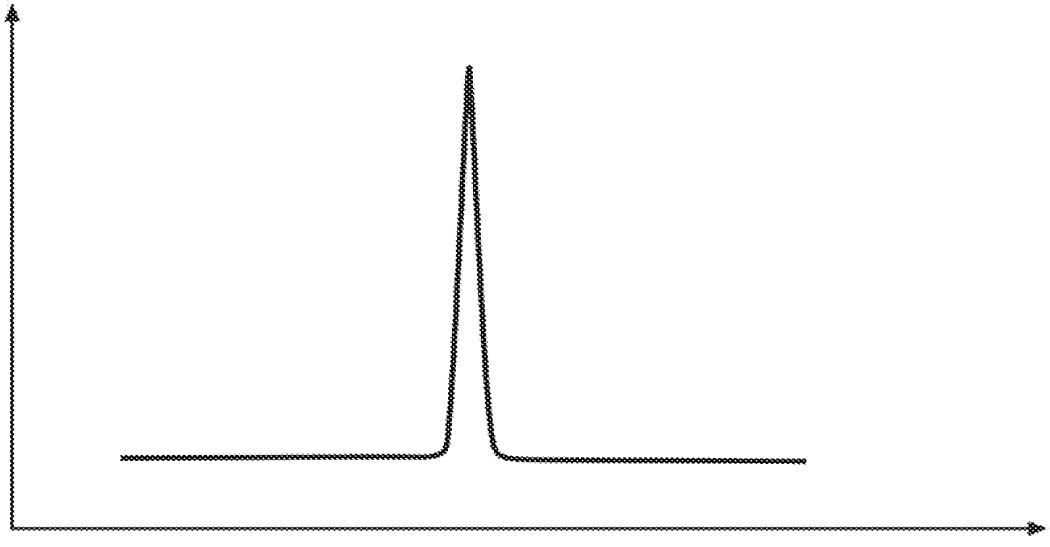


FIG. 5C

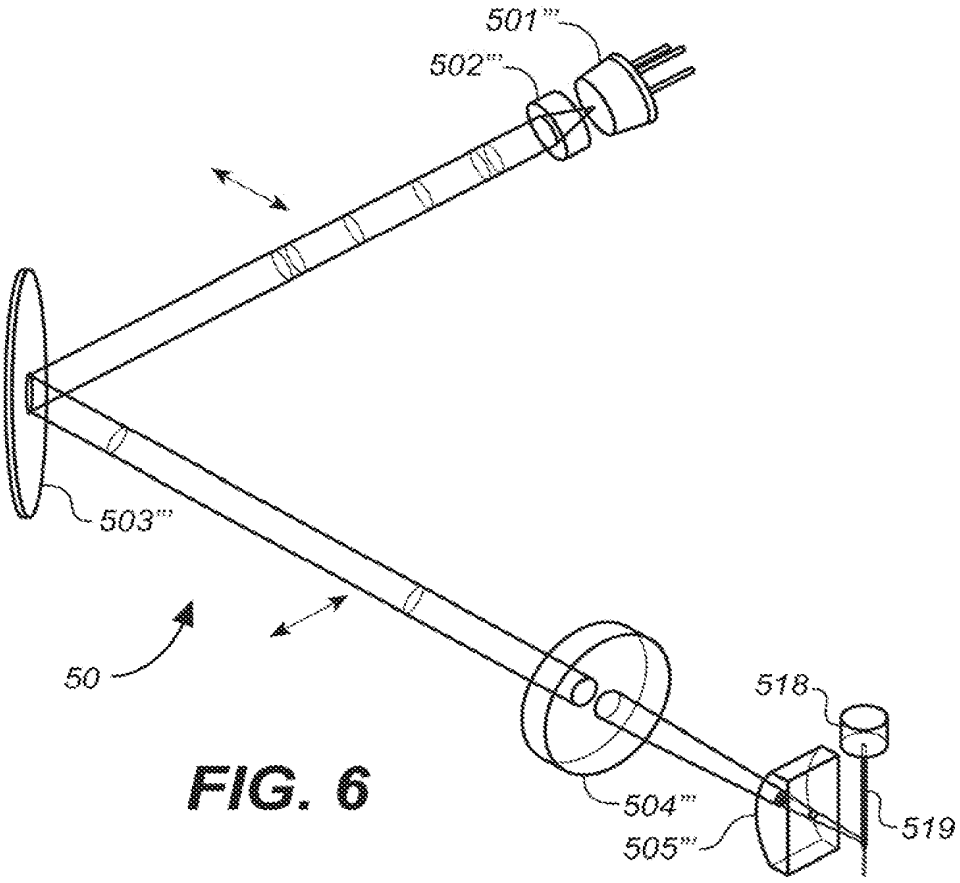


FIG. 6

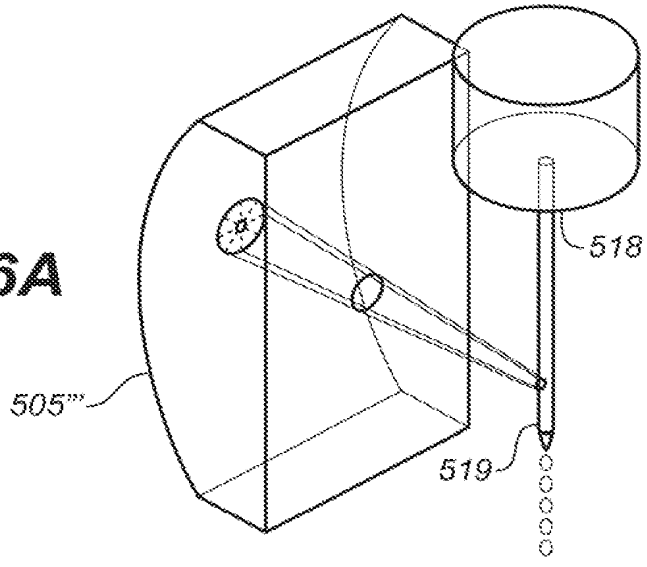


FIG. 6A

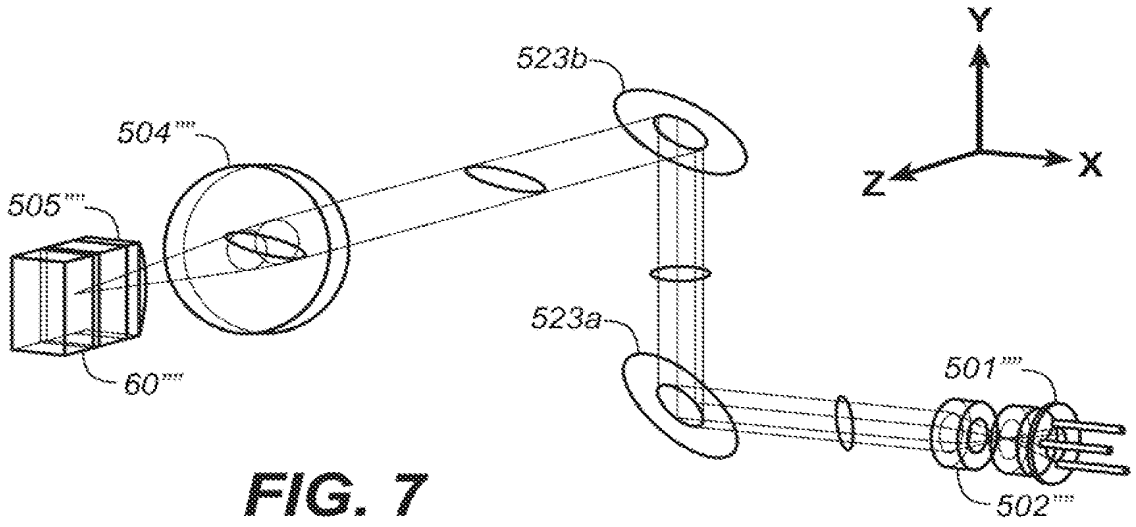


FIG. 7

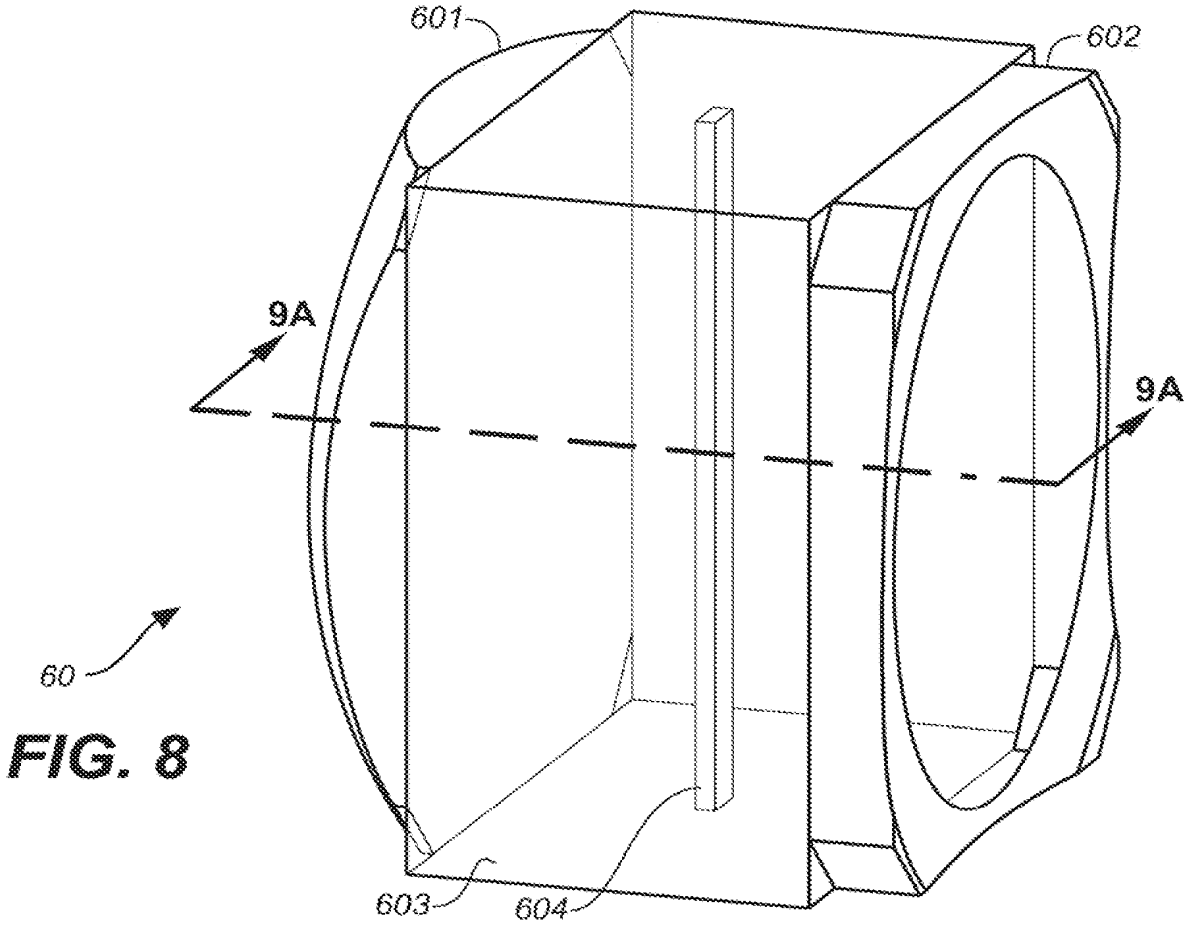


FIG. 8

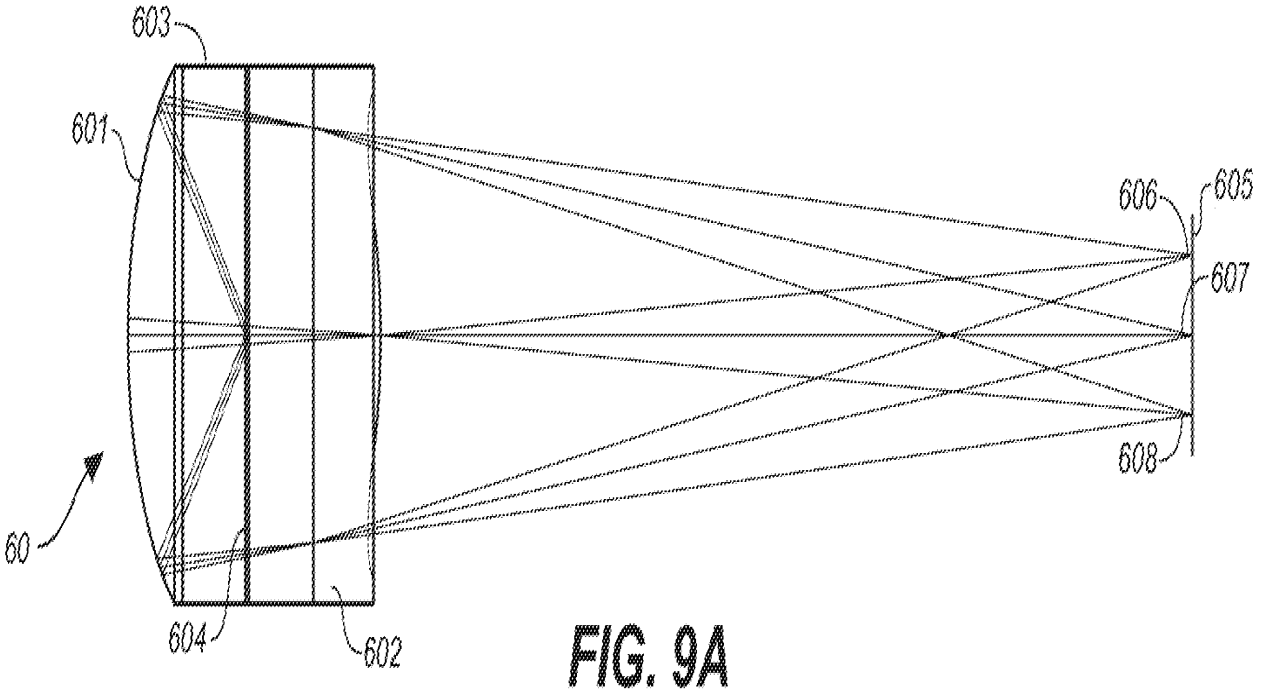


FIG. 9A

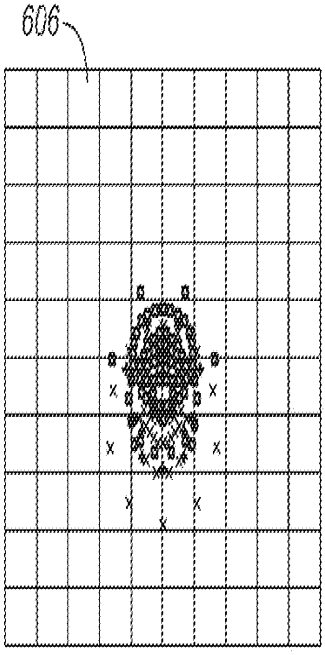


FIG. 9B1

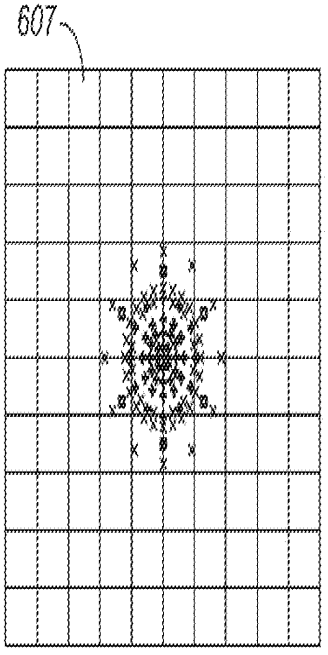


FIG. 9B2

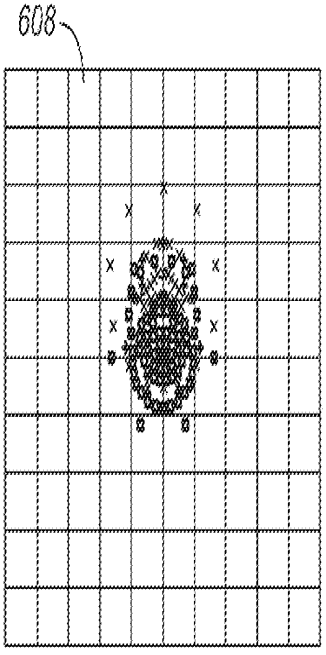


FIG. 9B3

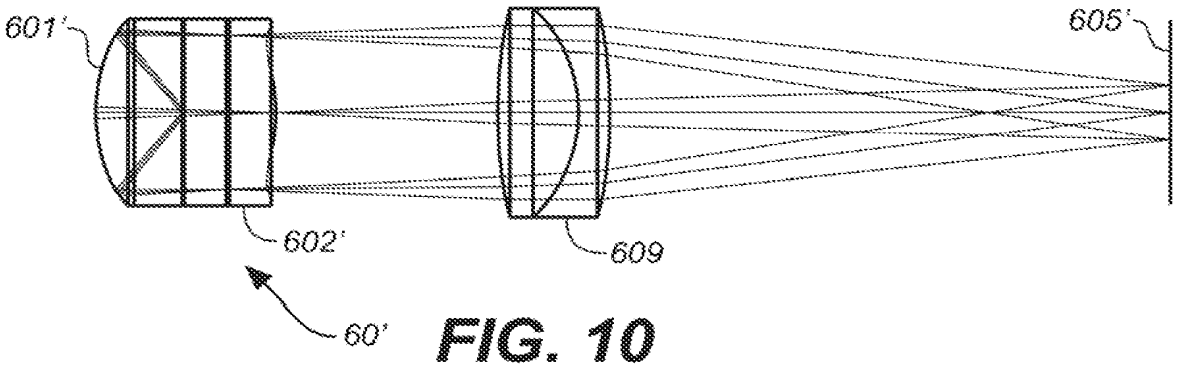


FIG. 10

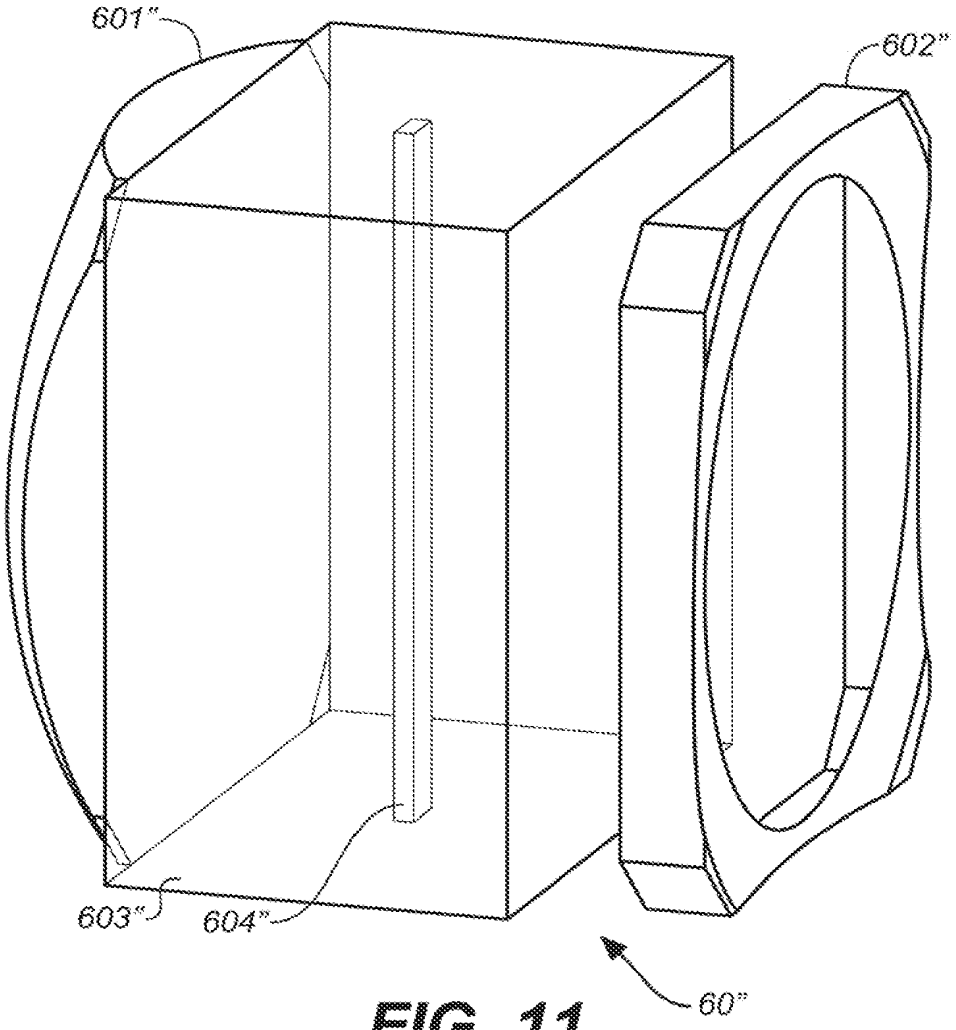


FIG. 11

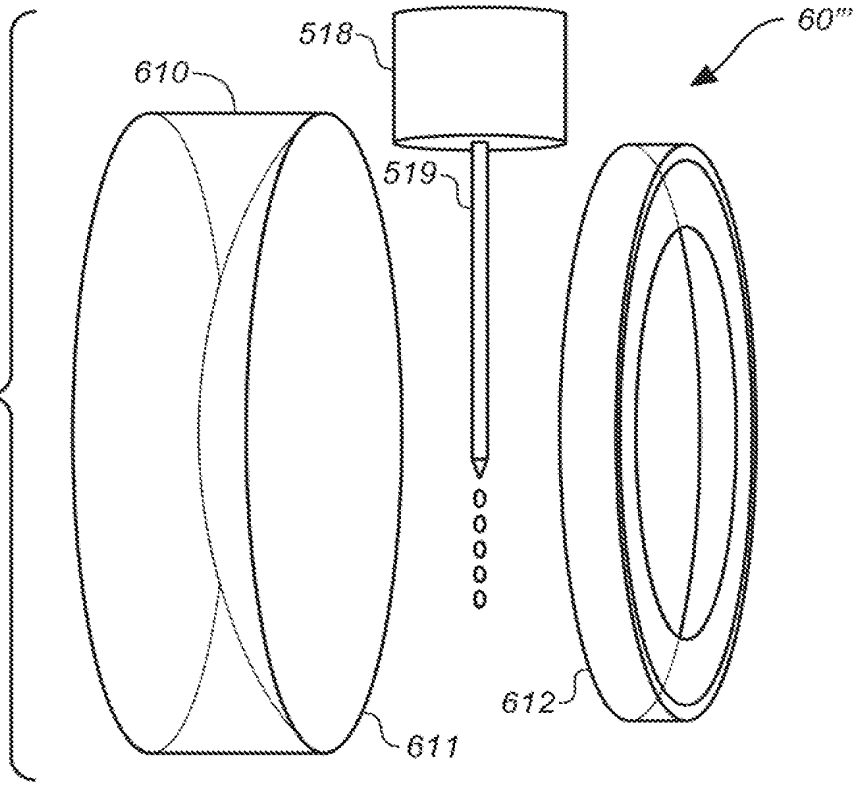


FIG. 12

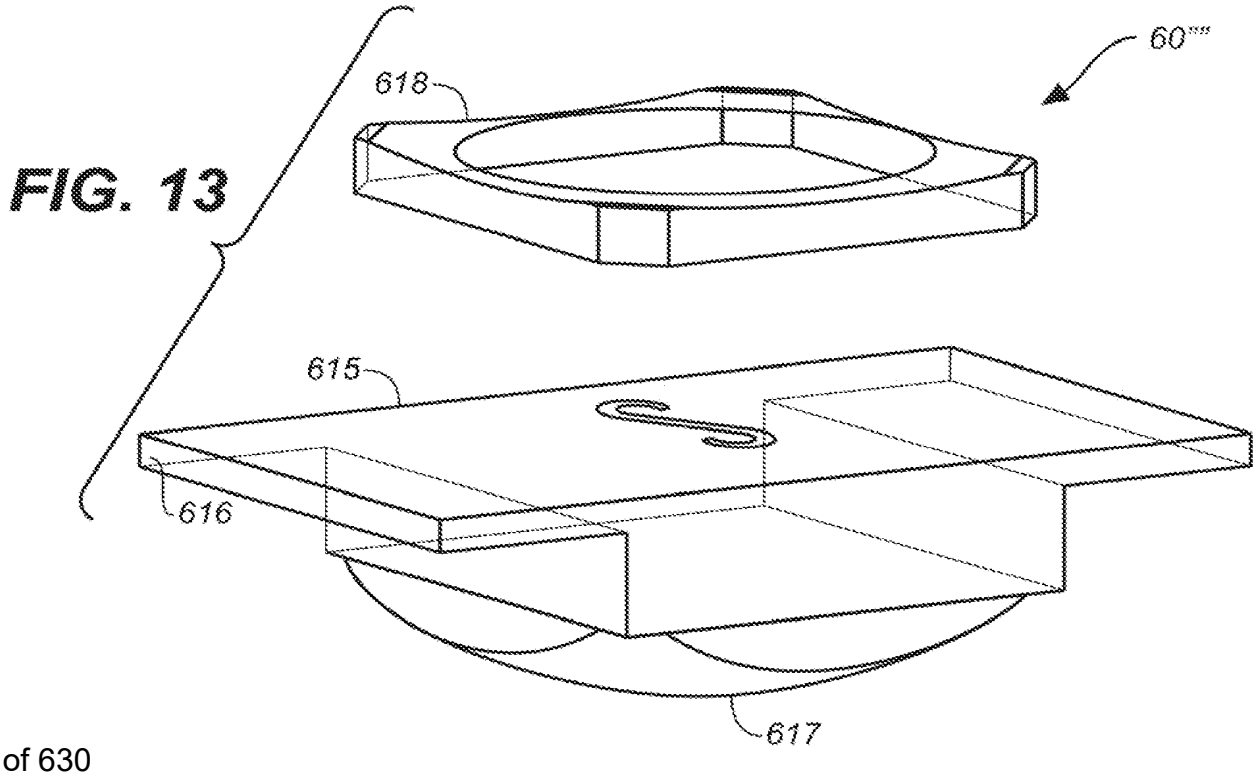


FIG. 13

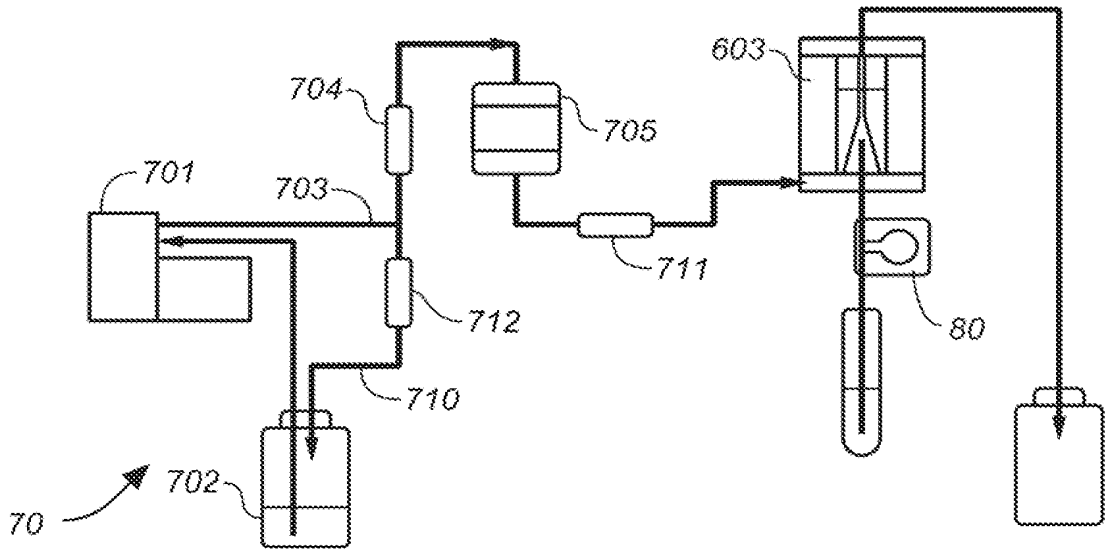

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FIG. 14

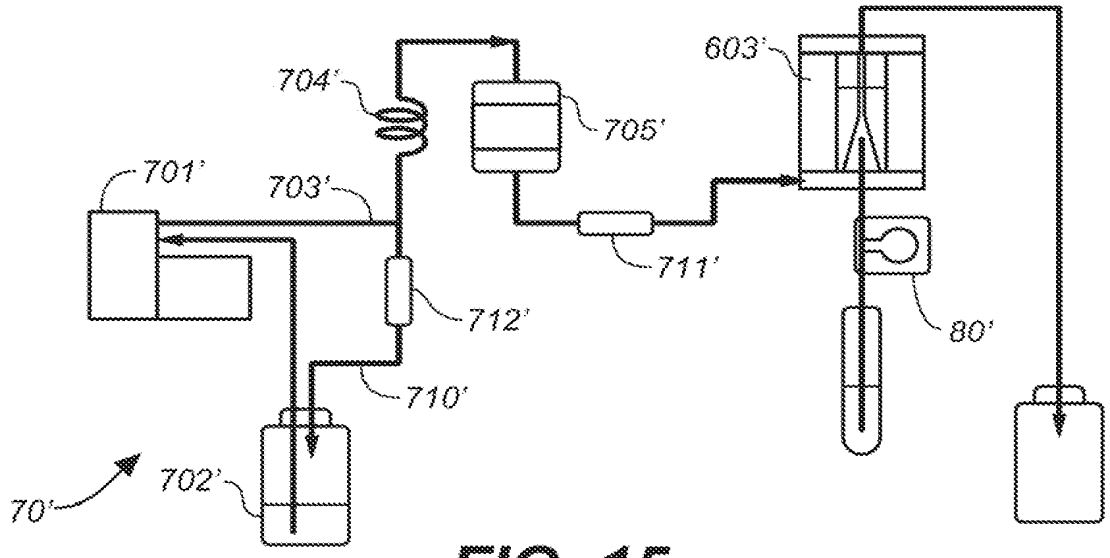

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711'
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80'
710'
702'
70'

FIG. 15

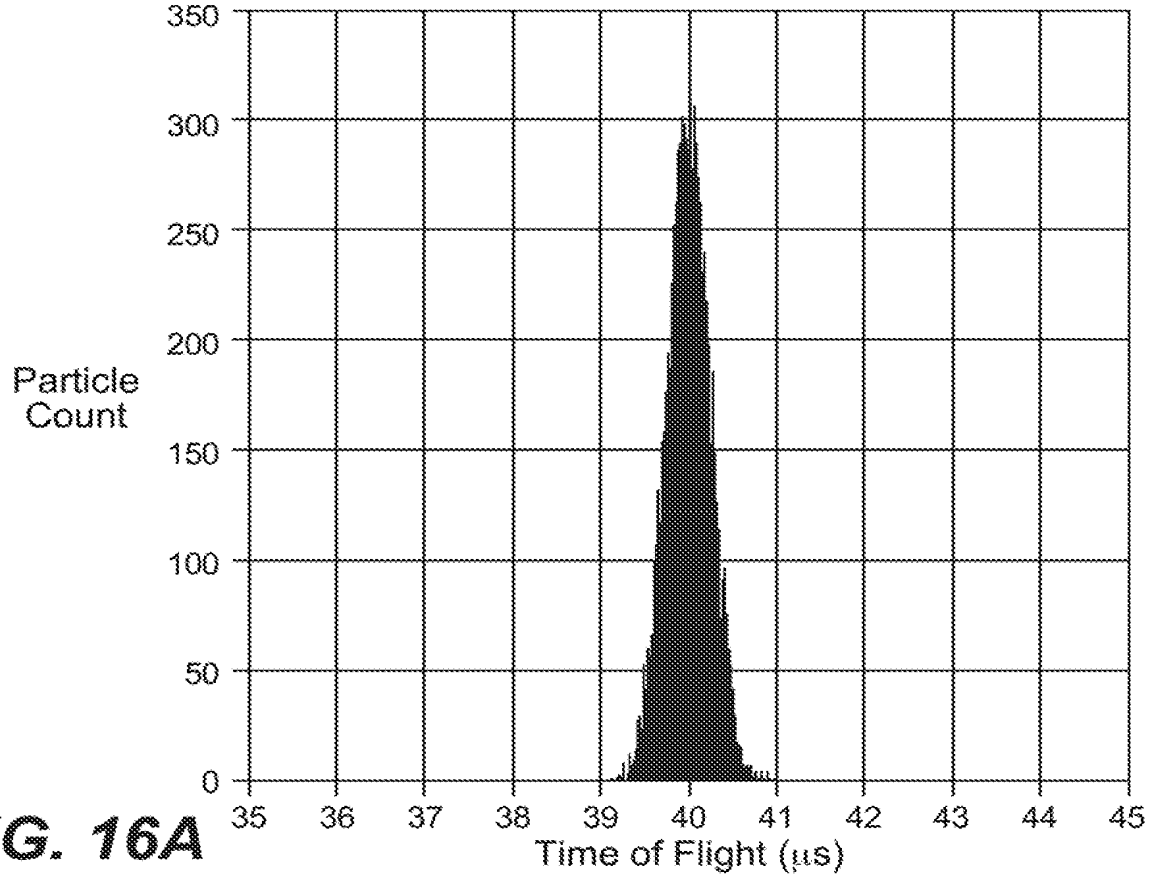


FIG. 16A

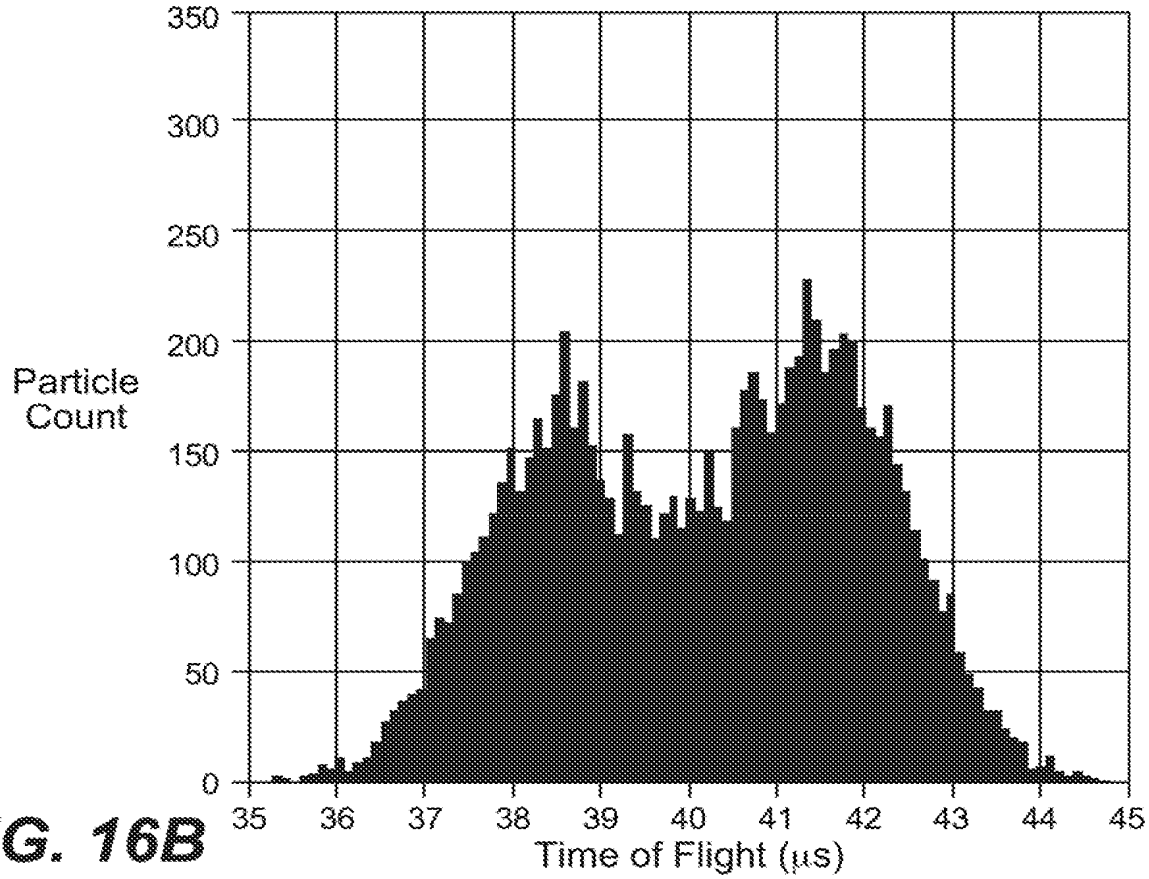


FIG. 16B

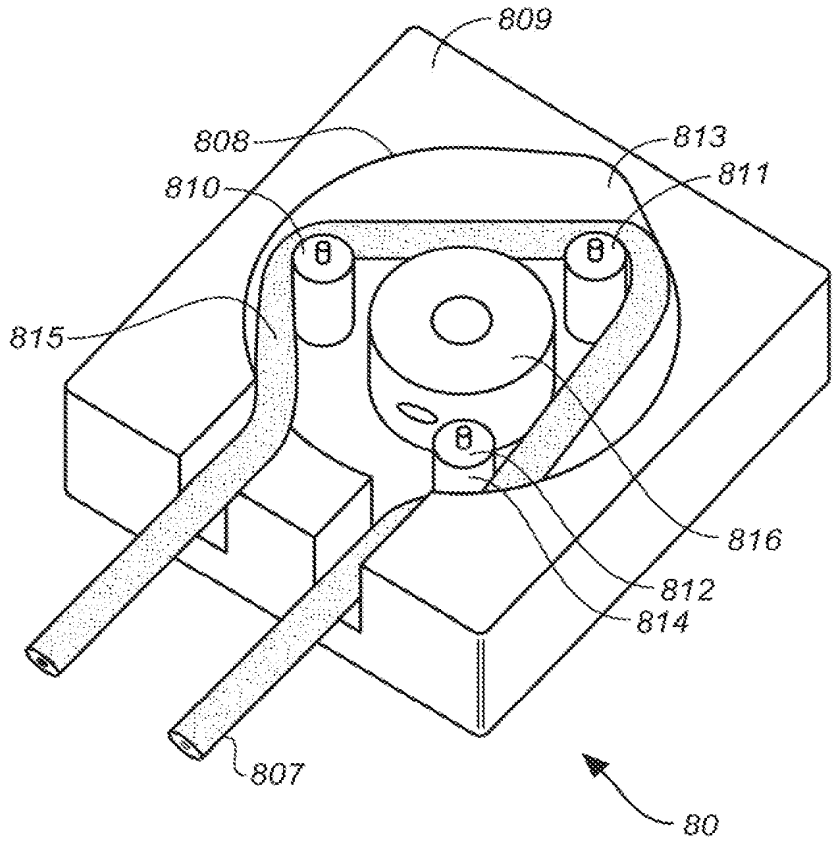


FIG. 17

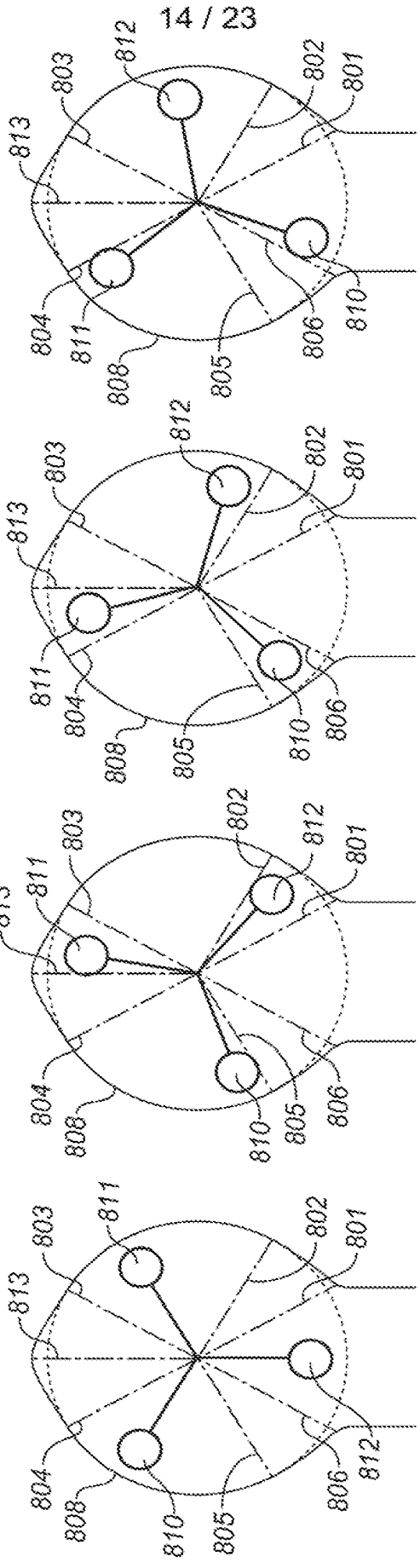


FIG. 18A FIG. 18B FIG. 18C FIG. 18D

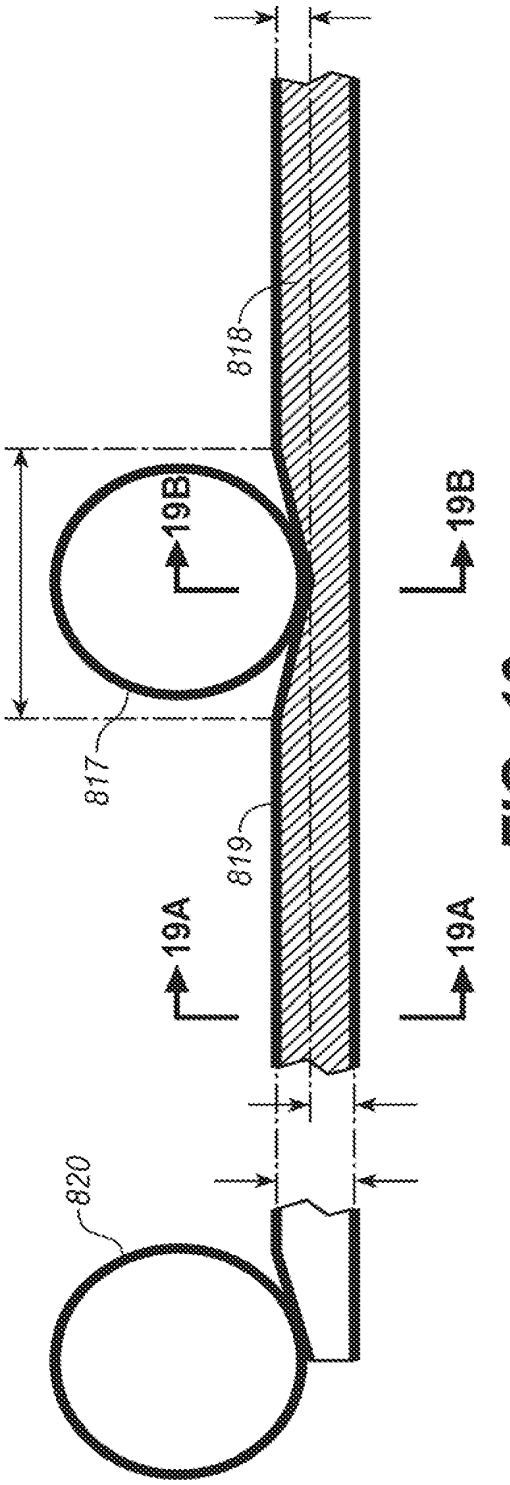


FIG. 19

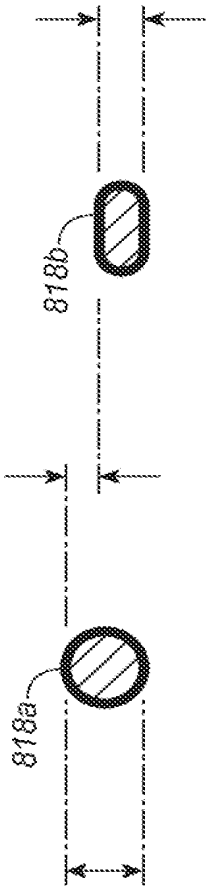


FIG. 19A

FIG. 19B

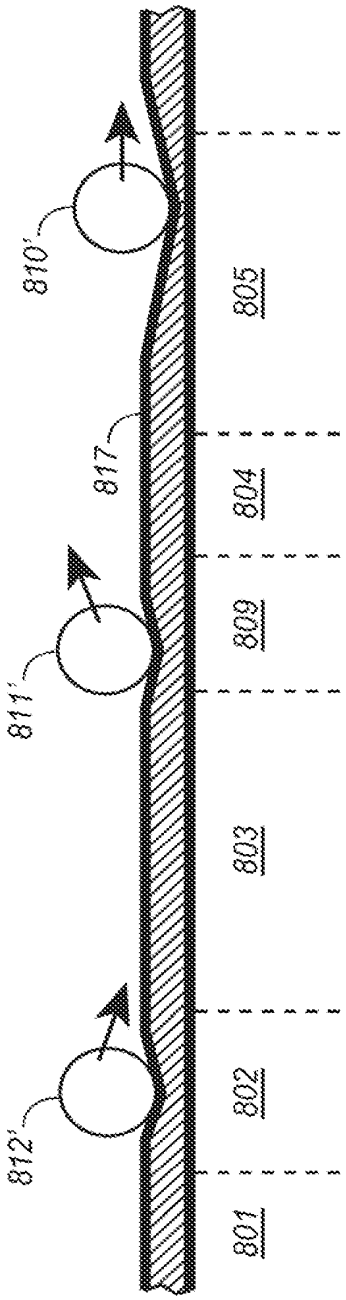


FIG. 20A

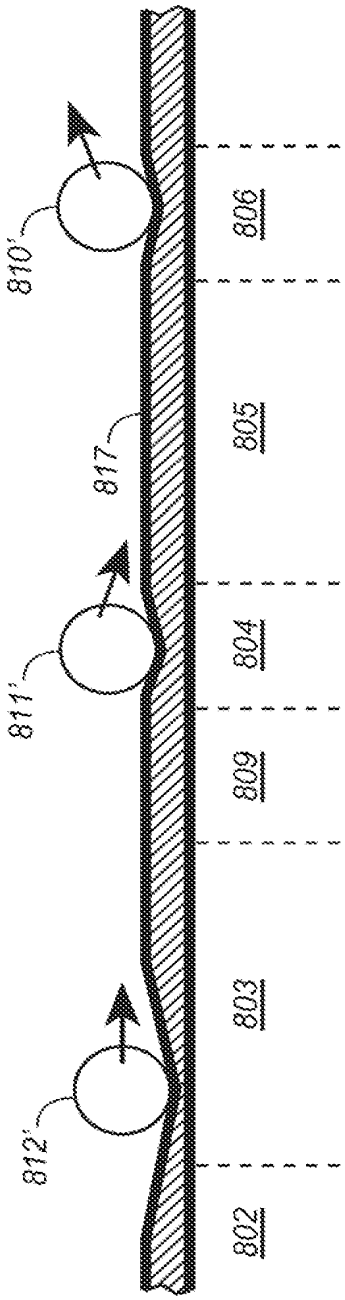


FIG. 20B

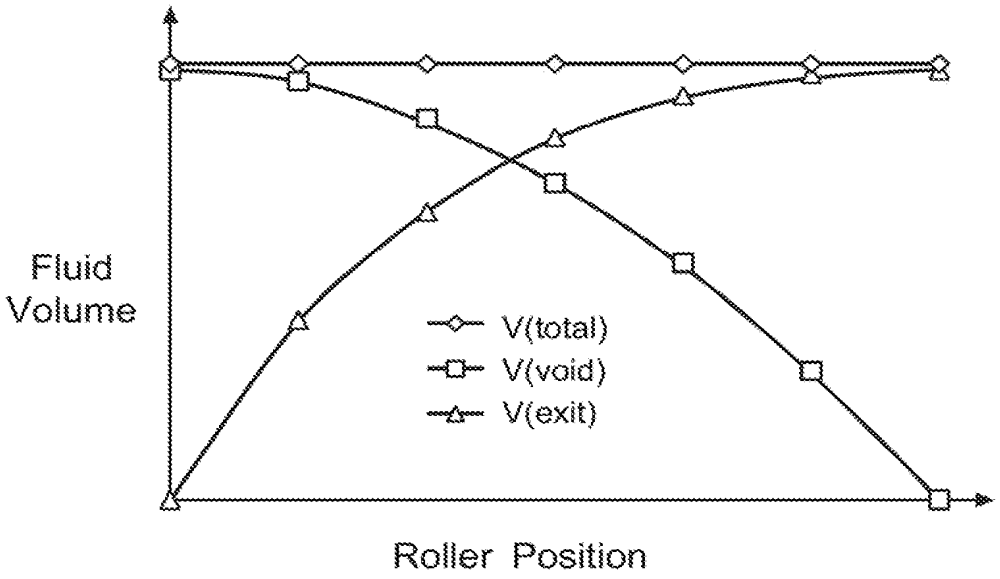


FIG. 21

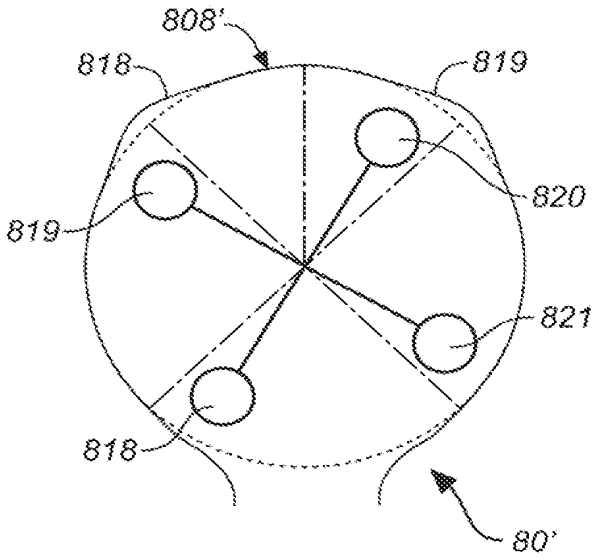


FIG. 22

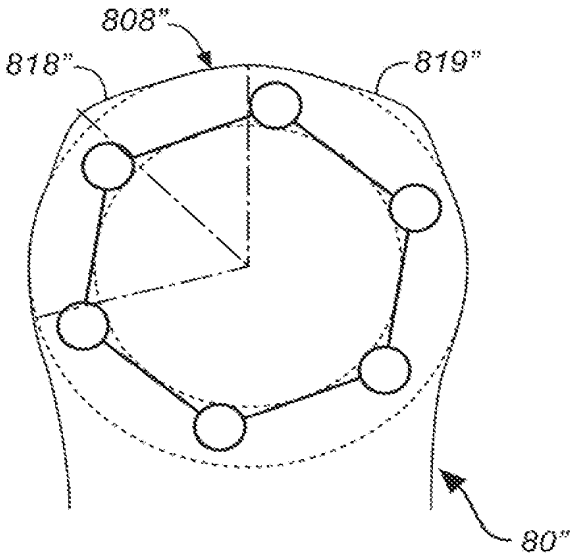


FIG. 23

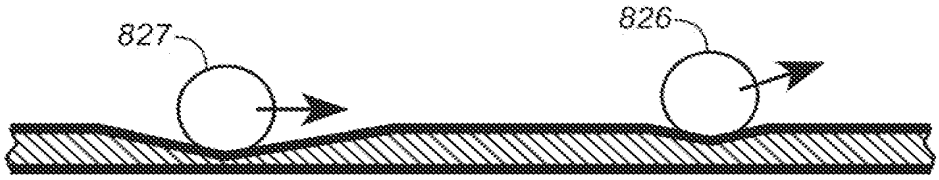


FIG. 24A

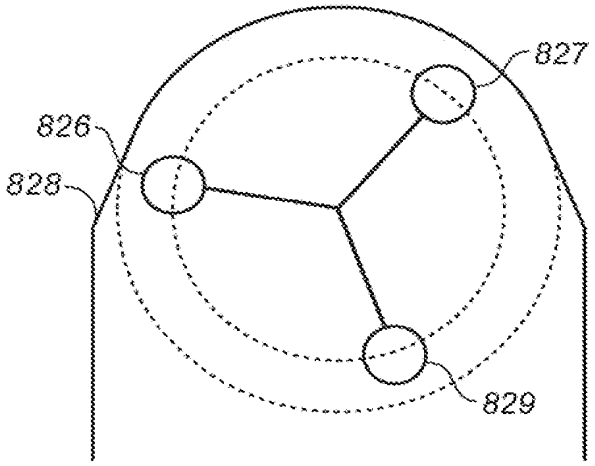


FIG. 24B

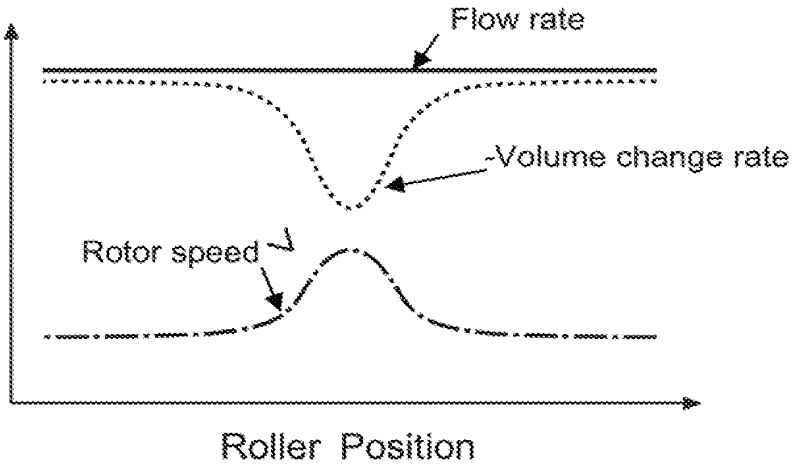


FIG. 24C

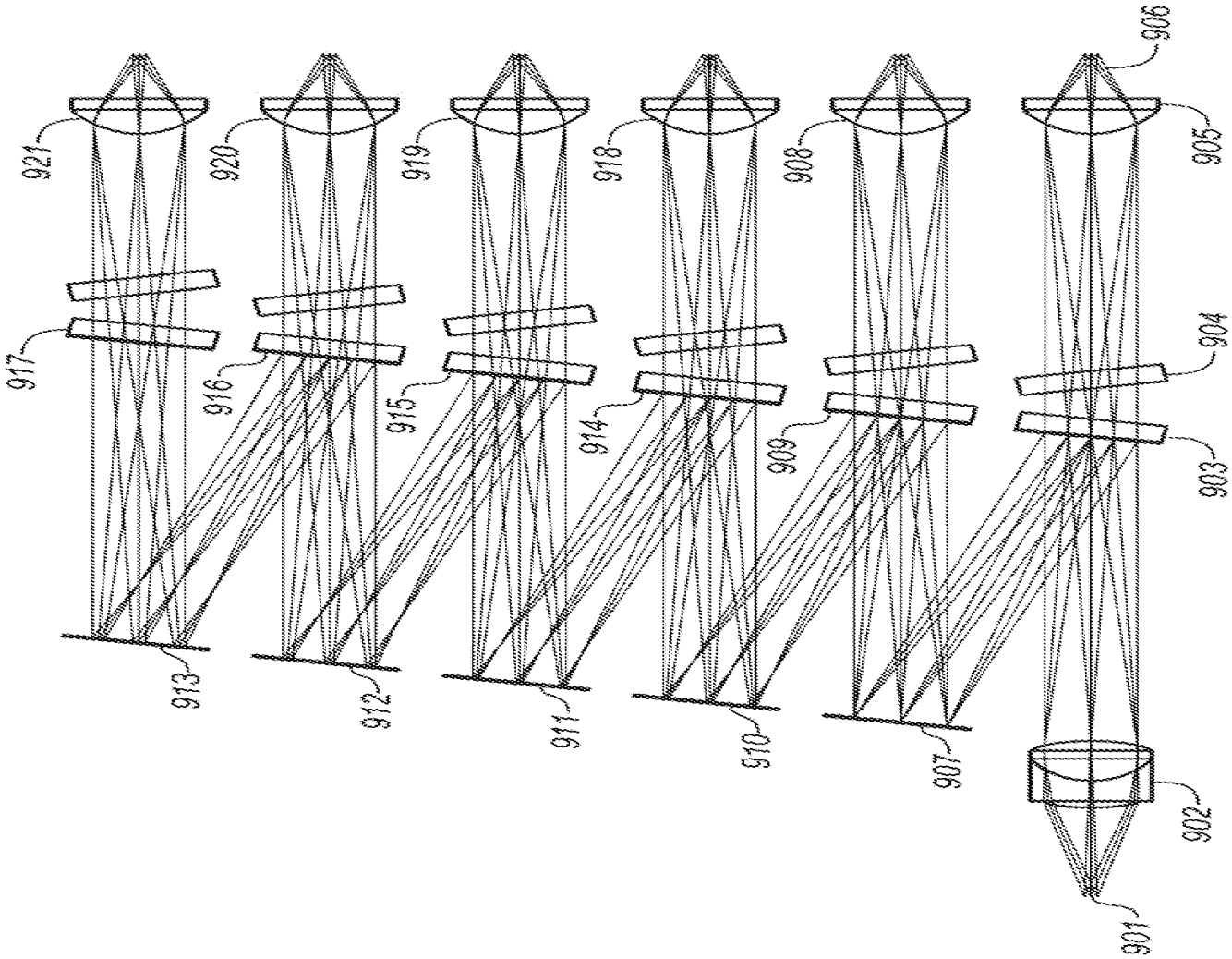

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FIG. 25

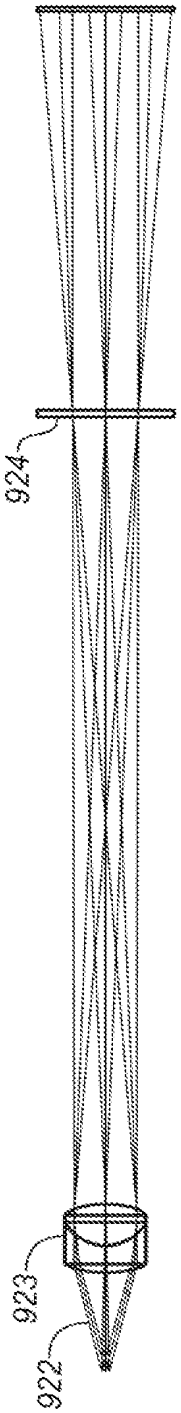


FIG. 26

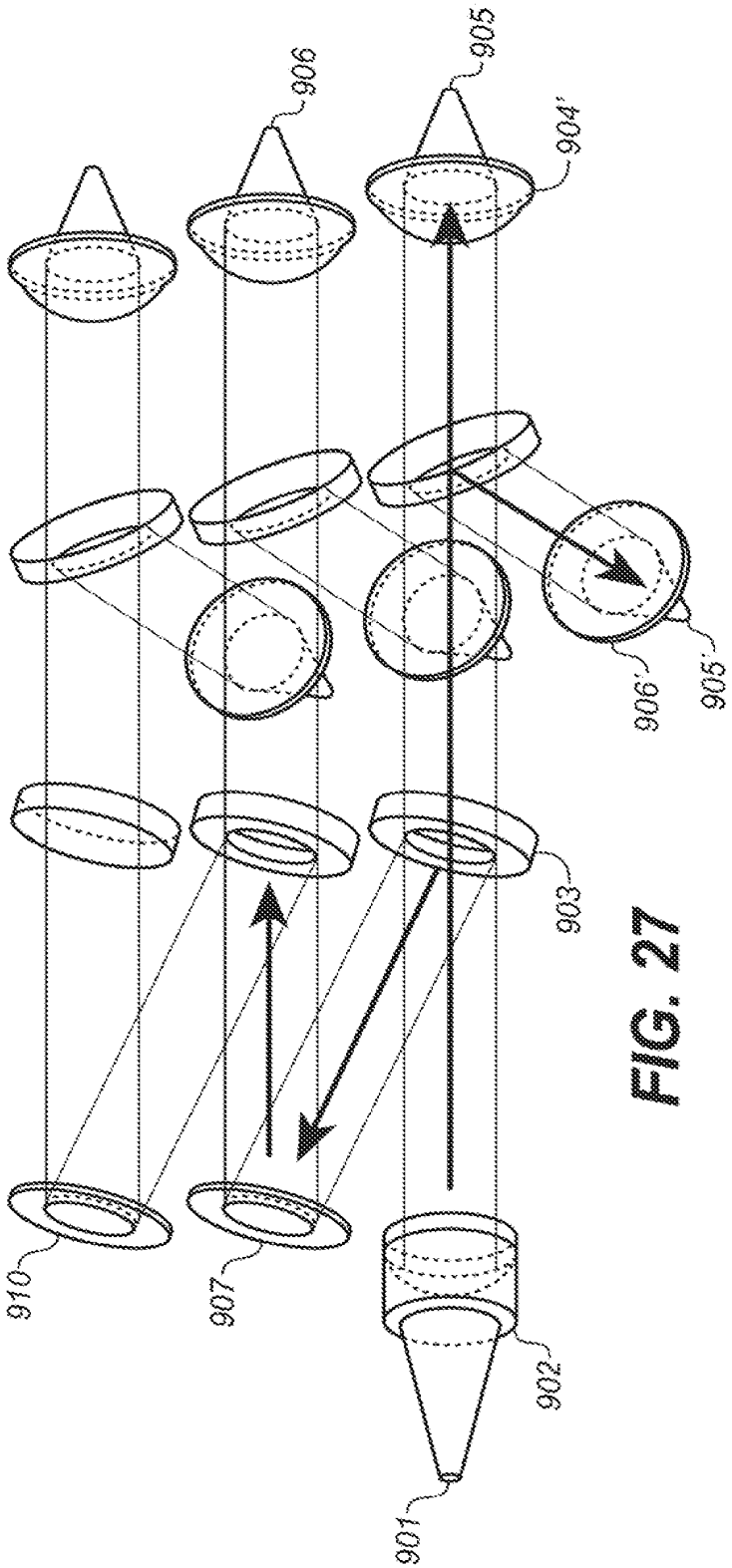


FIG. 27

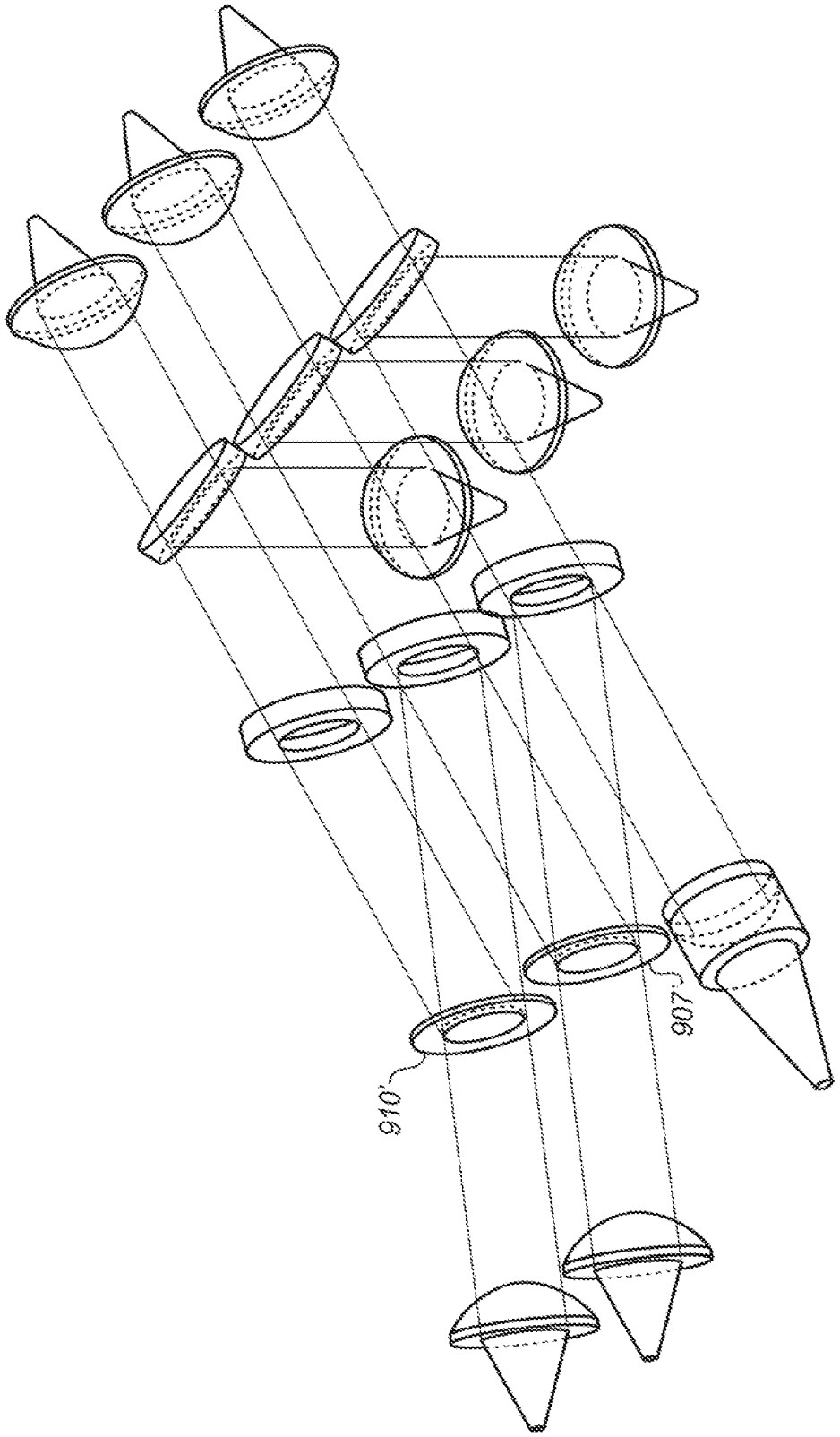


FIG. 28

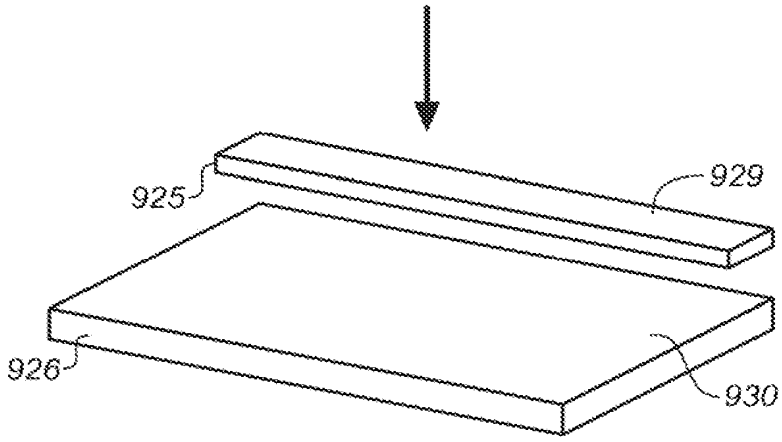


FIG. 29A

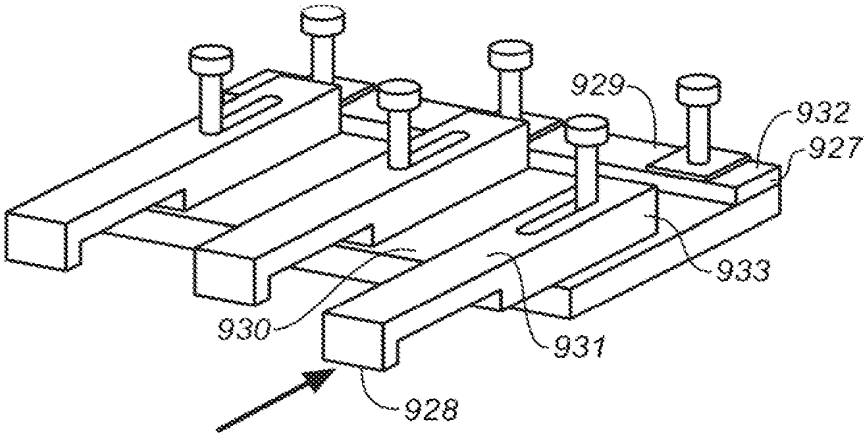


FIG. 29B

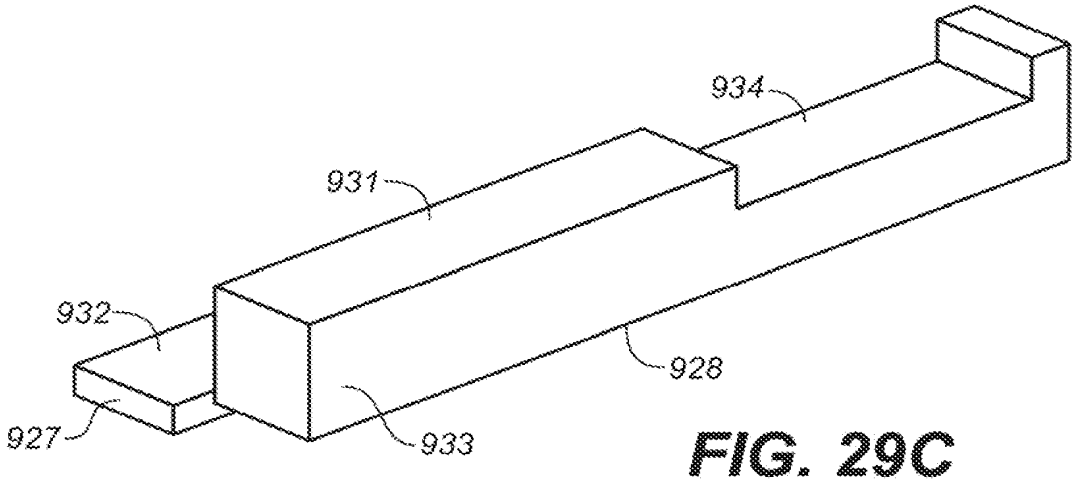


FIG. 29C

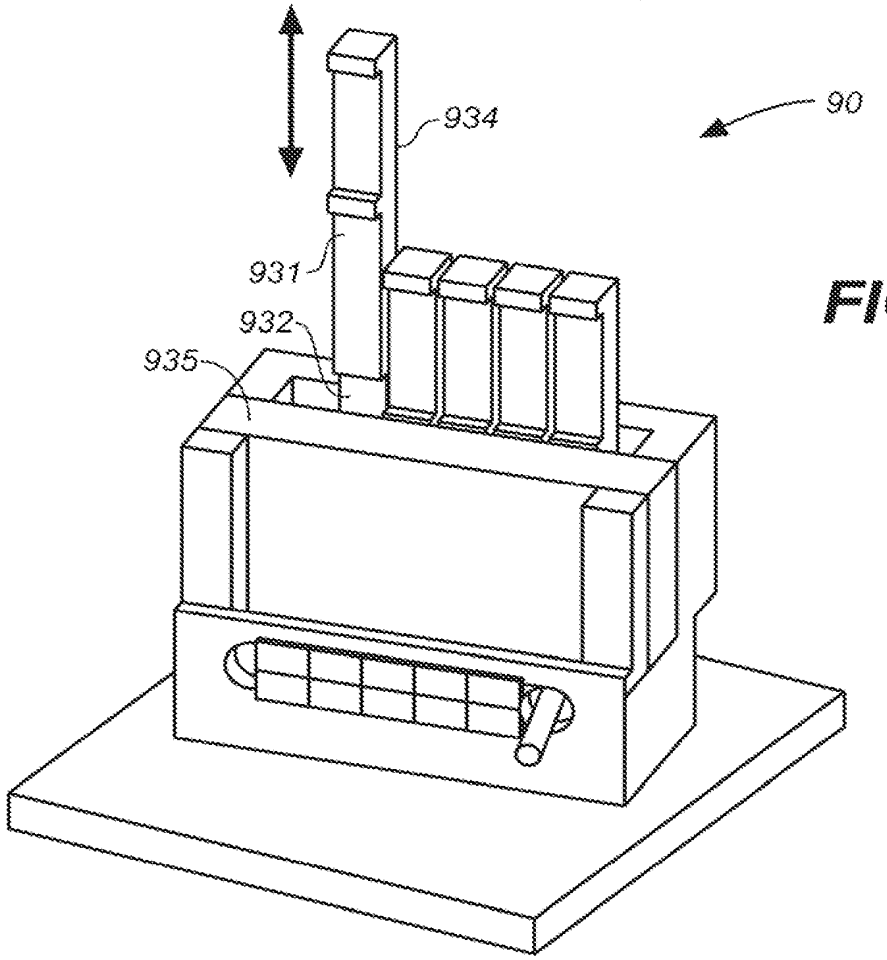


FIG. 30A

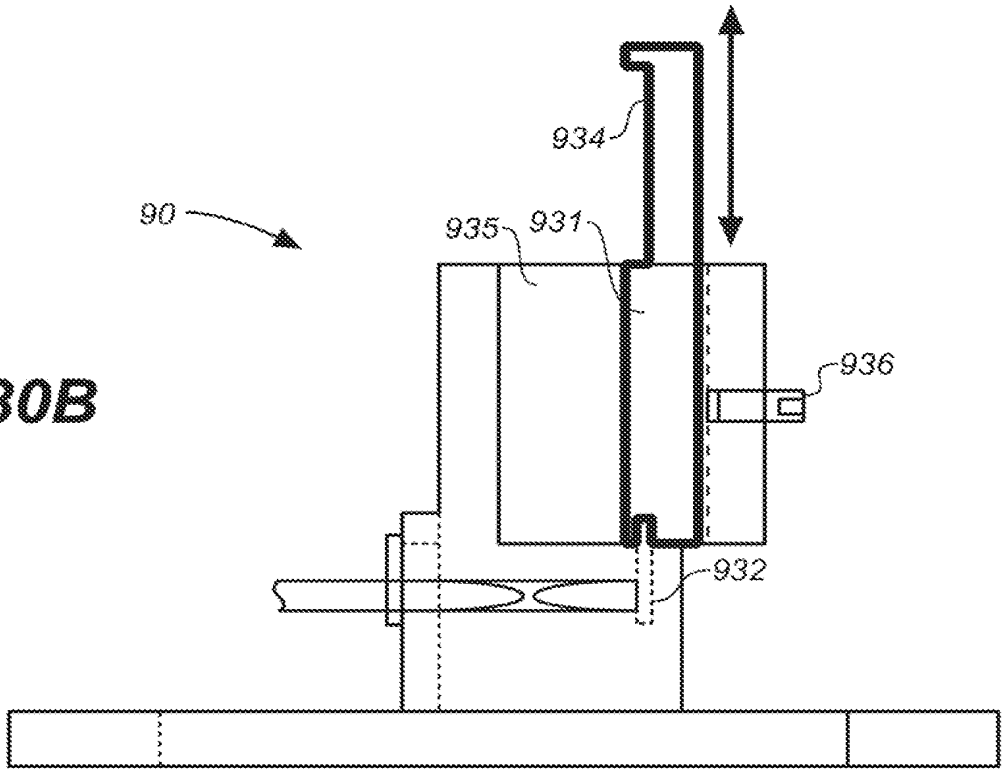


FIG. 30B

Espacenet

Bibliographic data: JP2003505707 (A) — 2003-02-12

SYSTEM FOR MICROVOLUME LASER SCANNING CYTOMETRY

Inventor(s):

Applicant(s):

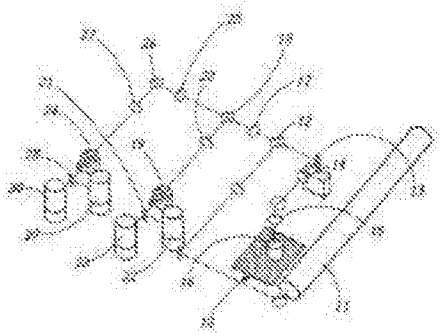
Classification: - international: ***G01N15/00; G01N15/14; G01N21/64; G01N33/483; G06K9/00;*** (IPC1-7): G01N15/00; G01N15/14; G01N21/64; G01N33/483
- cooperative: **G01N15/1475**; **G06K9/00127**; G01N15/1484

Application number: JP20010513096 20000426

Priority number (s): WO2000US11133 20000426 ; US19990144798P 19990721

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Abstract not available for JP2003505707 (A)
Abstract of corresponding document: WO0108081 (A1)



The present invention provides an improved integrated system for biological marker identification. The system uses Microvolume Laser (11) Scanning Microscopy (MLSC) (16) in order to measure patterns of expression of biological markers in biological fluids (10). The system includes improved instrumentation for performing MLSC, and also includes improved particle detection and analysis methods. The system further comprises an informatics architecture for the analysis of data obtained from the MLSC in tandem with other medical information.

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15/00		15/00	Ｂ	２Ｇ０４５
15/14		15/14	Ｃ	
			Ｄ	
33/483		33/483	Ｚ	

審査請求　未請求　予備審査請求　有　（全 69 頁）

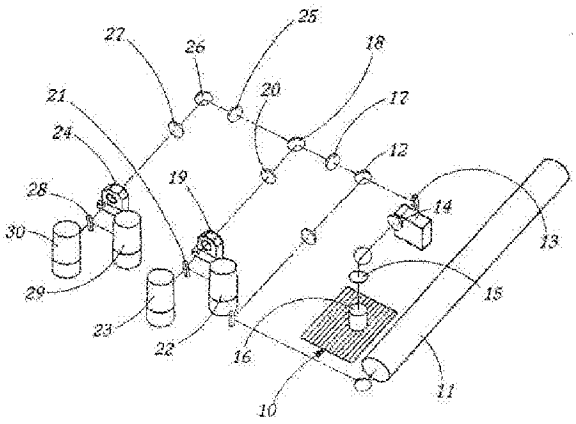
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(32)優先日　平成11年７月21日(1999.7.21)
(33)優先権主張国　米国（ＵＳ）

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最終頁に続く

(54)【発明の名称】　微小体積レーザ・スキャニング・サイトメトリのためのシステム

(57)【要約】
本発明は生物学的マーカ識別用に改良された統合システムを提供する。生物学的流体（１０）の中で生物学的マーカの表現のパターンを測定するために、微小体積レーザ（１１）スキャニング顕微鏡法（ＭＬＳＣ）（１６）を使用する。システムはＭＬＳＣを実行するために改良された計測器を含んでおり、また改良された粒子検出及び分析法も含む。さらにシステムは、他の医療情報と提携してＭＬＳＣから得られたデータを分析するための情報処理システム・アーキテクチャを含む。



【特許請求の範囲】

【請求項１】　蛍光性のバックグラウンドを保持し、バックグラウンド特性を示す固定容積の毛細管において複数の検知可能な特性を持つターゲット粒子を検知し特性を決定するために粒子を含有しているサンプルを分析するための方法であって、

（ａ）　データの各チャンネルは明瞭な検知可能な特性及び明瞭なバックグラウンド特性を含み、サンプルを含んでいる固定容積の毛細管を走査してデータの複数のチャンネルを生成するステップと、、

（ｂ）　データのチャンネルの各々をサンプリングして対応する組のピクセル値を作成するステップと、

（ｃ）　各組のピクセル値を個別に修正することによって増強されたピクセル値の組を生成し、ターゲット粒子を示す空間の特徴を選択的に増強するステップと、

（ｄ）　１つ以上の組の増強されたピクセル値から、対応するチャンネルの明瞭なバックグラウンド特性を取り除くステップと、

（ｅ）　増強された各組のピクセル値に対して、前記粒子を検出するためのノイズ閾値を別々に確立するステップと、

（ｇ）　増強された各組のピクセル値において、前記粒子の診断パターンに配置された上記閾値ピクセルのグループを別々に識別するステップと、

（ｈ）　特定の組の増強されたピクセル値において診断パターンに配置された上記閾値ピクセルの各グループに対して、残りの組の増強されたピクセル値における対応する閾値より下あるいは閾値のピクセルを別々に識別するステップと、

（ｉ）　ステップ（ｇ）及び（ｈ）において別々に識別されたピクセルの分析によって、サンプル内のターゲット粒子の特性を決定するステップと

を含み、

粒子は、前記粒子の診断に用いるパターンに配置された上記閾値ピクセルにより、チャンネルにおいて最初に識別され分析され、その後、最初に識別された上記閾値ピクセルと同じ位置にピクセルを配置することにより、残りのすべてのチャンネルにおいて前記粒子は別々に分析されることを特徴とする方法。

【請求項２】　励起光による照明において複数の波長成分を備えた光を発する粒子を含有しているサンプル上で微小体積レーザ・スキャニング・サイトメトリ（ＭＬＳＣ）を実行するための器具において、

（ａ）　前記励起光のための１つ以上の光源と、

（ｂ）　複数の所定の走査速度で前記励起光を前記サンプル上に走査するための手段と、

（ｃ）　前記励起光に応答して前記サンプルによって放射された光を収集し走査解除するための手段と、

（ｄ）　前記放射光線を複数の明瞭な成分波長に分け、各々の前記明瞭な成分波長を別々の光学通路に沿って方向付けるための複数のダイクロイック・フィルタ手段と、

（ｅ）　放射光線に反応し、明瞭な成分波長を収集するために配置され、可変帯域幅を備えたアナログ・フィルタ手段に動作的に連結される、複数の光検出器と、

（ｆ）　各アナログ・フィルタ手段に効果的に連結され、かつ各々の光検出器からのデジタル信号出力を与えるために複数の所定のデジタル・サンプリング・レートで操作することができる、デジタル・サンプリング手段と

を含み、

前記走査速度、各前記アナログ・フィルタ手段の帯域幅、及びデジタル・サンプリング・レートは、前記粒子の検出を最適化するために統合的に調整されることができることを特徴とする器具。

【請求項３】　励起光を備えた照明上に少なくとも４つの明瞭な波長成分を備えた光を発する微粒子を含有しているサンプル上で微小体積レーザ・スキャニング・サイトメトリ（ＭＬＳＣ）を実行するための器具であって、

（ａ）　前記励起光のための１つ以上の光源と、

（ｂ）　前記励起光を前記サンプル上に走査するための手段と、

（ｃ）　前記励起光に応答して前記サンプルによって放射された光を収集しスキャニング解除するための手段と、

（ｄ）　前記放射光線を少なくとも４つの明瞭な成分波長に分け、各々の前記

明瞭な成分波長を別々の光学通路に沿って方向付けるための複数のダイクロイック・フィルタ手段と、

（ｅ）　各光検出器が明瞭な成分波長を収集するために配置され、デジタル・サンプリング手段に効果的に連結される、放射光線に反応する少なくとも４つの光検出器と

を含むことを特徴とする器具。

【請求項４】　器官から得られるサンプル上で微小体積レーザ・スキャニング・サイトメトリ（ＭＬＳＣ）を実行するための統合システムであって、前記サンプルが、励起光を備えた照明上に複数の波長成分を備えた光を発する微粒子を含有する、統合システムにおいて、

（ａ）　前記励起光のための１つ以上の光源と、

（ｂ）　複数の所定の走査速度で前記励起光を、前記サンプル上に走査するための手段と、

（ｃ）　前記励起光に応じて前記サンプルによって照射された光を収集しスキャニング解除するための手段と、

（ｄ）　前記放射光線を複数の明瞭な成分波長に分け、各々の前記明瞭な成分波長を別々の光学通路に沿って方向付けるための複数のダイクロイック・フィルタ手段と、

（ｅ）　各光検出器が明瞭な成分波長を収集するために配置され、変更可能な帯域幅を備えたアナログ・フィルタ手段に効果的に連結される、放射光線に反応する複数の光検出器と、

（ｆ）　各アナログ・フィルタ手段に効果的に連結され、かつ各々の光検出器からのデジタル信号出力を提供するために多くの所定のデジタル・サンプリング・レートで操作することができる、デジタル・サンプリング手段と、

（ｇ）　１つ以上のコンピュータからなる制御及びデータ分析システムであって、

（ｉ）　各々の臨床プロトコルが、

前記粒子を最適に検出するために必要とされる走査速度と、

前記粒子を最適に検出するために必要とされる各々の前記アナログ・フィルタ

手段の帯域幅と、

前記粒子を最適に検出するために必要とされるデジタル・サンプリング速度と、

前記サンプルの分析条件と、

前記器官の医療歴と、

前記デジタル信号出力の分析用アルゴリズムと

に関する情報を含む臨床プロトコルを格納するステップと、

（ii）　前記臨床プロトコルからの情報を用いて、前記走査する手段、前記アナログ・フィルタ手段、及びデジタル・サンプリング手段を制御するステップと、

（iii）　前記デジタル信号出力を受け取り、格納するステップと、

（iv）　前記粒子データに関するデータを提供するために、前記臨床プロトコルからの情報を用いて、前記デジタル信号出力を分析するステップと、

（v）　医療条件に関する情報を、前記微粒子に関するデータに格納するステップと、

（vi）　（v）からの情報を用いて、前記器官の医療条件を決定するステップと、

（7）　前記粒子に関するデータに、医療条件に関係する新情報を生成するステップと

を行う能力がある、制御及びデータ分析システムと

からなることを特徴とする統合システム。

【請求項５】　蛍光性のバックグラウンドを保持し、バックグラウンド特性を示す、固定容積の毛細管内で、複数の検知可能な特性を持つターゲット粒子を検知し特徴を決定するために、粒子を含有しているサンプルを分析するための方法において、前記方法は

（ａ）　データの複数のチャンネルを生成するように、サンプルを含んでいる固定容積の毛細管を走査するステップであって、データの各チャンネルが明瞭な検知可能な特性、及び明瞭なバックグラウンド特性を含む、ステップと、

（ｂ）　対応する組のソース・ピクセル値を作成するように、データのチャン

ネルの各々をサンプリングするステップと、

（ｃ） 合成イメージを生成するためにソース・ピクセル値の組を合計するステップと、

（ｄ） 前記合成イメージ内の粒子検出用の閾値を計算するステップと、

（ｅ） 前記閾値を用いて、前記合成イメージ内の粒子検出を実行するステップと、

（ｆ） 前記合成イメージ内で識別された各粒子に対して、ソース・ピクセル値の組内の対応するピクセルを識別するステップと、

（ｇ） ステップ（ｆ）で識別されたピクセルを分析するステップと

を含み、改良点が、

（１） ソース・ピクセル値の各組内で、粒子検出用の閾値を別々に計算するステップと、

（２） ソース・ピクセル値の各組内で、対応する閾値を用いて、粒子検出を別々に実行するステップと、

（３） ステップ（２）におけるソース・ピクセル値の特定の組内で識別された各ピクセルに対して、ソース・ピクセル値の残りの組内の対応するピクセルを識別するステップと、

（４） ステップ（２）及び（３）で識別されたピクセルを分析するステップと

を含むことを特徴とする方法。

【請求項６】 蛍光性のバックグラウンドを保持し、バックグラウンド特性を示す、固定容積の毛細管内で、複数の検知可能な特性を持つターゲット微粒子を検知するために、微粒子を含有しているサンプルを分析するための方法において、前記方法が

（ａ） データの複数のチャンネルを生成するように、サンプルを含んでいる固定容積の毛細管を走査するステップであって、データの各チャンネルが明瞭な検知可能な特性、及び明瞭なバックグラウンド特性を含む、ステップと、

（ｂ） ソース・ピクセル値の対応する組を作成するように、データのチャンネルの各々をサンプリングするステップと、

（ｃ） 合成イメージを生成するためにソース・ピクセル値の組を合計するステップと、

（ｄ） 前記合成イメージ内の微粒子検出用の閾値を計算するステップと、

（ｅ） 前記閾値を用いて、前記合成イメージ内の微粒子検出を実行するステップと

を含み、改良点が、

（１） ソース・ピクセル値の各組内で、最初にソースイメージを合計せずに、微粒子検出用の閾値を別々に計算するステップと、

（２） ソース・ピクセル値の各組内で、対応する閾値を用いて、微粒子検出を別々に実行するステップと

を含むことを特徴とする方法。

【発明の詳細な説明】

【０００１】

発明の技術分野

本発明は、微小体積・レーザ・スキャニング・サイトメトリ（Ｍｉｃｒｏｖｏｌｕｍｅ Ｌａｓｅｒ Ｓｃａｎｎｉｎｇ Ｃｙｔｏｍｅｔｙ）（ＭＬＳＣ）を用いる、生物学的マーカ（ｂｉｏｌｏｇｉｃａｌ ｍａｒｋｅｒ）の分析に関係する。本発明は、ＭＬＳＣを実行するための計測器、その計測器から得られるイメージデータを分析するためのシステム、及び生物学的マーカ・データ及び医療情報の統合分析を行うための情報処理システムを含む。

発明の背景

遺伝子技術（ｇｅｎｏｍｉｃｓ）、組合わせ化学、及び高スループット・スクリーニングを含む薬品発見における新しい技術革新の結果、臨床試験に利用可能な薬品候補の数が、製薬産業の開発及び経済的許容量を超過している。１９９８年、世界トップの薬品会社及びバイオテクノロジ会社は、研究開発に５００億ドル以上を費やし、そのうちの３分の１以上が、臨床開発に直接費やされた。増大する競合、並びに管理医療組織及び他の支払人からの圧力を含む多くの要因の結果、製薬産業は、市場に出される新薬の安全性及び効率性を含めた品質向上、並びに臨床開発の効率の改善を求めている。

【０００２】

したがって、最近の薬品発見の技術革新が、臨床試験を困難にする一因となった。確認されている治療ターゲット、並びに生成されている鉛化合物の数は、現時点で実行されているような、製薬会社が臨床試験を行なう容量をはるかに越えている。さらに、新薬を開発する平均コストがほぼ５億ドルであると当産業が現時点で推定しているように、可能性のある薬品候補のすべてを開発することは法外に費用がかかる。

【０００３】

製薬産業は、薬品開発における相応の技術改善を求めるよう迫られている。臨床試験は非常に高価で、非常にリスクのある状態のままであり、多くの場合、意志決定が非常に主観的な分析に基づいてなされている。その結果、薬品が最も有

効な患者母集団、所定の薬品に対する適切な服用量、及びその使用に伴う副作用の可能性を、判定することは多くの場合困難である。このことは、臨床開発において、より多くの障害をもたらすだけでなく、不適切に服用され、処方され、あるいは危険な副作用を引き起こすかもしれない製品が承認されることになることもある。それらの進行中の薬品数が増加するにつれて、製薬会社は、薬品開発工程の初期において、薬品候補の安全性及び効能のプロフィールについての客観的な測定値を確認する技術を必要としている。

【０００４】

生物学的マーカは、測定されたり、あるいは評価される時に、通常の生物学的なプロセス、病原性のプロセス、又は治療の介入に対する薬理学的な反応、に関する指標として、離散関係あるいは相関を持つ特性である。治療の介入に対する薬理学的な反応は、以下に限定されるものではないが、介入に対する一般的な反応（例えば効能）、介入に対する服用量反応、介入による副作用のプロフィール、並びに薬品代謝の速さ及び薬品代謝の確認といったような薬物動態学の特性、を含む。反応は、特効があるか、又はその逆に作用する（例えば有毒な）変化のどちらかと相関する。生物学的マーカは、病理学的なプロセスと関連して変化し、診断値及び／又は予想値を持つ、セル又は分子のパターンを含む。生物学的マーカは、セル母集団及びそれらの関係する分子のレベル、可溶性要素のレベル、他の分子のレベル、遺伝子発現レベル、遺伝子突然変異、並びに病気の存在及び／又は進行と相関する臨床パラメータを含む。病気の進行又は再発といったような臨床エンドポイント、あるいは生活の質の測定（通常、評価に長時間を要する）とは対照的に、生物学的マーカは、薬品の臨床的なプロフィールについてより迅速な量的な測定を提供することができる。臨床業務及び薬品開発の両方において現時点で用いられる単一の生物学的マーカは、コレステロール、前立腺の特定抗原（「ＰＳＡ」）、ＣＤ４Ｔセル、及びウイルスＲＮＡを含む。高コレステロールと心臓病との間、ＰＳＡと前立腺癌との間、及びＣＤ４プラスＴセルの減少とエイズのウイルスＲＮＡとの間で、よく知られている相関とは異なって、他のほとんどの病気と相関する生物学的マーカは、まだ確認されていない。その結果、政府機関及び製薬会社の両方が、臨床試験で使用するための生物学的マーカの

開発をますます求めているが、薬品開発における生物学的マーカの使用は、現在まで制限されてきた。

【０００５】

病気、病気進行、及び治療法に対する反応、並びに生物学的マーカとの相関を確立するために必要とされる膨大な量の情報を分類することができる生物学的マーカ同定システムに対するニーズがある。そのような生物学的マーカ同定システムは、１９９９年4月２６日に提出された「Ｂｉｏｌｏｇｉｃａｌ Ｍａｒｋｅｒ Ｉｄｅｎｔｉｆｉｃａｔｉｏｎ Ｓｙｓｔｅｍ（生物学的マーカ同定システム）」と題された米国暫定特許出願第６０／１３１，１０５号、並びにこの出願と同時に提出された「Ｐｈｅｎｏｔｙｐｅ ａｎｄ Ｂｉｏｌｏｇｉｃａｌ Ｍａｒｋｅｒ Ｉｄｅｎｔｉｆｉｃａｔｉｏｎ Ｓｙｓｔｅｍ（フェノタイプおよび生物学的マーカ同定システム）」と題され共に所有されている米国実用出願において、記述されており、それらは共に、その全体がここに参考文献として明確に組み入れられる。この技術は、何百から何千という生物学的マーカを測定するのに必要とされる計測器、このデータが容易にアクセスされるようにする情報処理システム、マーカのパターンを臨床データと相関させるソフトウェア、及びその結果として生じる情報を薬品開発工程内に利用する能力を含む。そのシステムは微小体積・レーザ・スキャニング・サイトメトリ（ＭＬＳＣ）を広範囲に利用する。

【０００６】

マーカ同定システムの望ましい実施形態では、生物学的流体を、その流体内の特定の分子と結び付くことのできる１つ以上の蛍光標識付けされた検出分子と接触させる。通常、その生物学的流体は血液サンプルで、検出分子は、１つ以上のサブタイプの血液セルの上あるいは中に存在するセル会合分子に特有の蛍光染料で標識付けされた抗体である。その後、標識付けされたサンプルが毛細管に置かれ、その毛細管がＭＬＳＣ器具に取り付けられる。この器具は、顕微鏡の対物レンズを通って血液サンプルに向けられたレーザ光を走査する。サンプルから発っせられる螢光は、顕微鏡の対物レンズによって収集され、各蛍光チャンネル内のサンプルの画像が形成される一連の光電子増倍管に渡される。その後、そのシス

テムは、セルを識別するために各チャンネルからの生の画像を処理し、次に、蛍光抗体で標識付けられた各タイプのセルに対して、完全なセル数、及び相対的な抗原密度レベルを決定する。

【０００７】

またマーカＭＬＳＣは、生物学的流体内の可溶性要素の量を計るために、その要素に対する蛍光標識付けされた二次抗体に加えて、その要素に対する微小球体束縛一次抗体を用いることによって、使用されることができる。そうすることで、その要素は、微小球体に結び付けられ、その結び付けられた要素を、二次抗体の結合が蛍光で標識付けする。この実施形態におけるシステムは、溶性の各要素の濃度を決定するために、血液サンプル内の各ビードに関する蛍光信号を測定する。複数のビード・タイプ（各々が異なる一次抗体と結合する）を用いることにより、同じサンプル量で複数の分析を実行することが可能である。各ビード・タイプを識別するために、異なるビードが別個のサイズを持つか、又は異なる内部の色を持つか、あるいは各二次抗体が異なる蛍光体で標識付けされる。

【０００８】

本発明の望ましい実施形態は、生物学的マーカを検知するために抗体を用いるが、特に特定の生物学的マーカに結び付くことのできる任意の他の検出分子も意図される。例えば、様々なタイプの受容分子は、蛍光標識付けされた同系統の配位子との相互作用を通して検知されることができる。

【０００９】

分析の対象であったセル母集団及び可溶性要素に関するデータを作成するために、ＭＬＳＣ器具からの生データが、画像分析ソフトウェアによって処理される。その後、このデータがデータベースに転送される。生物学的マーカと病気又は医療条件との間の相関を確立する目的で、このセル母集団及び可溶性要素のデータに加えて格納されることができる他のデータは、それに制限されるものではないが、薬品投与と薬物生体反応（身体内での薬品の濃度とその代謝の測定）、並びに個人の年齢、性、体重、身長、体型、病歴（病的状態と同等のもの、投薬などを含む）、（もしあれば）病気又は医療条件の発現と分類、及び医師によってなされた他の標準的な臨床観察を含む臨床パラメータ、を含む。また臨床パラメ

ータに含まれるものは、それに制限されるものではないが、標準ＥＬＩＳＡテスト、酵素活性に対する比色機能検定、及び質量分光測定を含む、個人の体液の中に存在する特定分子の濃度を測定するための他の技術から得られる結果だけでなく、環境的な及び家系的な履歴要素である。またデータは、レントゲン写真、脳のスキャン、又はＭＲＩといったようなイメージ、あるいは個人の状態についての生体組織検査、心電図、ストレステスト又は他の測定から得られる情報を含む。

【００１０】

そうして情報処理システムは、ａ）そのデータを、（同じ個人から、病気の進行又は治療評価目的のいずれかから）格納されているプロフィールと、及び／又は（病気診断用の）他の個人からのプロフィールとを比較し、そしてｂ）新しいプロフィールを引き出すためにそのデータを「調べる」。このような方法で、診断及び前兆となる情報がデータベースから得られ、引き出されることができる。１９９９年４月２６日に提出された米国特許出願第６０／１３１，１０５号の「Ｂｉｏｌｏｇｉｃａｌ Ｍａｒｋｅｒ Ｉｄｅｎｔｉｆｉｃａｔｉｏｎ Ｓｙｓｔｅｍ（生物学的マーカ同定システム）」と、共通に所有される米国実用出願において、この出願と同時に提出された「Ｐｈｅｎｏｔｙｐｅ Ｂｉｏｌｏｇｉｃａｌ Ｍａｒｋｅｒ Ｉｄｅｎｔｉｆｉｃａｔｉｏｎ Ｓｙｓｔｅｍ（フェノタイプの生物学的マーカ同定システム）」と題された出願との両方が、参考文献としてその全体がここに組み入れられ、多くの様々なアプリケーションにおいて、ＭＬＳＣの用途が非常に詳しく記述される。システムは、従来の技術システムがドナー・サンプル中の変動によって妨げられる分析であっても、強い一貫した分析データを提供することができる。アプリケーションは、病気進行中及び治療中の、セルタイプ母集団の変化及び可溶性要素の変化を測定するためにＭＬＳＣの使用を含む。例えば、ＭＬＳＣは多発性硬化症及び慢性関節リウマチに対する斬新な生物学的マーカを識別するために用いられることができる。

発明の概要

本発明は微小体積・レーザ・スキャニング・サイトメトリ（ＭＬＳＣ）を実行するために改良されたシステムを提供する。システムはＳｕｒｒｏＳｃａｎシス

テムと名付けられる。それは、可変の走査速度で動作することができ、かつ、４つの異なる蛍光チャンネル内で同時にデータを収集することができる改良されたＭＬＳＣ器具を含む。本発明は、ＭＬＳＣ器具から得られた生データ上でイメージ処理を実行するための改良された方法、及び関連データベース内でこのデータを機能させる改良された方法を含む。ここで記述される改良点は、高速で多要素からなる病気データベースの構築及び使用を大いに促進する。このデータベースは、ユーザが、ａ）前兆となる結果又は診断に役立つ結果を得るために、レーザ・スキャニング・サイトメータで得られた血液プロフィールを、よく知られた病気で苦しむ個人について格納されたプロフィールと比較できるようにし、そしてｂ）ユーザが特定の病気に対する新しい前兆及び診断に役立つプロフィールを速やかに構築できるようにし、ｃ）生物学的マーカのパターンと任意の器官内の病気との間の新しい関連を発見できるようにする。

発明の詳細な記述

定義

ここで用いられているような用語「生物学的マーカ」又は「マーカ」あるいは「バイオマーカ」は、通常の生物学的工程、病原性工程、又は治療の介入に対する薬理学的反応の、インディケータとして測定され数値を求められる特性を意味する。治療の介入に対する薬理学的反応は、制限されるものではないが、一般的な介入に対する反応（例えば効能）、服用量による介入に対する反応、介入の副作用プロフィール、及び薬物動態学的特性を含む。反応は、有効であるか、又はその逆の（例えば有毒な）変化と相関する。生物学的マーカは、病理学的工程に関連して変化し、かつ診断及び／又は前兆となる値を持つ、セル又は分子のパターン又は集合を含む。

【００１１】

生物学的マーカは、制限されるものではないが、病気の存在及び進行、並びに治療に対する通常の生物学的工程及び反応と相関する、セル母集団の総数、並びに会合分子のレベル、可溶性要素のレベル、他の分子のレベル、遺伝子発現レベル、遺伝子突然変異、及び臨床パラメータを含む。臨床業務及び薬品開発の両方において現時点で用いられる単一の生物学的マーカは、コレステロール、ＰＳＡ

、ＣＤ４Ｔセル、及びウイルスＲＮＡを含む。高コレステロールと心臓病、ＰＳＡと前立腺がん、並びにＣＤ４プラスＴセル及びウイルスＲＮＡとエイズ、の間でよく知られている相関とは異なって、他のほとんどの病気と相関する生物学的マーカは、まだ確認されていない。その結果、政府機関及び製薬会社の両者が、臨床試験で使用するための生物学的マーカの開発を一層求めているけれども、薬品開発における生物学的マーカの使用は、現在のところ制限されている。

【００１２】

制限されるものではないが、例として、生物学的マーカは、通常の生物学的状態、病気、又は医療条件と個々別々の関係を持つとしばしば考えられていて、例えば、高コレステロールは心臓病の危険を増大させることに関連し、高いＰＳＡレベルは前立腺がんの危険を増大させることに関連し、ＣＤ４Ｔセルの減少及びウイルスＲＮＡの増大はエイズの存在／進行と関連する。しかしながら、様々な病気又は医療条件に対して有効なマーカが、非常に複雑なパターンから成ることは、かなりよくあることである。例えば、特定のセルタイプ上での１つ以上の特定のセル表面抗原レベルの低下が、高レベルの１つ以上の溶性タンパク質、恐らくサイトカインと共に発見される場合、それが特定の自己免疫疾患を示すものであると発見されることがある。したがって、本発明の目的のために、生物学的マーカは多くのインディケータのパターンを参照する。

【００１３】

ここで用いられる用語「生物学的マーカ同定システム」は、患者母集団から情報を得て、データの相関関係及び生物学的マーカの識別を可能にするやり方で、その情報を消化するためのシステムを意味する。患者母集団は任意の生物体を含むことができる。生物学的マーカ同定システムは、複数のデータカテゴリからなる統合データベースと、前記データカテゴリの各々に対応する複数の個人からのデータと、データカテゴリ内のデータを相互に関係づけるための処理手段とからなり、前記病気又は医療条件を持つ個人を、前記病気又は医療条件を持たない個人と区別することができるように、データカテゴリ又はカテゴリを識別するためのデータカテゴリの相関分析がなされることが可能であり、また前記識別されたカテゴリ（複数可）が前記病気又は医療条件に対するマーカである。さらにマー

カは、異なる時点、例えば薬品投与の前と後で、一人の個人に対する様々なデータ・カテゴリ内のデータを比較することにより識別されることができる。ＳｕｒｒｏＳｃａｎシステムと名付けられた本出願であるＭＬＳＣシステムは、生物学的マーカ同定システムの１つの例である。

【００１４】

ここで用いられる用語「データ・カテゴリ」は、個人の差分を識別することができる測定のタイプを意味する。本発明において有用であるデータ・カテゴリの例は、制限されるものではないが、個人の生物学的流体内での多くのそして何タイプものセル母集団及びそれらの関連する分子と、個人の生物学的流体内での多くのそして何タイプもの可溶性要素と、個人の臨床パラメータに関連する情報と、個人の生物学的流体内での１ｍｌ当たりのセルの容積測定カウントと、個人の生物学的流体内での多くのそして何タイプもの小さな分子と、個人のＤＮＡに関連するゲノム情報とを含む。例えば、単一のデータ・カテゴリは、個人の血液内のＩＬ－１の濃度に相当する。さらに、あるデータ・カテゴリは、血液又は尿内の薬品又はその代謝のレベルであってもよい。データ・カテゴリのさらなる例は、ＣＤ４Ｔセルの確実な総数である。

【００１５】

ここで用いられる用語「生物学的流体」は、限定されるものではないが、血液（全血、赤血球の溶解によって作られる白血球、周辺の血液単核セル、血しょう、及び血清を含む）、痰、唾液、尿、精液、脳脊髄液、気管支の吸引液、汗、便、関節液、リンパ液、涙、また任意の器官から得られる柔らかくされた組織を含む任意の生物学的物質を意味する。生物学的流体は、通常は、セルとそれらの会合分子、可溶性要素、小分子、及び他の物質を含む。血液は、多くの理由のために、本発明における望ましい生物学的流体である。まず第１に、血液は容易に利用可能で、複数回、抜き取ることができる。血液は、時間が経てば、骨髄内の前駆（セル）から部分的に補充される。血液は抗原投与に反応し、抗原投与についての記憶を保つ。血液は中心に配置され、再循環し、潜在的に身体全体にわたる変化について伝える。血液は、表面の分子、内部の分子、及び個々のセルに関連して分泌された分子を含む、多数のセル母集団を含む。また血液は、サイトカイ

ン、抗体、及び急性期タンパク質などのような自分自身のものと、伝染病に関する化学薬品及び製品のような外部からのものとの両方である、可溶性要素を含む。

【００１６】

ここで用いられる用語「セル母集団」は、共通の特性を備えたセル一式を意味する。その特性は、１、２、３、又はそれ以上のセル会合分子の存在及びレベル、サイズなどを含む。１、２、又はそれ以上のセル会合分子は、セル母集団を定義することができる。一般的には、いくつかの追加のセル会合分子が、１つのセル母集団をさらにサブ組化するために使用されることができる。セル母集団は、母集団レベルで識別されるのであって、タンパク質レベルで識別されのではない。セル母集団は、１、２、又はそれ以上のセルによって定義されることができる。いかなるセル母集団も潜在的なマーカである。

【００１７】

ここで用いられる用語「セル会合分子」は、セルに関連するいかなる分子もを意味する。これは、次のものに制限されるものではないが、１）タンパク質、糖タンパク質、脂質、及び糖脂質のような本来備わっているセル表面分子、２）その受容体に結び付くサイトカイン、Ｆｃ受容体に結び付く免疫グロブリン、Ｂセル又はＴセル受容器に結び付く外部からの抗体、及び自己抗原に結び付く自己抗体、のような外来的なセル表面分子、３）セル質のタンパク質、糖質、脂質、及びｍＲＮＡのような本来備わっている内部分子、並びに核のタンパク質及びＤＮＡ（ゲノム及び身体の核酸を含む）、４）ウイルス・タンパク質及び核酸のような外来的な内部分子、を含む。望ましいセル会合分子は、通常はセル表面のタンパク質である。一例として、白血球分化抗原（現時点でＣＤ－１６６を通してＣＤ抗原を含む）、（Ｂセル受容体及びＴセル受容体のような）抗原受容器、及び主要組織適合複合体を含む、何百もの白血球のセル表面タンパク質又は抗原がある。これらの部類の各々は、莫大な数のタンパク質を包含する。

【００１８】

ここで用いられる用語「可溶性要素」は、生物学的流体、通常は血液内で発見されるすべての可溶性の分子を意味する。可溶性要素は、制限されるものではな

いが、溶性タンパク質、糖質、脂質、リポタンパク質、ステロイド類、他の小分子、並びに前述の成分の任意の複合体、例えばサイトカインと溶性受容体、抗体と抗原、及び任意のものに複合する薬品、を含む。可溶性要素には、サイトカイン、抗体、急性期タンパク質などのような自分自身のものと、伝染病の化学薬品及び製品のような外部からのものとの両方がある。可溶性要素は、本来備わっているもの、つまり、個人によって作成されたものであるか、あるいはビールス、薬品又は環境上の毒素のような外来性のものである。可溶性要素はプロスタグランディン、ビタミン、代謝物質（鉄、糖類、アミノ酸などのような）、薬品及び薬品代謝のような小さな分子化合物でありうる。

【００１９】

ここで用いられる用語「小分子」又は「器官分子」あるいは「小器官分子」は、２～２０００の範囲内の分子量を持つ、可溶性要素あるいはセルに関連する要素を意味する。小分子は、制限されるものではないが、プロスタグランディン、ビタミン、代謝物質（鉄、糖類、アミノ酸などのような）、薬品、及び薬品代謝を含む。１つの重要な実施形態では、ＭＬＳＣシステムが、治療法制度中に他の生物学的マーカと力を合わせて、生物学的流体内での薬品濃度及び薬品代謝の変化を測定するために用いられる。

【００２０】

ここで用いられる用語「病気」又は「医療条件」は、任意の生体内での身体機能、システム、あるいは器官の、障害、休止、不調又は変化を意味する。病気又は医療条件の例は、制限されるものではないが、免疫欠乏及び炎症性の状態、癌、心臓血管の病気、伝染病、精神医学的状態、肥満症、また他のそのような病気を含む。図解の方法で、免疫及び炎症性の状態は、慢性関節リウマチ（ＲＡ）、多発性硬化症（ＭＳ）、糖尿病などをさらに含む自己免疫疾患を含む。

【００２１】

ここで用いられる用語「臨床パラメータ」は、病気又は医療条件に関連する臨床的なセッティングにおいて得られる情報を意味する。臨床パラメータの例は、制限されるものではないが、年齢、性、体重、身長、身体タイプ、病歴、民族性、家族の履歴、遺伝要素、環境要因、病気又は医療条件の発現と分類、並びに、

血圧、ＭＲＩ、レントゲン写真などのような任意の臨床的な実験テストの結果を含む。

【００２２】

ここで用いられる用語「臨床エンドポイント」は、患者がどのように感じ、機能し、生存しているかを測定する特性又は変数を意味する。

ここで用いられる用語「微小体積・レーザ・スキャニング・サイトメトリ」又は「ＭＬＳＣ」あるいは「ＭＬＳＣシステム」は、蛍光で標識付けされた検出分子を用いて、サンプルを蛍光放射が記録される光学スキャニングにさらして、小容積のサンプル内の組成の存在を検知する方法を意味する。ＭＬＳＣシステムは、他の技術とそれの相違を示すいくつかのキーとなる特徴を持っていて、１）多くの分析に対して、少量の血液（５－５０μｌ）だけが必要とされ、２）確実なセル数（１μｌ当たりのセル数）が得られ、３）分析を、全血上か、又は浄化された白血球上のどちらかでダイレクトに実行することができる。この技術が実現すれば、一回の血液採取から、数百の異なるセル母集団の測定が容易になされる。ＭＬＳＣ技術は、米国特許第５，５４７，８４９号及び第５，５５６，７６４号、及びディーツ等による（（１９９６年の）サイトメトリ２３：１７７－１８６）、並びに１９９８年８月２１日に提出された「Ｌａｓｅｒ－Ｓｃａｎｎｅｒ Ｃｏｎｆｏｃａｌ Ｔｉｍｅ－Ｒｅｓｏｌｖｅｄ Ｆｌｕｏｒｅｓｃｅｎｃｅ Ｓｐｅｃｔｒｏｓｃｏｐｙ Ｓｙｓｔｅｍ（レーザ・スキャナの焦点を共有する時間分解蛍光分光システム）」と題された米国暫定特許出願第６０／０９７，５０６号、及び１９９９年８月２０日に提出された「Ｎｏｖｅｌ Ｏｐｔｉｃａｌ Ａｒｃｈｉｔｅｃｔｕｒｅｓ ｆｏｒ Ｍｉｃｒｏｖｏｌｕｍｅ Ｌａｓｅｒ－Ｓｃａｎｎｉｎｇ Ｃｙｔｏｍｅｔｅｒｓ（微小体積・レーザスキャニング・サイトメータのための斬新な光学アーキテクチャ）」と題された米国特許出願第０９／３７８，２５９号に記述されており、それらの各々が、その全体がここに組み入れられる。微小体積毛細管を備えたレーザ・スキャニング・サイトメトリは、全血、処理された血液、及び生物学的流体を含む他の流体内の、蛍光で標識付けされたセル及び分子をモニタするための強力な方法を提供する。さらに本発明は、小量の血液から複数の生物学的マーカの同時測定を行うためのＭＬＳＣ

器具の容量を向上させることにより、ＭＬＳＣ技術を向上させる。本発明の改良されたＭＬＳＣシステムは、「ＳｕｒｒｏＳｃａｎシステム」と名付けられる。

【００２３】

ここで用いられる用語「検出分子」は、重要な分子、特にタンパク質に結び付くことができる任意の分子を意味する。望ましい検出分子は抗体である。抗体は単クローン性又は多クローン性でありうる。

【００２４】

ここで用いられる用語「色素」、「蛍光体」、「蛍光色素」、「蛍光標識」、又は「蛍光性のグループ」は、レーザによる励起で蛍光を発することができる分子を意味するために、交互に用いられる。間接的なリンクもまたここで包含されるが、色素は通常、本発明内の検出分子と直接的にリンクされる。多くの色素がこの技術分野ではよく知られている。特定の望ましい実施形態では、スペクトルの（６００ｎｍを越える）赤い領域で起動することができる蛍光体が用いられる。２つの赤い色素、Ｃｙ５及びＣｙ５．５が通常は用いられる。それらは、６６５及び６９５ナノメートルの放射ピークをそれぞれ持っており、抗体に容易に連結することができる。両方とも、ヘリウムネオンレーザでもって６３３ｎｍで起動することができる。使用されることができる３つの赤い色素の組は、Ｃｙ５、Ｃｙ５．５及びＣｙ７、あるいはＣｙ５、Ｃｙ５．５及びＣｙ７－ＡＰＣを含む。また、１９９９年７月６日に提出された「Ｂｒｉｄｇｅｄ Ｆｌｕｏｒｅｓｃｅｎｔ Ｄｙｅｓ、Ｔｈｅｉｒ Ｐｒｅｐａｒａｔｉｏｎ ａｎｄ Ｔｈｅｉｒ Ｕｓｅ ｉｎ Ａｓｓａｙｓ（ブリッジ蛍光色素、分析に際してのその準備及びその使用）」と題された米国仮特許出願第６０／１４２，４７７号を参照のこと。

【００２５】

ここで用いられる用語「微粒子」は、いくつかの実施形態中で、生物学的マーカに関する情報を得るためにＭＬＳＣによって検知される、すべての巨大分子の構造を意味する。いくつかの実施形態では、その検知される微粒子は、セルであり、他の実施形態では、検知される微粒子は、抗体に標識付けされたビードである。

【００２６】

本発明は、ＳｕｒｒｏＳｃａｎシステム又は単にＳｕｒｒｏＳｃａｎと名付けられた、改良された微小体積・レーザ・スキャニング・サイトメトリ（「ＭＬＳＣ」）システムを提供する。先のシステムは、米国特許第５，５４７，８４９号及び第５，５５６，７６４号、並びに１９９９年４月２１日に提出された「Ｂｉｏｌｏｇｉｃａｌ　Ｍａｒｋｅｒ　Ｉｄｅｎｔｉｆｉｃａｔｉｏｎ　Ｓｙｓｔｅｍ（生物学的マーカ同定システム）」と題された米国特許出願第６０／１３１，１０５号、１９９８年８月２１日に提出された「Ｌａｓｅｒ－Ｓｃａｎｎｅｒ　Ｃｏｎｆｏｃａｌ　Ｔｉｍｅ－Ｒｅｓｏｌｖｅｄ　Ｆｌｕｏｒｅｓｃｅｎｃｅ　Ｓｐｅｃｔｒｏｓｃｏｐｙ　Ｓｙｓｔｅｍ（レーザ・スキャナの焦点を共有する時間分解栄光分光システム）」と題された米国暫定特許出願第６０／０９７，５０６号、ディーツ等による（（１９９６年の）サイトメトリ２３：１７７－１８６）、及び１９９９年８月２０日に提出された「Ｎｏｖｅｌ　Ｏｐｔｉｃａｌ　Ａｒｃｈｉｔｅｃｔｕｒｅｓ　ｆｏｒ　Ｍｉｃｒｏｖｏｌｕｍｅ　Ｌａｓｅｒ－Ｓｃａｎｎｉｎｇ　Ｃｙｔｏｍｅｔｅｒｓ（微小体積・レーザスキャニング・サイトメータのための斬新な光学アーキテクチャ）」と題された米国特許出願第０９／３７８，２５９号に記述されており、それらの各々が、その全体がここに組み入れられる。バイオメトリック・イメージング（Ｂｉｏｍｅｔｒｉｃ　Ｉｍａｇｉｎｇ）社から市販されているＩｍａｇｎ２０００システムは、従来技術のＭＬＳＣシステムの例である。

【００２７】

本発明の改良されたＭＬＳＣシステムは、次のコンポーネントを含む、

（ａ）被検体サンプルから生データを得るための電子制御システムを含むＭＬＳＣ器具、

（ｂ）ＭＬＳＣ器具から生データを収集し向上させるための画像分析システム、

（ｃ）多重パラメータ分析設計、測定器制御、最終データ分析、及びデータ・アーカイビング用の統合された情報処理システム・アーキテクチャ。

【００２８】

現在の発明は、ＭＬＳＣシステムの操作のいくつかのキーとなる側面における

重要な改良、つまりａ）ＭＬＳＣ光学、ｂ）ＭＬＳＣシステム制御電子工学、ｃ）画像表示及び分析のアルゴリズム、並びにｄ）情報処理システム・アーキテクチャを提供する。また本発明は、業界標準であるフロー・サイトメトリ・スタンダード（．ＦＣＳファイル形式）に画像表示及びデータ変換するための改良された方法を提供する。

ＭＬＳＣ計器

ＳｕｒｒｏＳｃａｎシステムは、ＭＬＳＣ器具の光学アーキテクチャにおいて重要な改良を提供する。前のＭＬＳＣ器具は、通常は２本のチャンネルの蛍光信号を検知することができたが、そのために、単一の実験で同時に検知することができる被検体の数が制限された。いくつかのアプリケーションでは、特定のセルを識別するために、２つ以上の異なる蛍光信号を検知する必要がある。例えば、ＣＤ４、ＣＤ４５ＲＡ及びＣＤ６２Ｌを示す単純なＴセルのようないくつかのセル母集団を識別するには、３つ以上の抗原の同時測定が必要である。本発明の改良されたＳｕｒｒｏＳｃａｎ器具は、少なくとも４つの別個の蛍光信号を検知することができ、そのために、単一の実験内で少なくとも４つの別個の蛍光試薬を使用することができる。改良された光学的構成の１つの実施形態が図１に示される。毛細管アレイ１０は、分析のためのサンプルを含む。望ましい実施形態では、平行にされた励起光が、１つ以上のレーザによって提供される。特に望ましい実施形態では、６３３ｎｍの励起光がヘリウム－ネオン・レーザ１１によって提供される。この波長は、生体試料の自己蛍光に関連する問題を回避する。レーザの出力が３～１７ｍＷまで増大させられる。レーザ・パワーを高めると、２つの潜在的利点、つまり感度の増大及び走査速度の増大がもたらされる。平行にされたレーザ光は、励起ダイクロイック・フィルタ１２によって偏光させられる。反射して、光は検流計駆動の走査反射鏡１３上に入射する。走査反射鏡は、固定された範囲の角度上を、例えば＋／－２．５度、検流計によって急速に揺れ動かされることができる。走査反射鏡は、顕微鏡の対物レンズ１６の入射瞳孔上に、その走査反射鏡を映し出す２つのリレーレンズ１４及び１５の中にその入射光を反射する。この光学的構成は、反射鏡で走査された特定の角度を、顕微鏡の対物レンズの焦点での特定のフィールド・ポジションに変換する。＋／－角度の走査は

、対物レンズの焦点での１ミリメートルのスキャン幅になる。走査角度とフィールド・ポジションの関係は、この構成において、及びこの範囲の角度上では、本質的に線形である。さらに、顕微鏡の対物レンズは、入ってくる平行ビームを対物レンズの焦点平面で１つのスポットに集束させる。光学的解像度を設定するそのスポットの直径は、平行ビームの直径及び対物レンズの焦点距離によって決定される。

【００２９】

走査される励起ビームの経路に置かれる蛍光サンプルは、ストークスシフトされた光を発する。この光は対物レンズによって収集されコリメートされる。このコリメートにされた光は、２つのリレーレンズ１４及び１５から、コリメートされたままで現れ、走査反射鏡に突き当たり、反射鏡はそれを反射しスキャン解除する。その後、ストークスシフトされた光は、ダイクロイック励起フィルタ（より短い波長光を反映し、より長い波長光を通す）を通り抜け、そしてその後、さらに任意の反射された励起光を濾過する働きをする第１の長いパス・フィルタを通過する。

【００３０】

その後、本発明の改良された器具は、４つの異なる発光バンドにストークスシフトされた光を分けるために、さらに一連のダイクロイック・フィルタを用いる。第１の蛍光ダイクロイック・フィルタ１８は、２つの最も赤い蛍光色から、２つの最も青い蛍光色を分割する。その後、いかなる焦点の外れた蛍光信号をも著しく減少させるために、第１の集束レンズ２０を通して、２つの最も青い色が、第１のアパーチャ１９上に集束させられる。アパーチャを通過した後、第２の蛍光ダイクロイック・フィルタ２１がさらに、個々の青い色を互いに分離する。そうして、その個々の青い色が、２つの別個の光電子増倍管２２及び２３で分析される。最も赤い２つの色は、第１の蛍光ダイクロイック・フィルタ２８によって最も青い２色から分割された後、第２の長いパス・フィルタ２５、ミラー２６、及び第２の集束レンズ２７を通して、第２のアパーチャ２４上に集束させられる。アパーチャ２４を通り抜けた後に、最も赤い色は、第３の蛍光ダイクロイック・フィルタ２８によって互いに分離される。その後、個々の赤い色が、光電子増

倍管２９及び３０で分析される。このようにして、４つの別個の蛍光信号が、毛細管の中に保持されたサンプルから、同時に個々の光電子増倍管に伝送されることができる。この改良によって、初めて、別個の４つの被検体が同時にモニタされることが可能になる。各光電子増倍管は、入ってくる蛍光光子束に応じて、電子の流れを生成する。これらの個々の電流は、検出電子工学で、１つ以上のプリアンプによって個々の電圧に変換される。その電圧は、走査されたイメージに対して、ピクセルの輝度を決定するために、アナログ・デジタル変換器によって規則的な間隔でサンプルされる。本発明の４チャンネルは、０、１、２及び３と名付けられる。

【００３１】

意味のあるデータが、単一の励起波長、例えばヘリウム－ネオン・レーザからの６３３ｎｍを用いて、得られるようにするためには、単一の励起波長から励起されることができ、オーバーラップが最小で識別可能な波長を発する、色素が必要である。ヘリウム－ネオン・レーザを用いる３チャンネル検出システムに対して、３種の色素の適切な１つの組合せは、Ｃｙ５（６７０ｎｍで放射ピーク）、Ｃｙ５．５（６９４ｎｍで放射ピーク）、及びＣｙ７（７６７ｎｍで放射ピーク）である。代案となる実施形態では、アロヒコシアニン（ａｌｌｏｐｈｙｃｏｃｙａｎｉｎ（ＡＰＣ））がＣｙ５と置換される。Ｃｙ７（７４３ｎｍ）に対する吸収ピークが、ヘリウム－ネオン励起レーザの波長（６３３ｎｍ）からずっと離れているので、通常、この分野の技術を持つ者であれば、ヘリウム－ネオン励起システムにおいてＣｙ７が有用であるとは見なさない。しかしながら、本発明者は、全血内の特定のセルを数え上げるために、Ｃｙ７が６３３ｎｍで十分に励起させられることが可能であることを発見した。この励起は、おそらく長い励起テールの存在に起因し、それはＭｕｊｕｍｄａｒ，Ｒ．Ｂ．、Ｌ．Ａ．Ｅｒｎｓｔ、Ｓ．Ｒ．Ｍｕｊｕｍｄａｒ、Ｃ．Ｊ．Ｌｅｗｉｓ、及びＡ．Ｓ．Ｗａｇｇｏｎｅｒによって１９９３年に「Ｃｙａｎｉｎｅ ｄｙｅ ｌａｂｅｌｉｎｇ ｒｅａｇｅｎｔｓ：ｓｕｌｆｏｉｎｄｏｃｙａｎｉｎｅ ｓｕｃｃｉｎｉｍｉｄｙｌ ｅｓｔｅｒｓ（試薬にラベルを付けるシアニン色素であるスルホインドシアニン・スクシンイミディル・エステル）」と題されたＢｉｏｃｏｎｊｕｇＣｈｅｍ

．４：１０５－１１に記述されていて、その全体がここに参考文献として組み入れられる。Ｃｙ７の励起及び検出は、レーザーパワーを増大させること、及びスペクトルの赤色領域に対してより敏感な検出器を用いることにより改良されることができる。

【００３２】

他の実施形態では、ＡＰＣ励起波長で励起可能であるが、Ｃｙ７放射波長で放射する、縦並びの色素を作るために、Ｃｙ７がＡＰＣに連結される。この縦並びの色素は、アクセプタ（Ｃｙ７）を励起させるために、ドナー（ＡＰＣ）からのエネルギー移動を用いるが、そのことは、Ｂｅａｖｉｓ，Ａ．Ｊ．、Ｋ．Ｊ．Ｐｅｎｎｌｉｎｅによって１９９６年に「Ａｌｌｏ－７：ａ ｎｅｗ ｆｌｕｏｒｅｓｃｅｎｔ ｔａｎｄｅｍ ｄｙｅ ｆｏｒ ｕｓｅ ｉｎ ｆｌｏｗ ｃｙｔｏｍｅｔｒｙ（Ａｌｌｏ－７：流動セル分析において使用するための新しい蛍光の縦並び色素）」と題されたＣｙｔｏｍｅｔｒｙ，２４：３９０－５、並びにＲｏｅｄｅｒｅｒ，Ｍ．、Ａ．Ｂ．Ｋａｎｔｏｒ、Ｄ．Ｒ．Ｐａｒｋｓ、及びＬ．Ａ．Ｈｅｒｚｅｎｂｅｒｇによって１９９６年に「Ｃｙ７ＰＥ ａｎｄ Ｃｙ７ＡＰＣ：ｂｒｉｇｈｔ ｎｅｗ ｐｒｏｂｅｓ ｆｏｒ ｉｍｍｕｎｏｆｌｕｏｒｅｓｃｅｎｃｅ（Ｃｙ７ＰＥ及びＣｙ７ＡＰＣ：螢光抗体法のための鮮やかな新しいプローブ）」と題されたＣｙｔｏｍｅｔｒｙ，２４：１９１－７において記述されており、その両方ともその全体がここに参考文献として組み入れられる。

【００３３】

本発明のいくつかの実施形態では、１つ以上の励起波長が使用される。１つ以上の励起波長を用いることによって、各色素が同じ励起要件を持つ必要がないように、種々様々の蛍光色素を用いることが可能である。複数の励起波長が、少なくとも３つの方法、（１）４８８ｎｍ、５６８ｎｍ、及び６４７ｎｍの励起波長を備えた励起光源としてＡｒ－Ｋｒレーザを用い、そのために、３つの異なる蛍光性のグループ（例えばフルオレセイン、ローダミン、及びＴｅｘａｓ Ｒｅｄ（登録商標））からの３種類の励起に対する使用が可能となる方法、（２）コリメートされた励起光の異なる波長を各々が供給する１つ以上のレーザ・ソースを

用いる方法、（３）多光子の蛍光励起用のＴｉ－Ｓレーザ（～７００ｎｍの励起光）又はＮｄ：ＹＬＦレーザ（１０４７ｎｍの励起光）といったようなフェムト秒のパルスを生成することができるレーザを用いる方法、で得られる。

【００３４】

上に記述された本発明の実施形態は、別個の４本のチャンネルを用いるが、ここで開示される光学アーキテクチャは、より多くの偶数個のチャンネルを備えた器具の設計を考慮に入れる。

【００３５】

望ましい実施形態では、走査されるサンプルが、Ｘ、Ｙ及びＺ平面に自動翻訳可能なステージに取り付けられる。検流計で操作される反射鏡が、Ｙ軸における励起ビームを走査するが、その時、ステージはＸ軸においてサンプルを等速度で移動させる。走査されたビーム速さで乗算された各アナログ・デジタル変換器のサンプル採取間隔が、イメージのＹ軸にけるピクセル間隔を決定する。線レートで割り算されたＸステージ走査速度は、イメージのＸ軸におけるピクセル間隔を決定する。

【００３６】

ステージはＸ軸において個々のサンプルを走査するだけでなく、顕微鏡の対物レンズに対して多くのサンプルを左右に動かすことができる。このようにして、いかなるオペレータも介入せずに、コンピュータ制御によって、多くの個々のサンプルを順次走査することができる。このことが、器具のスループットを大幅に増大させ、器具による臨床セッティング内の血液サンプルの高速での自動分析をより行いやすくする。

【００３７】

本発明の望ましい実施形態では、ＳｕｒｒｏＳｃａｎのＭＬＳＣのステージが、１つ以上の毛細管アレイを保持し、その各々が９６－ウェル・プレートのフットプリントを持つ。各毛細管は、分析されるべきサンプルを保持する。多重チャンネル・ピペットと互換性をもつ各々３２本の固定された毛細管及び間隔を持つ使い捨ての毛細管アレイは、１９９９年４月２３日に提出された「Ｄｉｓｐｏｓａｂｌｅ　Ｏｐｔｉｃａｌ　Ｃｕｖｅｔｔｅ　Ｃａｒｔｒｉｄｇｅ（使い捨ての

光学キュベット・カートリッジ）」、「Ｓｐｅｃｔｒｏｐｈｏｔｏｍｅｔｒｉｃ Ａｎａｌｙｓｉｓ Ｓｙｓｔｅｍ Ｅｍｐｌｏｙｉｎｇ ａ Ｄｉｓｐｏｓａｂｌｅ Ｏｐｔｉｃａｌ Ｃｕｖｅｔｔｅ Ｃａｒｔｒｉｄｇｅ（使い捨ての光学キュベット・カートリッジを使用する分光光度分析システム）」、及び「Ｖａｃｕｕｍ Ｃｈｕｃｋ ｆｏｒ Ｔｈｉｎ Ｆｉｌｍ Ｏｐｔｉｃａｌ Ｃｕｖｅｔｔｅ Ｃａｒｔｒｉｄｇｅ（薄膜の光学キュベット・カートリッジ用の真空チャック）」と題された米国仮特許出願の代理人登録第０３２５１７－００４号、００５号及び００６号、並びに２０００年４月２１日に提出された「Ｄｉｓｐｏｓａｂｌｅ Ｏｐｔｉｃａｌ Ｃｕｖｅｔｔｅ Ｃａｒｔｒｉｄｇｅ（使い捨ての光学キュベット・カートリッジ）」と題された共有される米国特許出願、において記述されていて、その全体がここに参考文献として組み込まれる。各アレイは、毛細管の内側の大きさを規定するように型抜きされている、２倍の粘質を持つ粘着層で共に間にはさまれた２層のマイラーから造られる。その結果生じるカートリッジは、フレックス－３２と呼ばれ、大量に低価格で製造されることができる。カートリッジは柔軟であり、そのために、真空圧力によって光学的に平らな底板上に保持されることが可能であり、製造工程において平らであることに対する要件が除去される。毛細管の間隔は、多重チャンネル・マイクロプレート・ピペッタ及びロボット工学との互換性を保持するように設計された。

【００３８】

望ましい実施形態では、オペレータが、３２本の毛細管を持つ２つのプレートを一度に取り付けることができる。プレートが走査され、イメージが処理されている間は、オペレータの介入は必要ない。代案として、Ｉｍａｇｎ２０００（ＶＣ１２０）用に設計された１６本の個別の毛細管が、別のホルダに取り付けられる。

【００３９】

ステージのＺ運動は、対物レンズの焦点平面に各サンプルを置くための手段を提供する。またＺ運動は、各々の個別のサンプルに対する焦点平面イメージのスタックを捕捉することができるよう走査されることが可能である。オペレータ介入の必要性を回避するために、コンピュータ制御システムによって、各サンプル

に対する最適の焦点位置を、この走査されたＺイメージから決定できることが望ましい。さらに、サンプルの２つの両端に対して、最適の焦点を決定することができる。サンプルがＸ軸において走査されている間、ステージは、２つの両端の間の焦点差を通じて等速度で動かされ、そうしてサンプルあるいは取付具に存在するかもしれないすべての傾斜を修正する。

【００４０】

レーザ光線の走査速度は、各ピクセルで光信号を統合するのに費やされる時間量を決定するが、統合時間が長いほど、信号対雑音比が良くなる。また走査速度は、システムのスループット速度に比例する。以前のＭＬＳＣ器具は、単一の速度でサンプルを走査した。これは多くのアプリケーションにとって適切であるが、本発明は走査速度が変更可能なシステムの使用を意図する。そのように走査速度が変更可能なシステムであれば、各々の個別のサンプルに対してシステム感度を最適化することが可能である。例えば、ある種の分析は、サンプル内に非常に低濃度で存在する検体の検出を必要とするかもしれない。そのような低濃度の検体からの蛍光信号は、バックグラウンド・ノイズに比べて、相当低い可能性がある。このような場合、走査をゆっくり行うことによりシステム感度を増大させることができ、各ピクセルで光信号を統合するための時間をより多くすることが可能である。このことにより、信号対雑音比内が大きく改善されることになる。対照的に、ある種の分析では、サンプル内に検出される特定の検体の濃度が比較的高いために、はるかに明るい蛍光信号の検出を伴うかもしれない。この場合、より速い走査速度が望ましく、満足な信号対雑音比を達成する各ピクセルの信号を統合するために必要な時間は少しでよい。また走査速度が速ければ、それだけ大きなサンプル・スループットが得られる。したがって、ここで考えられている走査速度が変更可能なシステムは、従来技術である走査速度が固定されたシステムにとって大きな改善である、なぜならば、それは、ａ）各検体の信号対雑音比を最適化することができ、そのために、各検体にとって可能な最も高い品質のデータを収集でき、そして、ｂ）可能な最も効率的なスループット速度でシステムを機能させることができるからである。すべての場合に、検流計に取り付けられた反射鏡の走査速度を調整することによって、及びサンプル・イメージング中にス

テージがＸ軸において移動する速さを調整することによって、その走査速度は変更されることができる。

【００４１】

またＳｕｒｒｏＳｃａｎシステムは、各走査速度でシステム感度を最適化するために、アナログ処理回路に組み込まれる斬新な切り替え可能フィルタ・スキームを提供する。低域通過フィルタは、一般に重要な信号を通過させるため、及び測定工程で作られる不必要な高周波ノイズを拒絶するために用いられる。ＳｕｒｒｏＳｃａｎシステムでは、各走査速度に対する最適フィルタ帯域幅が、異なっていて、通常は走査速度に比例する。望ましい実施形態では、少なくとも２つの帯域幅が、切り替え可能なフィルタによって各チャンネルに提供される。特に望ましい実施形態では、４つの帯域幅が提供される。図２Ａ及び２Ｂは、４、８、１２、及び１６ｋＨＺ（６４、１２８、１９２、及び２５６ヘルツの走査速度用の最適帯域幅にそれぞれ対応する）の帯域幅を提供する切り替え可能なフィルタ・スキームのための回路図を示す。望ましい実施形態では、そのようなフィルタ帯域幅スイッチング・スキームが、各光電子増倍管チャンネルと関係する。

【００４２】

したがって、本システムは、１）システムの走査速度が信号対雑音比を最適化するように変更可能であり、２）各信号チャネルでの各アナログ・フィルタの帯域幅もまた信号対雑音比をさらに最適化するために変更されるという、２つの別個の方法で最適化されるので、本発明は従来技術のＭＬＳＣシステムを越える大きな改良である。この斬新な組合せは、相乗してＭＬＳＣ器具及びシステムの感度及び効率を向上させる。

【００４３】

本発明の望ましい実施形態では、ＳｕｒｒｏＳｃａｎシステムの最適の走査速度及びフィルタ帯域幅が、各々の実行される特定の分析に対して決定される。これらの変数は、後にオペレータが同じ分析の再実行を選択する場合に、これらの設定値を自動的に選択することができる臨床プロトコル・データベース（以下を参照）に格納される。このようにして、同じステージ上で、多くの様々な分析を行うことが可能であり、コンピュータは、各サンプルに対して、前もって定義し

た最適の走査速度及びフィルタ設定を自動的に選択することができる。この利点が、ＳｕｒｒｏＳｃａｎシステムの柔軟性に大きく寄与している。

【００４４】

上に記述されたすべての実施形態は、微粒子を検知するために、可視か、あるいは電磁スペクトルの赤外線部分の近くで発する蛍光体のレーザ励起を使用することに注目すること。しかしながら、本発明はまた、赤外線のような他のタイプの電磁放射及び放射プローブの使用を意図する。さらに、本発明は、ただの単一のプローブだけではなく、プローブのアセンブリの使用を意図する。また本発明は、制限されるものではないが、ラマン散乱、ミー散乱、ルミネセンス、及び燐光を含む、蛍光以外の光散乱モードの使用を意図する。

ＳｕｒｒｏＩｍａｇｅ画像分析ソフトウェア

イメージ処理は、レーザ・スキャニング・サイトメトリにとって、重大な要件である。イメージ処理プログラムは、セルの蛍光又は他の微粒子の蛍光（チャンネル）の異なるスペクトル領域を描写する複数のバイナリ・イメージを処理する必要がある、また、各チャンネルのバックグラウンド蛍光レベルを決定する必要がある、また、ノイズからセル又は他の微粒子を数えあげることができるような、各チャンネルの全体のノイズ、また、バブル、ゴミ微粒、及び他の「汚れ」や「ほこり」の源といったような外来の信号を無視する必要がある、また、制限されるものではないが、加重フラックス、サイズ、楕円率、並びに同じ位置にある他チャンネル内の信号間の比率及び相関を含むパラメータをレポートするために、各々の識別されたセル又は微粒子の特性を決定する必要がある。ＳｕｒｒｏＳｃａｎシステムは、上記の基準を満たし、テキスト・リストモード・フォーマットでの分析結果を出力するＳｕｒｒｏＩｍａｇｅシステムと呼ばれるイメージ処理及び微粒子検出システムを含む。

【００４５】

ＳｕｒｒｏＩｍａｇｅシステムについての次の説明は、バイナリ・イメージ入力ファイル（．ｓｍｌ）で始まり、関連する様々なアルゴリズムの記述及び説明を備えたテキスト・リストモード出力ファイル（．ｌｓｍ）までの、関数の形式で与えられる。図３は、ＳｕｒｒｏＩｍａｇｅシステムによって実行される操作

のフローチャートを描く。後に続く有効にする記述において、ＳｕｒｒｏＩｍａｇｅシステムは、セル検出のコンテキストで記述されることにも注意すること。しかしながら、上に記述されるように、ＳｕｒｒｏＩｍａｇｅシステムは、抗体標識付けされたビードのような、定義済みの物理パラメータを備えるいかなる構造をも検知することができる。ＳｕｒｒｏＩｍａｇｅシステムは、制限されるものではないが、「Ｂｉｏｌｏｇｉｃａｌ Ｍａｒｋｅｒ Ｉｄｅｎｔｉｆｉｃａｔｉｏｎ Ｓｙｓｔｅｍ（生物学的マーカ同定システム）」と題された米国暫定特許出願第６０／１３１，１０５号、及びこの出願と同時に提出された「Ｐｈｅｎｏｔｙｐｅ ａｎｄ Ｂｉｏｌｏｇｉｃａｌ Ｍａｒｋｅｒ Ｉｄｅｎｔｉｆｉｃａｔｉｏｎ Ｓｙｓｔｅｍ（フェノタイプの生物学的マーカ同定システム）」と題された共有される米国実用出願において記述されている実施形態を含めて、従来技術において記述されるＭＬＳＣのいかなる実施形態での使用に対しても意図される。

入力

本発明の望ましい実施形態では、バイナリの組み合わせられたフォーマットが、イメージデータを格納するために用いられる。図４に図解されたフォーマットで、いかなる数の１６ビット・データ・チャネル（イメージ）でも組み合わされることができる。チャンネル・イメージ・アレイは、各行に沿って格納される：（行０：列０、列１、列２，．．．．、列ｎ列；行１：．．．行ｎ行へ）、ただし、ｎ列は通常２５０ピクセルで、ｎ行は通常１００００ピクセルである。図４に示されるようなＳＭ１ヘッダは、記述子当たり４バイトを備えたヘッダ内に２８バイトを持つ。各ファイル記述子は、下位－上位ワードの形式で配列される。「４文字記述子」は「ＳＭ０１」のようなユニークなイメージ・タイプを記述する任意の４文字でありうる。

【００４６】

本発明の１つの実施形態で、システムはピクセル当たり２バイトあるいは１６ビットを使用しているので、各ピクセルは、６５５３６の値のうちのいずれかを持つことができる。しかしながら、フィールド記述子「ピクセル当たりのバイト」が、ワードからフロートまで、又は他のデータフォーマットまで、イメージタ

イプを拡張する柔軟性を可能にする。さらに、変数フィールド「ヘッダ内のバイト」が、さらなるフィールド記述子を追加する能力を見込む。例えば、このフォーマットを利用する４バイトのフロート・イメージは、１ピクセル当たりのバイト＝４を設定することになり、次いで恐らく、さらなる記述子フィールドが、フロートとしてフォーマット・タイプを記述するために追加される。その「インターリーブ」フィールドは、シーケンシャルモードでチャンネルを書くオプションを与える。例えば、本発明のいくつかの実施形態では、走査システムが、チャンネル情報を同時にではなく、順次に集める、例えば、最初にチャンネル０のすべてのデータを格納し、次にチャンネル１が続くといった具合である。図４は、望ましいファイル・フォーマットの図式を示す。

【００４７】

望ましい実施形態では、＊．ＳＭ１ファイルが、ＳｕｒｒｏＩｍａｇｅから読み取られ、各チャンネルがハンドル記述子を備えたメモリに格納される。データの各チャンネルに関する情報は、イメージ・ハンドル特性と共に、ＳｍＩｍａｇｅＩｎｆｏと称されるクラスに格納されるが、ｈＩｍはその構造の１メンバである。

実行：任意パラメータ

望ましい実施形態では、ＳｕｒｒｏＩｍａｇｅは実行可能なコマンド行である。プログラムを実行するために、次のフォーマットが使用されることができる。

Ｃ：＞ＳｕｒｒｏＩｍａｇｅ｛ＳＭ１入力ファイル｝　｛任意のＬＳＭ出力ファイル｝　｛任意パラメータ・リスト｝

ただし、

ＳＭ１入力ファイル：　＊．ｓｍ１ファイルへのフルパス、

任意のＬＳＭ出力ファイル：　＊．ｌｓｍの出力位置を指定する任意のフルパス。このパラメータが省略される場合、ベース名＊を含む＊．ｓｍ１と同じパスが使用される。

任意パラメータ・リスト：　スペースで区切られた複数のパラメータが割り当てられる。フォーマットの例は、

ＳｕｒｒｏＩｍａｇｅ　Ｃ：／ＳＭ１＿Ｆｉｌｅｓ＼Ｉｍａｇｅ１．ｓｍ１　Ｃ

：＼ＬＳＭ＿Ｆｉｌｅｓ＼Ｉｍａｇｅ１．ｌｓｍ ＴｈｒｅｓｈＲａｔｉｏ＝１．２ Ｗｒｉｔｅ ＲＡＷＦｉｌｅｓ

である。

【００４８】

任意パラメータは、制限されるものではないが、以下のものを含む。

ＴｈｒｅｓｈＲａｔｉｏ： セル検知閾値レベルを決定するために使用される倍数因子。

ｉＮｕｍＣｏｒｒｅｌａｔｉｏｎｓ： チャンネルのｉＮｕｍＣｏｒｒｅｌａｔｉｏｎｓ数への相関を提供する。

ＵｓｅＢａｎｄＰａｓｓＦｏｒＢｌｏｂ： １の場合、セルを検知するために濾過されたイメージを使用する（ＵｓｅＰｅａｋｓＦｏｒＢｌｏｂｓと相互排除でなければならない）。

ＵｓｅＰｅａｋｓＦｏｒＢｌｏｂｓ： １の場合、セルを検知するために、５ｘ５核の中心と、外部ピクセルとの間の差分を使用する。

ＵｓｅＦｕｌｌＰｅｒｉｍＤｅｔｅｃｔ： １の場合、セルを見つけるために中心と関連する外部のすべての周囲ピクセルを使用する。

Ｂｌｏｂａｒｅａｌｏ： 検知すべき最小のセル直径。

ＭａｘＣｅｌｌＳｉｚｅ： ＭａｘＣｅｌｌＳｉｚｅへのセルの直径を直径＞ＭａｘＣｅｌｌＳｉｚｅに設定する。

ＲｏｗｓＰｅｒＮｏｉｓｅＢｌｏｃｋ： ピークーピーク・ノイズ計算において、

ブロック当たりに使用する行の数

ＳａｍｐｌｅＲｏｗｓＰｅｒＮｏｉｓｅＢｌｏｃｋ： ノイズ計算用に各ブロック内でサンプリングすべき行の数

ＭａｘＢｌｏｂＰｉｘ： 閾値化されたメジアン減算ソースイメージが、特定のセグメントをイメージ・マスクに追加されるべき「ブロブ」として指定する、隣接ピクセルの数

ＭａｘＢｕｂｂｌｅＰｉｘ： 負に閾値化されたメジアン減算ソースイメージが、

特定のセグメントをイメージ・マスクに追加されるべき「バブル」として指定する、隣接ピクセルの数

ＢｕｂｂｌｅＴｈｒｅｓｈＦａｃｔｏｒ： バブル検出のためにメジアン減算ソースイメージに適用される－ｔｈｒｅｓｈｏｌｄ＊Ｎｏｉｓｅｆａｃｔｏｒ。代わりに、Ｎｏｉｓｅｆａｃｔｏｒは、ベースライン値（本文参照）に置き換えることができる。

ＢｌｏｂＴｈｒｅｓｈＦａｃｔｏｒ： 汚れ検出のためにメジアン減算ソースイメージに適用されるｔｈｒｅｓｈｏｌｄ＊Ｎｏｉｓｅｆａｃｔｏｒ。代わりに、Ｎｏｉｓｅｆａｃｔｏｒは、ベースライン値（本文参照）に置き換えることができる。

ＭａｓｋＤｉｌａｔｉｏｎＰｉｘ： 最終マスク・イメージは、拡大したＭａｓｋＤｉｌａｔｉｏｎＰｉｘピクセルである。

ＷｒｉｔｅＲＡＷＦｉｌｅｓ： すべての中間の画像ファイルがＣ：＼Ａのディレクトリに書き込まれるべきかどうかを示す診断に用いるブール変数

ＳａｍｅＣｅｌｌＲａｄｉｕｓ： それらの重心間の距離（フロート・フォーマットにおける）が、ＳａｍｅＣｅｌｌＲａｄｉｕｓ以下である場合、代替チャネル内のセルは、同じセルと見なされる。

ＮｏｍＣｅｌｌＭｉｃｒｏｎｓ： 次の３つのパラメータは、すべてのセル計算のために用いられる核・サイズを決定する。

ＢｅａｍＭｉｃｒｏｎｓ： ＮｏｍＣｅｌｌＰｉｘ＝ｈｙｐｏｔ（ＮｏｍＣｅｌｌＭｉｃｒｏｎｓ、ＢｅａｍＭｉｃｒｏｎｓ）／ＭｉｃｒｏｎｓＰｅｒＰｉｘ

ＭｉｃｒｏｎｓＰｅｒＰｉｘ： ｉＮｏｍＣｅｌｌＰｉｘ＝（ｉｎｔ）（ＮｏｍＣｅｌｌＰｉｘ＋１．）で、ｉＮｏｍＣｅｌｌＰｉｘは（ＫｅｒｎｅｌＳｉｚｅ－１）／２である。

ＰｒｉｎｔＭｏｄｅ： ＬＳＭファイルのテキスト出力フォーマットを決定するための数えられる変数で、０の場合は人間に読み取り易く、１の場合はタブで区切られ、２の場合はカンマで区切られる。

各チャンネルからのソースイメージの処理

ＳｕｒｒｏＩｍａｇｅ内の主要ルーチンは、ＳＭＰｒｏｃｅｓｓＩｍａｇｅｓ（）と称される。望ましい実施形態では、制限されるものではないが、ＳｕｒｒｏＩｍａｇｅシステムが、各ソースイメージ、すなわち各チャンネルからのイメージ上に、濾過を行い、マスクをし、汚れ及びバブルを見つけ、最初のセルリストを確立することを含めて、多くの機能を実行する。ＳｕｒｒｏＩｍａｇｅシステムの主要な機能は、発生している個々のチャンネルを合計することなく、各チャンネルが別々に分析されるということである。手短かに言えば、ＳｕｒｒｏＩｍａｇｅシステムは、ノイズ及びバックグラウンド機構（バブルや汚れのような）を除去し、かつ識別される微粒子の空間特性を備えた特徴を増強させるために、各ソースイメージ上で多くの操作を別々に実行する。またシステムは、各チャンネルの微粒子決定用の閾値を決定し、この閾値及び微粒子パラメータに基づいて、各チャンネルの微粒子を別々に識別し分析する。その後、システムは、微粒子がそのチャンネルに対する閾値未満であったために微粒子が検知されなかった残りのチャンネルにおいて、同じピクセルを発見し、それらのチャンネル内の微粒子のパラメータも測定する。この方法で、ＳｕｒｒｏＩｍａｇｅシステムは、微粒子が最初は識別されなかったチャンネルでさえ、各識別された微粒子のデータを収集する。

【００４９】

望ましい実施形態では、１）濾過されたソースイメージ（たたみこみ核のアプリケーション）、２）メジアン減算ソースイメージ、及び３）一時記憶用に使用される作業イメージ、を格納するために用いられる、多くの浮動小数点イメージにハンドルを開くことによって、ＳｕｒｒｏＩｍａｇｅシステムが始動する。さらに、多くのＢＹＴＥイメージが、後で説明されるマスク・イメージを含む、上記の浮動小数点イメージの閾値バージョンを格納するために作成される。

【００５０】

各チャンネルに対して、ベースラインとなる分析を実行することにより、ルーチンが始動することが望ましい。このサブルーチン・コールは、ｙ（注釈：後の参考のために、ｘは、通常は４０ミリメートルあるいはｎ行＝１００００ピクセルである長い毛細管の方向であり、ｙは、通常は１ミリメートルあるいはｎＣｏ

ｌｓ＝２５０ピクセルである検流計走査の方向である）に関するベースラインの全体的な変動に対する統計値を返す。統計値は、ベースラインが、定義済みの制限（一般に、最大値－最小値＞０．３メジアン値）を越えて変化したことを示すブーリアン値のＢａｓｅｌｉｎｅＥｒｒｏｒＦｌａｇを含めて、全体的に格納されることができる。図５は、フローチャート形式でこのプロセスを描く。

【００５１】

それから望ましい実施形態では、１５ｘ１５のメジアン核が、ＴｕｒｂｏＭｅｄｉａｎ（）と称される高速のメジアン・アルゴリズムを用いて、各ソースイメージに適応される。核は、１５ｘｌ５核内の中心ピクセルを、核内の全ピクセルのメジアン値に置き換えることにより作動する。各ピクセルにこのメジアン核を適応することは、Ｙ軸におけるイメージに沿って生じる、ピクセル輝度内の緩やかな変動を「スムーズにする」ように作用する。スムーズにする作業の第一の役割は、セルによる輝度への寄与分を取り除くことであり、その結果、イメージのバックグラウンド表示を得る。メジアンイメージは、ソースイメージから差し引かれ、ｈＩｍｂｇｎｄと称されるグローバル・ハンドルに格納される。制限されるものではないが、全フラックス、楕円率、及びセル直径（フィット・エリアとも呼ばれる）を含むセルパラメータを決定するために、セルリストが生成された後に、このイメージは使用されることができる。

【００５２】

その後、望ましい実施形態では、複数のイメージが、定義済み核でたたみみこまれ、ｉｍＢｌｏｂＳｒｃと称されるグローバル・ハンドルに格納される。そのようなたたみこみ核は、この技術分野ではよく知られている。選択される核構造（核の大きさ及び核内の加重値）は、検知されることになっている微粒子に依存する。例えば、血液セルの決定であれば、７ｘ７核が、ほぼ血液セルの大きさであるので、通常はこの核が用いられる。本説明のためのたたみこみ核は７ｘ７核であると仮定されるが、他の核が他の実施形態において有用であることが理解されるべきである。このたたみこみの結果は、検知されようとしているセルタイプに対応する定義済みの空間成分でもってそれらの特徴を増強するフィルタ補正されたイメージである。このイメージの閾値バージョンは、セル検出に対して、及

びさらに加重フラックス計算に対して、使用されることができる。

【００５３】

いくつかの実施形態では、検知されようとするセルタイプに対応する定義済みの空間成分でもって、最初にそれらの特徴を増強するために、上に記述されたたたみこみ法ではなく「ペリメータ」法が用いられる。ペリメータ法は、差分ソースイメージである「差分」イメージを作成し、異なる２つの方法で実行されることができる。ペリメータ法のいくつかの実施形態では、すべてのピクセルが、７ｘ７核のそれと、外部の４つのピクセルとの間の最小差分に設定される。他の実施形態では、各ピクセルが、７ｘ７核のその値と、外部のすべてのピクセルとの間の最小差分に設定される。畳み込まれたイメージではなく、これらの「差分」イメージを使用することは、ＵｓｅＰｅａｋｓＦｏｒＢｌｏｂｓと称されるブーリアン・コマンド行の引数を介して指定することができる。この場合もやはり、増強されたイメージは、グローバル・ハンドルｉｍＢｌｏｂＳｒｃに格納される。図６は、フローチャートの形式で、ペリメータ法及びたたみこみフィルタ法の使用を示す。

【００５４】

どちらの方法が最初の増強に対して用いられても、生じるイメージは閾値化され、細分化分析がセル位置を決定するために実施される。セル検出用の閾値を確立するには、各ソースイメージ内のノイズを突き止めなければならない。望ましい実施形態では、イメージのセグメントあるいはブロック上のピークピーク・ノイズを計算するアルゴリズムが使用される。図７は、フローチャートの形式でこのプロセスを示す。各ブロックは、ｎ列幅（イメージの全幅）で、ＲｏｗｓＰｅｒＮｏｉｓｅＢｌｏｃｋ（コマンド行の引数）の長さである。各ブロックに対する各ノイズ値は、（ｉｎｔ）（ｎＲｏｗｓ／ＲｏｗｓＰｅｒＮｏｉｓｅＢｌｏｃｋ）エレメントを持つアレイに格納される。その後、このアレイは、ｔｈｒｅｓｈｒａｔｉｏ（コマンド行の引数）で乗算され、閾値判定に使用されるｎ行長さのアレイに挿入される。閾値のサブルーチンは、閾値判定されたＢＹＴＥイメージであるｉｍＢｌｏｂＳｅｇを生成するために、畳み込まれたイメージ、あるいは「差分」イメージのどちらかを使用する。

【００５５】

望ましい実施形態では、ソースイメージがチャンネル０と関連したものである場合、ＭａｓｋＧｒｕｎｇｅＡｎｄＢｕｂｂｌｅｓ（）と呼ばれるサブルーチンが、ｉｍＢｌｏｂＳｅｇ上で細分化又はセル検出を実行する前に呼び出される。図８は、フローチャートの形式で、このサブルーチンを示す。チャンネル０は、バブル及び汚れを発見するために用いられ、それらの領域がマスク・イメージに追加される。これは、サンプルからの最短の放射波長に対応するこのチャンネル内で、サンプル内のゴミが一貫して発生する傾向があるからである。しかしながら、他の実施形態では、他のチャンネル（１つ以上）がマスク・イメージのために使用されることができる。

【００５６】

マスク・バイト・イメージは、３つの異なる状態を介して付加される。ＭａｓｋＧｒｕｎｇｅＡｎｄＢｕｂｂｌｅｓ（）が、これらの状態をテストする。それは、バブルとボルブ閾値であるＢｕｂｂｌｅＴｈｒｅｓｈＦａｃｔｏｒ及びＢｌｏｂＴｈｒｅｓｈＦａｃｔｏｒ（ピークピーク・ノイズ値を乗算された）をそれぞれ適用するために、メジアン減算ソースイメージであるイメージｈＩｍｂｇｎｄを用いる。例えば、バブルに関しては、ソースイメージの特定のブロックに対してｈＩｍｂｇｎｄのいかなる部分も、－１＊ＢｕｂｂｌｅＴｈｒｅｓｈＦａｃｔｏｒ＊ｐ－ｐＮｏｉｓｅ以下であり（バブルはバックグラウンド蛍光が無い事によって示される）、かつ隣接のピクセル総数がＭａｘＢｕｂｂｌｅＰｉｘを超過する場合、それらの対応するピクセルがマスク・イメージ内で「バブル」を示す特定の値に設定される。同様に、ボルブ検出は、ＢｌｏｂＴｈｒｅｓｈＦａｃｔｏｒ＊ｐ－ｐＮｏｉｓｅ及びＭａｘＢｌｏｂＰｉｘを用いてなされる。別の望ましい実施形態では、バブル及びボルブの閾値判定が、ピークピーク・ノイズレベルの因数ではなく、平均ベースライン値の百分率に基づく。したがって、バブル及びボルブの閾値レベルは、それぞれＢｕｂｂｌｅＴｈｒｅｓｈＦａｃｔｏｒ＊ＢａｓｅＬｉｎｅ（ｙ）、及びＢｌｏｂＴｈｒｅｓｈＦａｃｔｏｒ＊ＢａｓｅＬｉｎｅ（ｙ）によって与えられ、その場合のＢａｓｅＬｉｎｅ（ｙ）とは、与えられたｙ値に対してピクセルのｘ範囲を越えた（つまり毛細管の幅を越える）

ベースラインのメジアン値である。マスクへの最終追加は、セグメント化されフィルタ補正されたｉｍＢｌｏｂＳｅｇイメージに基づいてなされる。またそれは、コマンドラインにおいて与えられたのと同じＴｈｒｅｓｈＲａｔｉｏを用いるが、ＭａｘＢｕｂｂｌｅＰｉｘを超過する場合にだけ、マスクに追加する。最終的に、ｎ＝ＭａｓｋＤｉｌａｔｉｏｎＰｉｘのピクセルの拡張（バイナリ拡張とは、そのピクセルが、領域の一部である別のピクセルに既に触れている場合、その任意のバックグラウンド・ピクセルを「オン」に設定する）がマスク上でなされ、セルがバブルの縁で識別されたりすることがないよう予防する。たたみこみフィルタのアーティファクトは、バブルの周縁が、誤ってセルとして識別されることがある輪に畳み込まれる傾向があるということである。拡張は、この誤差を抑制するのに役立つ。

【００５７】

その後、望ましい実施形態では、ｉｍＢｌｏｂＳｅｇイメージ内のセルが、８ポイント接続性ルールを用いて、検数される。図９は、フローチャート形式でこのプロセスを示す。いかなる数の隣接ピクセルもセルリストに追加され、基本パラメータが各々に対して決定される。これらのパラメータは、制限されるものではないが、インデックス、最大のｘとｙのピクセル値、ピクセルの総数、均一に閾値化されるセル領域に基づくｘ－ｙ重心値、並びに閾値を超過する同じピクセルを用いるけれども、ソースイメージ内のピクセル値でそれらの位置に重みを加える加重重心、を含む。この重心値は、すべての後の計算に対して用いられる浮動小数点値である。重心値がマスク内の０でない領域にある場合（マスクに追加された各々は、バブルのために追加されたものが、ブロブのために追加されたものと区別されるように、異なる「識別子」でそれらのピクセルに標識付けがなされていることを思い出すこと）、そのセルはセルリストから削除される。ＳＭＰｒｏｃｅｓｓＩｍａｇｅｓの中でなされる計算の最後の部分は、前述の要素（汚れ、バブル、及びフィルタ・アーティファクト）の各々のために見えなくさせられるイメージの百分率を決定するマスク・イメージのヒストグラムである。またイメージがマスクされたパラメータの全合計が計算される。これは、重要な部分がマスクされる場合に、毛細管の容積を再計算できるようにする。

【００５８】

上で説明されたように、ＭＬＳＣシステムはまた、ＭＬＳＣ器具の操作のためのパラメータ、例えば走査速度、フィルタ帯域幅値などを臨床プロトコル・データベースに格納する。ＳｕｒｒｏＩｍａｇｅシステムを備えたＭＬＳＣ器具の操作パラメータを、見事に調整する能力によって、各分析ができる限り最も効率的で最も敏感な方法で、実行される

セル分析及びｌｓｍ出力

望ましい実施形態で、ＳｕｒｒｏＩｍａｇｅシステム内の大多数のセル分析及びファイル出力は、ＷｒｉｔｅＬｓｍＦｉｌｅ（）ルーチン内で生じる。このルーチンの目的は、任意のチャンネルで検知されたすべてのセルイベントについてのテキストベースのリストファイルを出力することである。さらに、＊．ＬＳＭファイルのヘッダ部分は、全体的なセル統計値（各チャンネルに検知されたセルの数、及び最小サイズと最大サイズ）だけでなく、イメージ統計値（ベースライン・レベルで測定された雑音レベル、平均、メジアン値、及び標準偏差統計値、バブル及びブロブのためにマスクされたイメージの百分率、並びにイメージ作成日）を保持する。その所定のチャンネル用の検知閾値を超過する「ブロブ」を１本のチャンネルが持っていても、セル特性情報がすべてのチャンネルに対して出力される。例えば、１つの「ブロブ」がチャンネル１に検知され、そのブロブが（ｘ＝２２．４、ｙ＝２３４２．３）という加重重心値を持っていたならば、７ｘ７核の中心は（２２、２３４２）となり、どのチャンネルが閾値を超過したセルを実際に持っていたかにかかわらず、その７ｘ７アレイ上で計算されるセル統計値が、すべてのチャンネル・イメージにおいて決定される。各チャンネルのこのように統合された分析が、各セルに対するすべての蛍光データの収集を保証するので、ＭＬＳＣシステムの精度を非常に向上させる。このようにして、例えば検知された分子が非常に低濃度であった場合でも、意味ある情報を供給する非常に弱い蛍光性の信号は無視されない。２本のチャンネル走査用の＊．ＬＳＭファイルの一部の例が、テーブル１で示される。テーブル１内の例は、２つの独立したセルイベント用のセルデータを一覧にする。この特定の例において、パラメータ・イベントソースで分かるように、第１のセルが両方のチャンネルで検知され

た（１＝ＣＨ０、２＝ＣＨ１、４＝ＣＨ２等々で、多重チャネル検出がその値の合計によって示されることに注目すること）。しかしながら、第２のセルは、チャンネル０でのみ検知されたが、それでもパラメータは、チャンネル１の同じ位置に対して計算された。そのことはこの例からは明白ではないけれども、＊．ＬＳＭ内のデータ出力がｙ－重心値によって完全に分類される。個々のチャンネルセルリストからの望ましい実施形態の中で、このデータがどのようにして生成されているかについての説明が続く。

【００５９】

そのルーチンは、各チャンネル内のセルリストの分類により始まる。「ＦｉｎｄＣｅｌｌ」ルーチンが、ｙ方角へ「少しずつ移動する」ことによって、それが最初に見つけるすべてのセルペリメータをセルリストに添付するので、それはｙ－重心値によって必ずしも並び替える必要はない。したがって、バブルの並び替えが、このリストを生成するために用いられる（少ない数の再配列を行う必要がある場合に、バブルの並び替えが最良のソート・アルゴリズムである）。

【００６０】

次のステップは、チャンネル内のセルを一緒にし、ｙ－重心によって並べ替えた汎用セルリストを作成することである。このルーチンの詳細は、以下のとおりである。処理されるべき次の利用可能なセル向けのインデックスは、ＣｅｌｌＦｉｒｓｔＡｖａｉｌＩｎｄｅｘ［チャンネル］と呼ばれ、各チャンネルに対して作成される。そのルーチンは、まだ印字されていない最低のｙ－重心値を備えたセルを見つけるために、チャンネル上をループする。その後、このセルインデックス、及びそれの対応するチャンネル番号が、一時的な設定の変数に保管される。リストであるＣｅｌｌＰｒｉｎｔＬｉｓｔＩｎｄｅｘ［ＣｈａｎｎｅｌｓＭａｘ］は、これまで見つけられたセルのＳａｍｅＣｅｌｌＲａｄｉｕｓ内にその重心がある、別のチャネル内のセルのインデックスを含めて、作成される。このリストのｎＣｈａｎエレメントを満たすために、そのルーチンはすべてのチャンネル内のセルをループする。しかしながら、別のチャネル内のセルが、分析済みであるという「マーク」を既に付けられていた場合、それは読み飛ばして、その指定されたチャンネル内の残りのセルへ移動する。（このループに入ると、ソース

セルインデックスは、ＣｅｌｌＰｒｉｎｔＬｉｓｔＩｎｄｅｘ［ｓｏｕｒｃｅ＿ｃｈａｎｎｅｌ］エレメントに最初に追加される（つまり分析される「予定である」というマークが付けられる）ことに注意すること）。それの重心が、元のセルからのＳａｍｅＣｅｌｌＲａｄｉｕｓ距離未満であるいかなるセルも、ＣｅｌｌＰｒｉｎｔＬｉｓｔＩｎｄｅｘアレイにそのインデックスが追加される。

【００６１】

いったん単一のセルイベントが、関連するチャンネルに適合すると、テキストベースの．ＬＳＭファイルに出力される準備が整う。このサブルーチンＰｒｉｎｔＣｅｌｌ（）は、ＷｒｉｔｅＬｓｍＦｉｌｅ（）ルーチンからコールされ、２つの引数、セルチャンネル・リストへのインデックス、及び現在のセルイベント・カウントを保持するＣｅｌｌＰｒｉｎｔＬｉｓｔＩｎｄｅｘアレイを得る。そのルーチンはすべてのチャンネルを通してループし、ＣｅｌｌＰｒｉｎｔＬｉｓｔＩｎｄｅｘアレイ中でインデックスを付けられたセルの重心値にアクセスする。その後、ルーチンは、数値が求められようとしている特定のセルに対して、チャンネル間のｘ及びｙにおける平均重心値を計算する。その結果はＸ、Ｙにおいて最も近い全ピクセルに丸められ、Ｘ、Ｙに関して中心にある７ｘ７ピクセル領域内のセルパラメータを計算するＡｎａｌｙｚｅＣｅｌｌ（）と呼ばれる別のルーチンをコールするために用いられる。このルーチンはチャンネル番号を通じてループ内でコールされる。セル構造ＡｎａｌｙｚｅＣｅｌｌ（）が満たすＣ＋＋は、以下のとおりである。

【００６２】

【表１】

```
typedef struct
{
  double        x,      y,
                Area,
                TotalFlux,
                WeightedFlux,
                Diameter,
                Ellipticity,
                Brightest;

  int           Printed; /* TRUE if printed already */
} CELLINFO;
```

【００６３】

ＡｎａｌｙｚｅＣｅｌｌ（）は、ｉｍＢｌｏｂＳｒｃイメージへのポインタを得て、セルのＸ、Ｙ位置へそのポインタを再配置することにより始まる。Ｘ、Ｙ位置及び呼出しチャンネル番号以外に、ＡｎａｌｙｚｅＣｅｌｌ（）に渡されるパラメータのうちの１つは、この特定チャンネルが「ソース」チャンネルだったかどうか（つまり、セルがこのチャンネルで実際に検知されたかどうか）を示すブーリアン・フラグである。それがソース・チャンネルである場合、ｉｍＢｌｏｂＳｒｃイメージ内の７ｘ７関心領域（ＲＯＩ）で見つけられた最大値の位置が、返される。これが核の中央Ｘ、Ｙ位置と一致しない場合、この特定チャンネルに対してグローバル・パラメータｎＢｌｏｂｓＯｆｆｓｅｔＦｒｏｍＰｅａｋが増加させられる。このようにして、中央セル位置を判定するために使用される方法が評価される。さらに、重複を明瞭にする手段として、セル構造それ自身にこのパラメータを追加することができる。

【００６４】

ＡｎａｌｙｚｅＣｅｌｌ（）をコールしたチャンネル内で、セルが検知されよ

うが検知されまいが、加重フラックスは、ｉｍＢｌｏｂＳｒｃイメージ内のＸ，Ｙ位置で単にピクセル値を評価することにより計算される。このピクセル値は、下のテーブル２で与えられる定義済みの７ｘ７核によって加重される、７ｘ７領域内のすべてのソースイメージ・ピクセル値の加重合計を表す。

【００６５】

別の実施形態では、ＡｎａｌｙｚｅＣｅｌｌ（）内で数値が求められる他のパラメータは、制限されるものではないが、全フラックス、楕円率、及び平均直径を含む。全フラックスと平均直径は、別の機能コールＣｏｍｐｕｔｅＭｅａｎＲａｄｉｕｓ（）によって、数値が求められる。図１０はこの機能コールを、フローチャート形式で示す。ＣｏｍｐｕｔｅＭｅａｎＲａｄｉｕｓ（）は平均直径を計算するだけでなく、全フラックスがメジアンを差し引いた同じイメージｈＩｍｂｇｎｄから計算されるので、このルーチンにも含まれる。ｈＩｍｂｇｎｄを引き出すために、１５ｘ１５ピクセル・メジアン・フィルタがソースイメージに適用され、その結果がソースイメージから減算されたことを思い起こすこと。平均直径を決定するために、重心値が最初に計算される（注釈：この重心は、そのチャンネル用の閾値を超過するピクセルから計算された以前の重心と対比して、７ｘ７正方形内のピクセルから計算されるので、セルの中心を決定するために計算された重心値とは異なる）。そうして数学的には次の式によって、示されるように、各ピクセルの重心からの距離は、ピクセル値に対して加重される。

【００６６】

【数１】

$$D = 2 \cdot \frac{\sum_{n=1}^{N} P_{x_n,y_n} \sqrt{(C_x - x_n)^2 + (C_y - y_n)^2}}{\sum_{n=1}^{N} P_{x_n,y_n}} \qquad (1)$$

【００６７】

ただし、重心値C_x及びC_yは、次式により与えられる。

【００６８】

【数２】

$$C_x = \frac{\sum_{n=1}^{N} x_n P_{x_n,y_n}}{\sum_{n=1}^{N} P_{x_n,y_n}} \quad \text{and} \quad C_y = \frac{\sum_{n=1}^{N} y_n P_{x_n,y_n}}{\sum_{n=1}^{N} P_{x_n,y_n}}, \tag{2}$$

【００６９】

Px_n，y_nは位置ｘ，ｙでのピクセルの値であり、Ｎは７ｘ７核に対して４９である。

小さな微粒子の直径を計算するためのこのモーメント法のアルゴリズムは、２次元のガウス・フィット・ルーチン上でより良い性能を提供することが発見された。ガウス・フィット・ルーチンは、図１１に示されるように、低輝度のセルに対する実際の直径を小さく見積もる傾向がある。この偏向は、モーメント法のアルゴリズムで発見されたが、非常に少ししか公言されていない。

【００７０】

全フラックスは、方程式（１）及び（２）の分母によって簡単に与えられる。全フラックスがゼロ以下である場合（それはバックグラウンドから差し引かれたイメージ中で起こることがある）、合計はオーバーフローを防ぐために値１．０に指定され、平均直径は０に設定される。

【００７１】

ＰｒｉｎｔＣｅｌｌ（）ルーチンで数値が与えられる他の２つのセルパラメータは、チャンネル間の比率及び相関の値を含む。比率（テーブル１の例を参照）は、次式により与えられる。

【００７２】

【数３】

$$R_{m/n} = \frac{Wtd.Flux_m}{Wtd.Flux_n}, \qquad \text{where } m>n. \tag{3}$$

【００７３】

ピアソンの相関、$\rho_{m,n}$ 係数は次式により計算される。

【００７４】

【数４】

$$\rho_{m,n} = \frac{\sum_{n=1}^{N}(P_{x_n,y_n}^{(m)} - \bar{P}^{(m)})(P_{x_n,y_n}^{(n)} - \bar{P}^{(n)})}{(N-1)S_m S_n}, \qquad (4)$$

【００７５】

ここでＳｍ及びＳｎは、それぞれチャンネルｍ及びｎにおけるソースイメージ（ｉｍＳｒｃ）ピクセル値の標準偏差であり、バーは平均ピクセル値を表す。各セルがチャンネルを横切ってグループ化するように、これらのセルパラメータの各々は、シーケンシャルな方式で、＊．ＬＳＭファイルに書き込まれる。

【００７６】

ＷｒｉｔｅＬＳＭＦｉｌｅ（）ルーチンは、ｐｒｉｎｔＣｅｌｌ（）及びサブルーチンＡｎａｌｙｚｅＣｅｌｌ（）をコールするたびに、すべてのセルを一定の順序に配列する。セル数の合計は検数され、＊．ＬＳＭファイルのヘッダ部分に書き込まれる。その後、ファイルが閉じられ、プログラムは終了する。

【００７７】

ここで記述されるＳｕｒｒｏＩｍａｇｅシステムは、レーザ・スキャニング・サイトメトリのコンテキストにおいて微粒子検出を行う従来技術システム上の実質的な進歩である。１つのそのような従来技術システムは、その全体がここに参考文献として組み入れられる、米国特許第５，５５６，７６４号（’７６４特許）に記述されている。’７６４特許に記述されたシステムは、まず第１に個々のチャンネルからのイメージを合計し、そして次に、その結果生じる合成イメージ上の粒子を実行するが、’７６４システムは、いかなるブロブとバブルのマスキングも実行しない。さらに、’７６４システムは、例えば、特定の粒径範囲内のセルを検知するといったように、分析の対象である特定のタイプのセルに対し、非常に選択的となるように設計されている。これとは対照的に、本システムはそれほど限定的でなく、このようにもっと様々なタイプのセルを検知する。ここで記述されたブロブ及びバブルマスキング技術によって結び付けられた別々のチャ

ンネル分析によって、ＳｕｒｒｏＩｍａｇｅは、’７６４システムより多くの本当のセルを、正確に識別することができ、かつそこからデータを収集することができる。従って、本システムは従来の技術システムより正確で、かつより感度がよい。

【００７８】

ＳｕｒｒｏＩｍａｇｅシステムの別の利点は、セル会合分子の蛍光について種々の形態構造、及び／又は異なるパターンあるいは輝度を備えた様々な異なるセルの検出に対して、容易に最適化されることができるということである。さらに、ＳｕｒｒｏＩｍａｇｅシステムは、セル以外の微粒子の検出に対して速やかに最適化されることができる。例えば、本発明のいくつかの実施形態では、ＳｕｒｒｏＩｍａｇｅシステムは毛細管内のマイクロビーズを検知するために用いられ、そのマイクロビーズは血液内に存在する特定の試薬に結び付く。ＳｕｒｒｏＩｍａｇｅシステムとは対照的に、従来の技術システムは特定のセルだけを検知することができ、重要なオペレータの介入なしでは、他の構造を検知するための型の変更ができない。たたみこみ核の構造のようなＳｕｒｒｏＩｍａｇｅシステムの個々のサブルーチンのパラメータは、これらの微粒子の検出を最適化するために速やかに変更されることができる。これらのパラメータは臨床プロトコル・データベースに格納されることができる（以下を参照）。したがって、ＳｕｒｒｏＩｍａｇｅシステムは、ＭＬＳＣシステムの柔軟性を増大させ、感度において妥協することなく種々の分析を実行することができる。

情報処理システムのアーキテクチャ

本発明は、多くの重要な機能を実行する斬新な情報処理システム・アーキテクチャを含む。システムの心臓部は、マルチパラメータ分析を設計し、測定計測器を制御し、イメージ及びデータ分析を実行し、並びに結果をアーカイブするために必要とされる情報のすべてを統合するために用いられる関連データベースである。システムは、別々の機能を実行する多くの連結されたモジュールを含む。図１１は、このシステムが望ましい実施形態の中で作用する方法についてフローチャートで表現したものを示す。手短かに言えば、計器制御ソフトウェアは、ＳｕｒｒｏＳｃａｎハードウェア（ＭＬＳＣ器具）を制御し、そうすることで、サン

プルを走査し、生の画像ファイル（．ＳＭ１ファイル）を作成する。その後、．ＳＭ１ファイルは、（上の）ＳｕｒｒｏＩｍａｇｅ画像分析ソフトウェアによって処理され、増強される。このモジュールは各イメージを増強し、各イメージ内の各セル（あるいはいくつかのアプリケーション内の蛍光ビード）の位置及びサイズを決定し、次に、各チャンネル内での各セル（あるいはビード）の蛍光輝度を計算する。その結果生じるＳｕｒｒｏＩｍａｇｅデータは、テキストファイル（．ＬＳＭファイル）として格納され、次に、ＦＣＳ変換ソフトウェア（ＦＣＳ Ｃｏｎｖｅｒｓｉｏｎ Ｓｏｆｔｗａｒｅ）によって、業界標準の．ＦＣＳフォーマットに変換されるか、又はその後の分析のための適切な他のファイル形式に変換されることができる。測定器制御ソフトウェア、画像分析ソフトウェア及びＦＣＳ変換ソフトウェアはすべて、臨床プロトコルの実行の中で用いられる各タイプの分析のためのパラメータを格納する臨床プロトコル・データベースによって制御される。そのようなパラメータは、制限されるものではないが、ＭＬＳＣ器具の走査速度、ＭＬＳＣ器具の中で用いられるフィルタ帯域幅の値、及びＳｕｒｒｏＩｍａｇｅシステムの中で用いられる核構造を含む。その後、その形式、例えば．ＦＣＳ及び．ＬＳＭのファイル内のデータは、例えば市販のＦｌｏｗＪｏソフトウェアを用いて、データをさらに処理するために、サーバに送出されることができる。またそのデータは、アーカイブするため、及び第３次メディアに周期的に送出するための、実験データファイル・サーバに送信され、またオラクルデータベースのような中央のデータベースにも送信される。中央のデータベースは、制限されるものではないが、臨床プロトコル・データベースの整合性を維持するため、器具結果、ファイル名、較正情報のための中央の貯蔵所として、（ＭＬＳＣシステムを通して得られようが、又は従来のＥＬＩＳＡ分析を通して得られようが）セルの分析測定値及び可溶性要素の測定値を格納するため、並びに臨床アンケート情報を維持するために、使用される。

【００７９】

望ましい実施形態では、ＳｕｒｒｏＳｃａｎ情報処理システムが、（適切な関連データベース・スキーマのこれまでの設計、及び較正された器具が使用可能であること、を仮定して）臨床研究のための次の方法で用いられる。まず第１に、

ユーザは、患者の数及び識別情報、患者当たりのサンプル数などのような情報を含む、臨床研究プロトコルを定義する。臨床研究は、何十から何百人もの患者を関与させ、何週間から何ヶ月も続く。またユーザは、分析プロトコルを定義し、それが各特定患者のサンプルで実行される分析の各々を詳細に定義する。各分析は、制限されるものではないが、使用される蛍光体、ターゲット分子、希釈、及び蛍光補正パラメータを含めて、試薬の各々に対する詳細な識別子及び説明を含む。サンプル調製方法及びサンプル希釈もまた含まれる。またプロトコルは、ＳｕｒｒｏＳｃａｎ器具及びデータ分析ソフトウェアを自動的に制御するのに必要な情報を含む。患者サンプルが（データベース制御の下で自動化されることができる）各分析に対して処理され、ＳｕｒｒｏＳｃａｎ上の測定カートリッジに取り付けられた後、ユーザが、スキャナ・ソフトウェア（その後、それは詳細な走査パラメータ例えばを決定するためにデータベースに質問する）にプロトコルＩＤ及びサンプルＩＤパラメータを入れ、そうして、そのソフトウェアが、詳細な走査パラメータ、例えば、走査速度、フィルタ帯域幅設定値、ステージ移動速度などを決定するために、データベースに質問する。走査が完了した後、器具は適切な分析パラメータを学習するために再びデータベースに質問し、そうしてＦＣＳ出力ファイルを生成して、ＳｕｒｒｏＩｍａｇｅ及びＳｕｒｒｏＦＣＳのソフトウェア・モジュールでもって正しいタイプの分析を自動的に実行する。ＦＣＳ出力ファイルは、市販のＦＣＳ分析ソフトウェアを用いて、さらに分析される。その後、各患者サンプルに対するＦＣＳ出力データの要約が、ＦＣＳソフトウェアによって生成され、リレーショナル・データベース内のストレージを有効にするためにさらに処理される。その後、測定結果及び患者の臨床情報は、対象となる生物学的マーカを示すパターン及び相関を識別するための様々な統計的手法及びビジュアル化の手法でさらに処理される。サンプル及び分析情報が、生のイメージから、関連データベースへのリスト・モード・フォーマットまで、分析の全体に渡って、そのデータと関連する。

【００８０】

また本発明は、増強されたデータを如実に表示するためにイメージ・システムの使用を意図する。このシステムは、ＳｕｒｒｏＶｉｅｗと名付けられるが、Ｓ

ｕｒｒｏＩｍａｇｅソフトウェアによって識別された個々のセルを表示し、そのイメージ内の真実のセルを他のセルの形をした擬似信号と区別するために、各識別されるセルの周りに、１つのボックスを置くことができる。ＳｕｒｒｏＶｉｅｗソフトウェアは特に、すばやく様々なタイプのシステム障害モードを診断するのに役立つ。ＳｕｒｒｏＳｃａｎ器具の通常操作中に、セルのそのようなイメージをオペレータが常に見ている必要はないことが指摘されるべきである。

テーブル１：リスト・モード出力の例。データは２チャンネル走査に対応する。

【００８１】

【表２】

セルNo.	チャンネル:0		全流束	加重流束	セル直径	セル楕円率	最輝度ピクセル	相関0-1	比率1/0	相関0-2	比率2/0
	X	Y									
1	191	23	4141	427	3.2	0.90	3798	0.91	0.56	0.48	0.08
2	196	26	2282	501	1.3	0.95	4124	0.917	0.36	0.71	0.11
3...											

セルNo.	チャンネル:1		全流束	加重流束	セル直径	セル楕円率	最輝度ピクセル	相関1-2	比率2/1	イベント・ソース
	X	Y								
1	191	23	1824	241	2.5	0.88	1962	0.41	0.14	3
2	196	26	1294	181	1.9	0.93	2027	0.77	0.29	1
3...										

【００８２】

テーブル２：フィルタ補正されたイメージｉｍＢｌｏｂＳｒｃを作成するために使用されるたたみこみ核。ｉｍＢｌｏｂＳｒｃは、セル検出及びセルの加重フラックス評価の両方に用いられる。

【００８３】

【表３】

-3.54	-3.54	-3.54	-3.54	-3.54	-3.54	-3.54
-3.54	2.00	2.00	3.00	2.00	2.00	-3.54
-3.54	2.00	4.00	6.00	4.00	2.00	-3.54
-3.54	3.00	6.00	9.00	6.00	3.00	-3.54
-3.54	2.00	4.00	6.00	4.00	2.00	-3.54
-3.54	2.00	2.00	3.00	2.00	2.00	-3.54
-3.54	-3.54	-3.54	-3.54	-3.54	-3.54	-3.54

【図面の簡単な説明】

【図１】　本発明の１つの望ましい実施形態におけるＭＬＳＣ器具の光アーキテクチャを示す。

【図２】　図２Ａは切り替え可能なフィルタ・スキームの部分的な回路図である。

図２Ｂは切り替え可能なフィルタ・スキームの部分的な回路図である。

【図３】　ＳｕｒｒｏＩｍａｇｅプロセスのフローチャートである。

【図４】　本発明によって意図される１つのファイル・ストレージの実施形態を図式的に示す。Ｎチャンネルのデータが、拡張子＊．ｓｍ１で指定されるバイナリ・ファイルにインタリーブ・フォーマットで格納される。様々なデータフォーマットが可能になるように、ヘッダが選定される。

【図５】　ベースライン分析プロセスのフローチャートである。

【図６】　セル検出プロセスのフローチャートである。

【図７】　ノイズ分析プロセスを示す。

【図８】　マスク生成プロセスのフローチャートである。

【図９】　セルを見つけるための８ポイント接続性ルールを図示するフローチャートである。

【図１０】　本発明によって意図される、可能ないくつかのタイプのセル分析を示す。

【図１１】　ガウス・フィット・アルゴリズムを、直径モーメント計算と比較したプロットである。イメージについて、各ポイントが、２５０カウントに等しいＲＭＳノイズで１０００個の粒子（セル）の擬似イメージの中から検出された微粒子の平均直径値である。

【図１２】　ＳｕｒｒｏＳｃａｎシステムの情報処理システム構成のフローチャートである。

【図１】

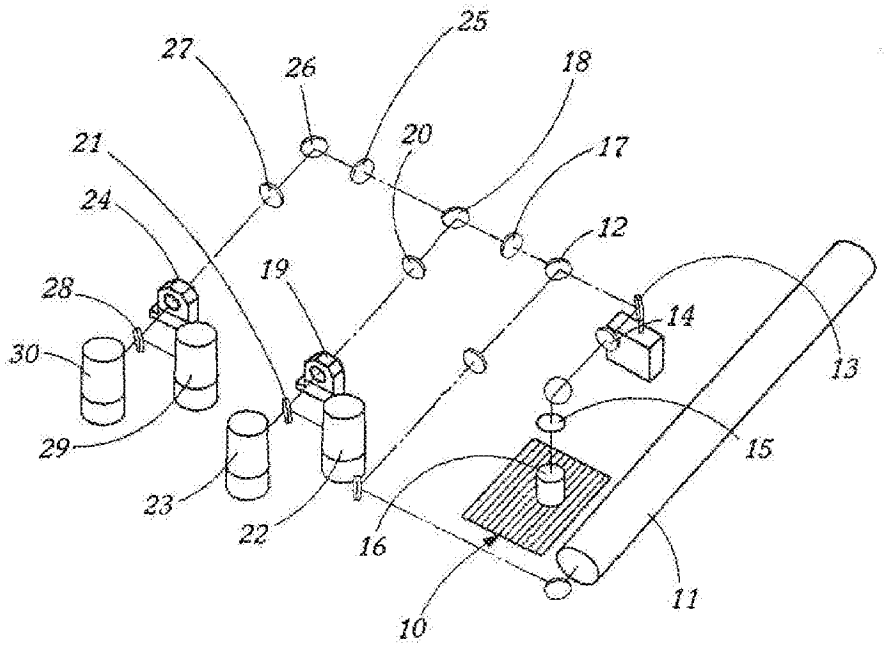


Figure 1

【図２Ａ】

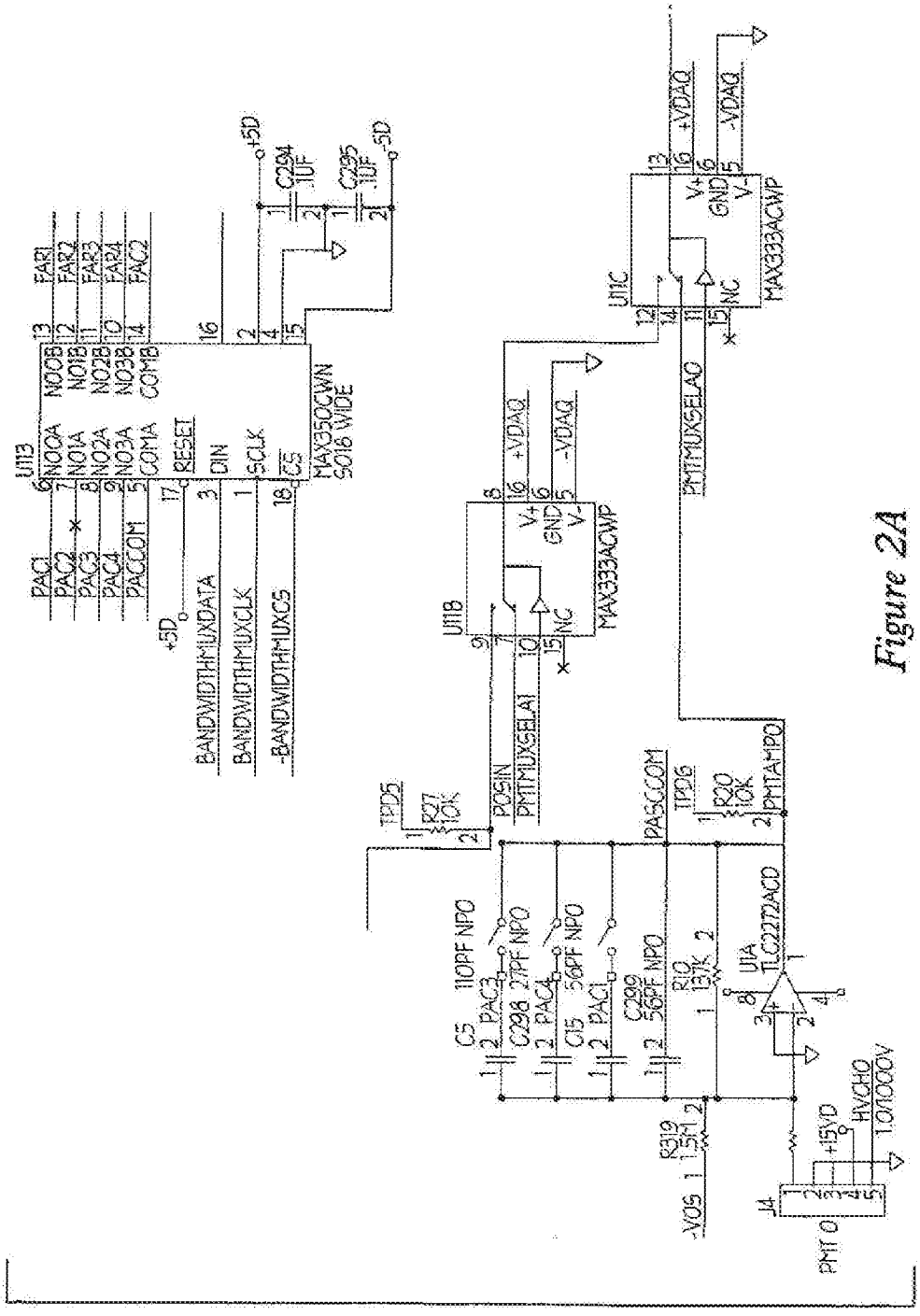


Figure 2A

【図２Ａ－１】

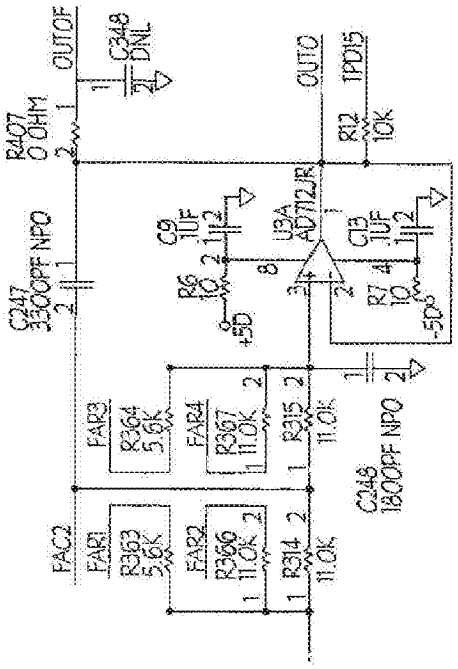


Figure 2A (cont.)

【図２Ｂ】

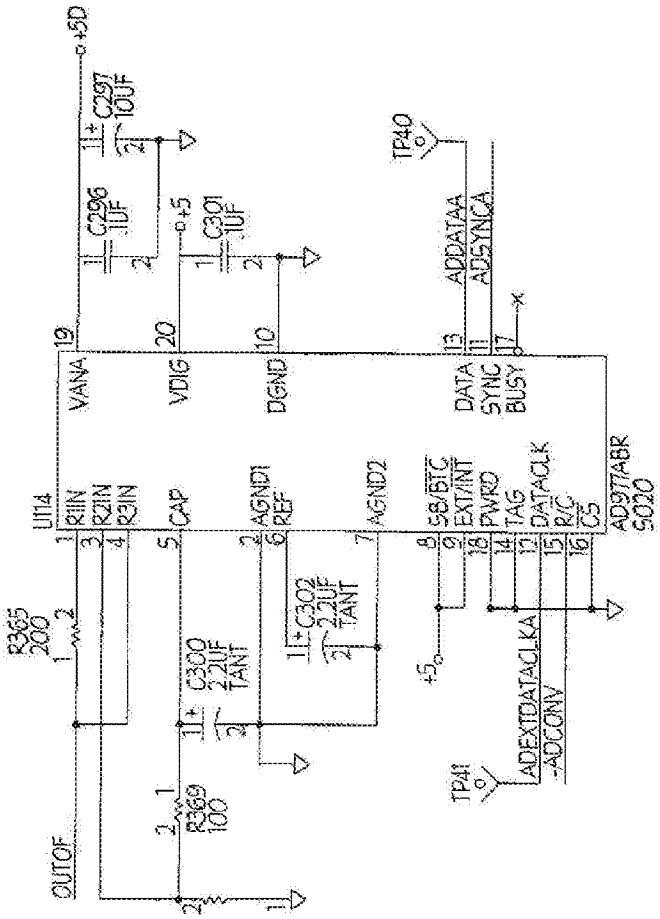
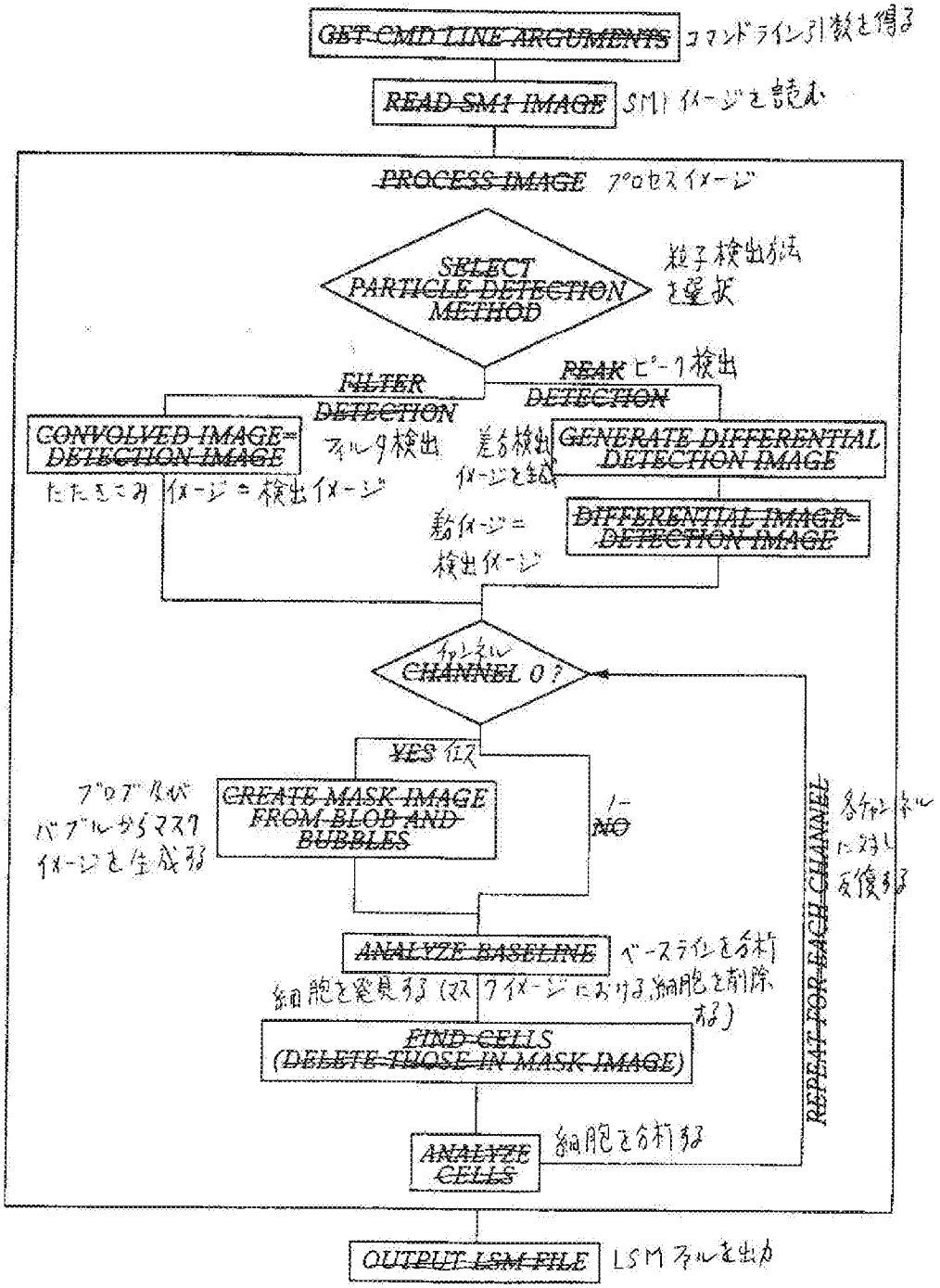
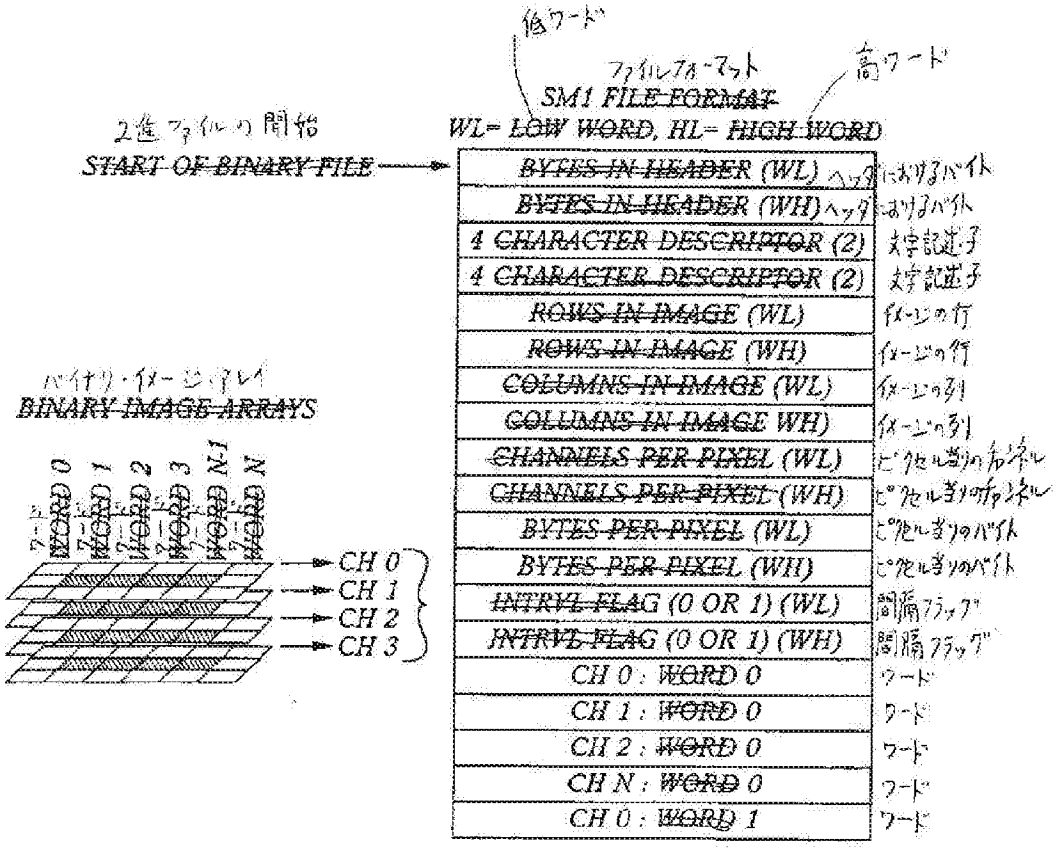


Figure 2B

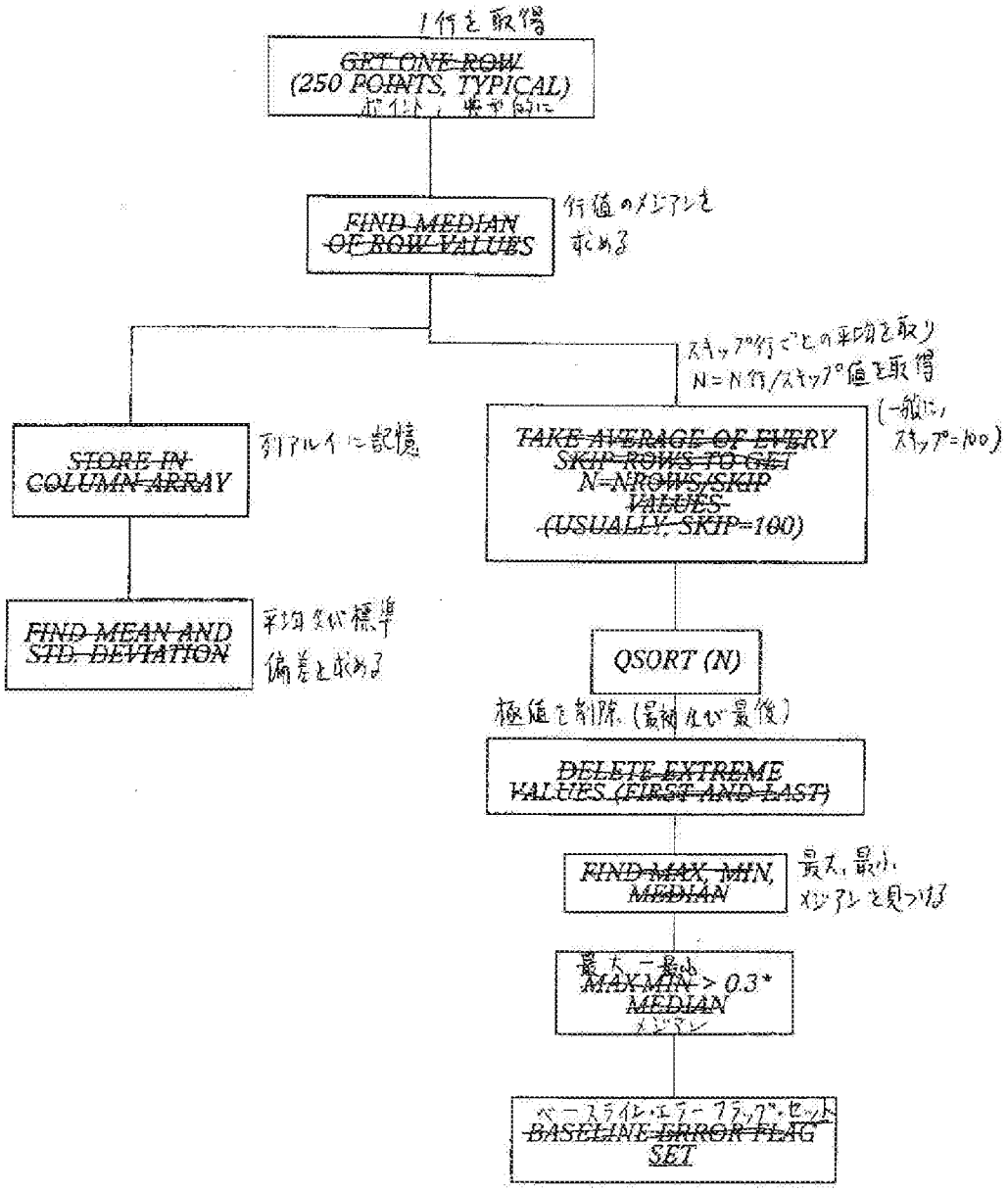
【図３】


GET CMD LINE ARGUMENTS
コマンドライン引数を得る
READ SM1 IMAGE
SM1 イメージを読む
PROCESS IMAGE
プロセスイメージ
SELECT PARTICLE DETECTION METHOD
粒子検出法を選択
FILTER DETECTION
フィルタ検出
PEAK DETECTION
ピーク検出
CONVOLVED IMAGE= DETECTION IMAGE
たたみこみイメージ＝検出イメージ
GENERATE DIFFERENTIAL DETECTION IMAGE
差分検出イメージを生成
DIFFERENTIAL IMAGE= DETECTION IMAGE
差イメージ＝検出イメージ
チャンネル CHANNEL 0?
YES 真
NO 偽
CREATE MASK IMAGE FROM BLOB AND BUBBLES
ブロブ及びバブルからマスクイメージを生成する
ANALYZE BASELINE
ベースラインを分析
細胞を発見する（マスクイメージにおける細胞を削除する）
FIND CELLS (DELETE THOSE IN MASK IMAGE)
ANALYZE CELLS
細胞を分析する
REPEAT FOR EACH CHANNEL
各チャンネルに対し反復する
OUTPUT LSM FILE
LSM ファイルを出力

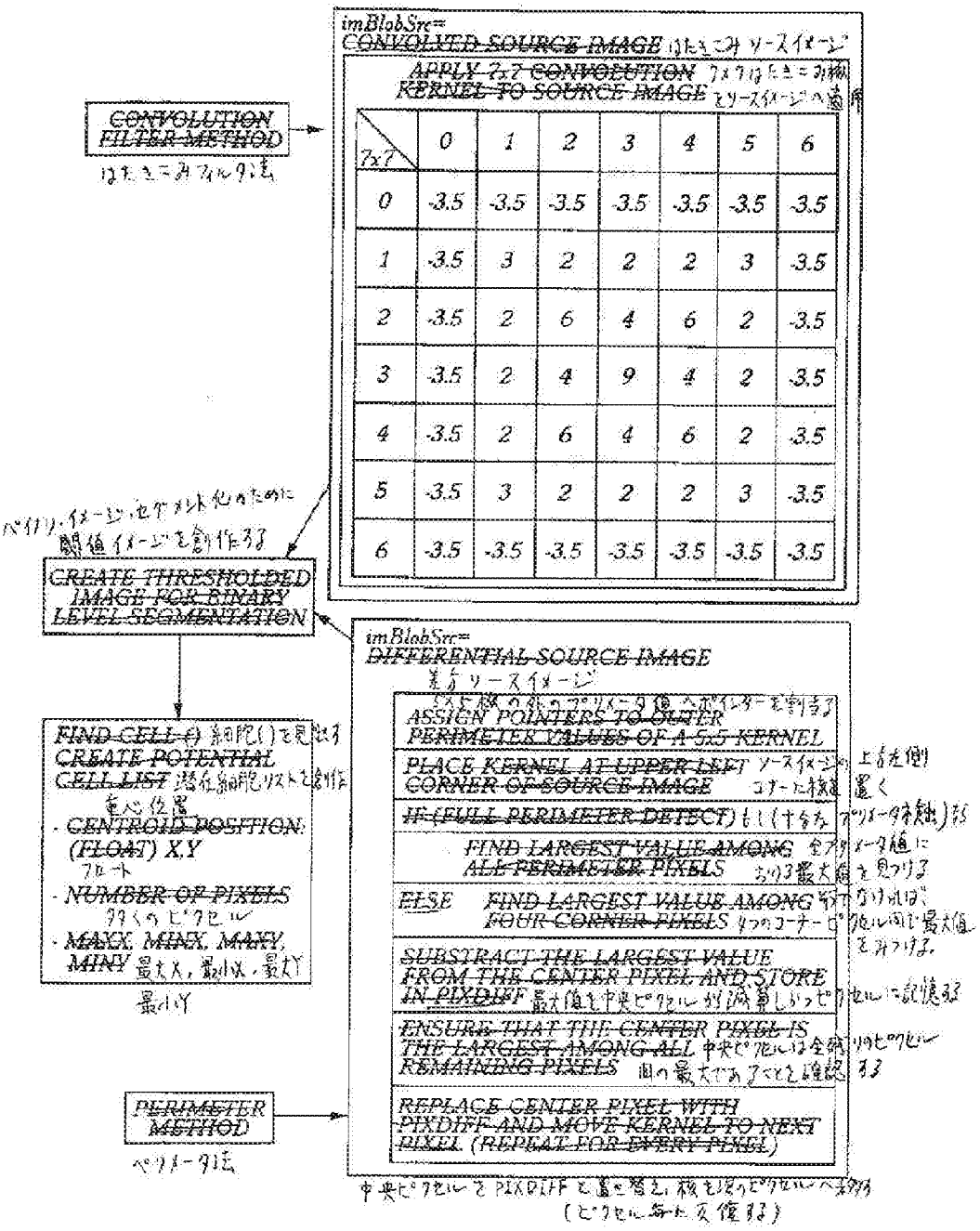
【図４】


低ワード
ファイルフォーマット
SM1 FILE FORMAT
高ワード
2進ファイルの開始
START OF BINARY FILE
WL= LOW WORD, HL= HIGH WORD
BYTES IN HEADER (WL) ヘッダにおけるバイト
BYTES IN HEADER (WH) ヘッダにおけるバイト
4 CHARACTER DESCRIPTOR (2) 文字記述子
4 CHARACTER DESCRIPTOR (2) 文字記述子
ROWS IN IMAGE (WL) イメージの行
ROWS IN IMAGE (WH) イメージの行
COLUMNS IN IMAGE (WL) イメージの列
COLUMNS IN IMAGE WH) イメージの列
CHANNELS PER PIXEL (WL) ピクセル当りのチャネル
CHANNELS PER PIXEL (WH) ピクセル当りのチャネル
BYTES PER PIXEL (WL) ピクセル当りのバイト
BYTES PER PIXEL (WH) ピクセル当りのバイト
INTRVL FLAG (0 OR 1) (WL) 間隔フラッグ
INTRVL FLAG (0 OR 1) (WH) 間隔フラッグ
CH 0 : WORD 0 ワード
CH 1 : WORD 0 ワード
CH 2 : WORD 0 ワード
CH N : WORD 0 ワード
CH 0 : WORD 1 ワード
バイナリ・イメージ・アレイ
BINARY IMAGE ARRAYS
WORD 0
WORD 1
WORD 2
WORD 3
WORD N-1
WORD N
CH 0
CH 1
CH 2
CH 3

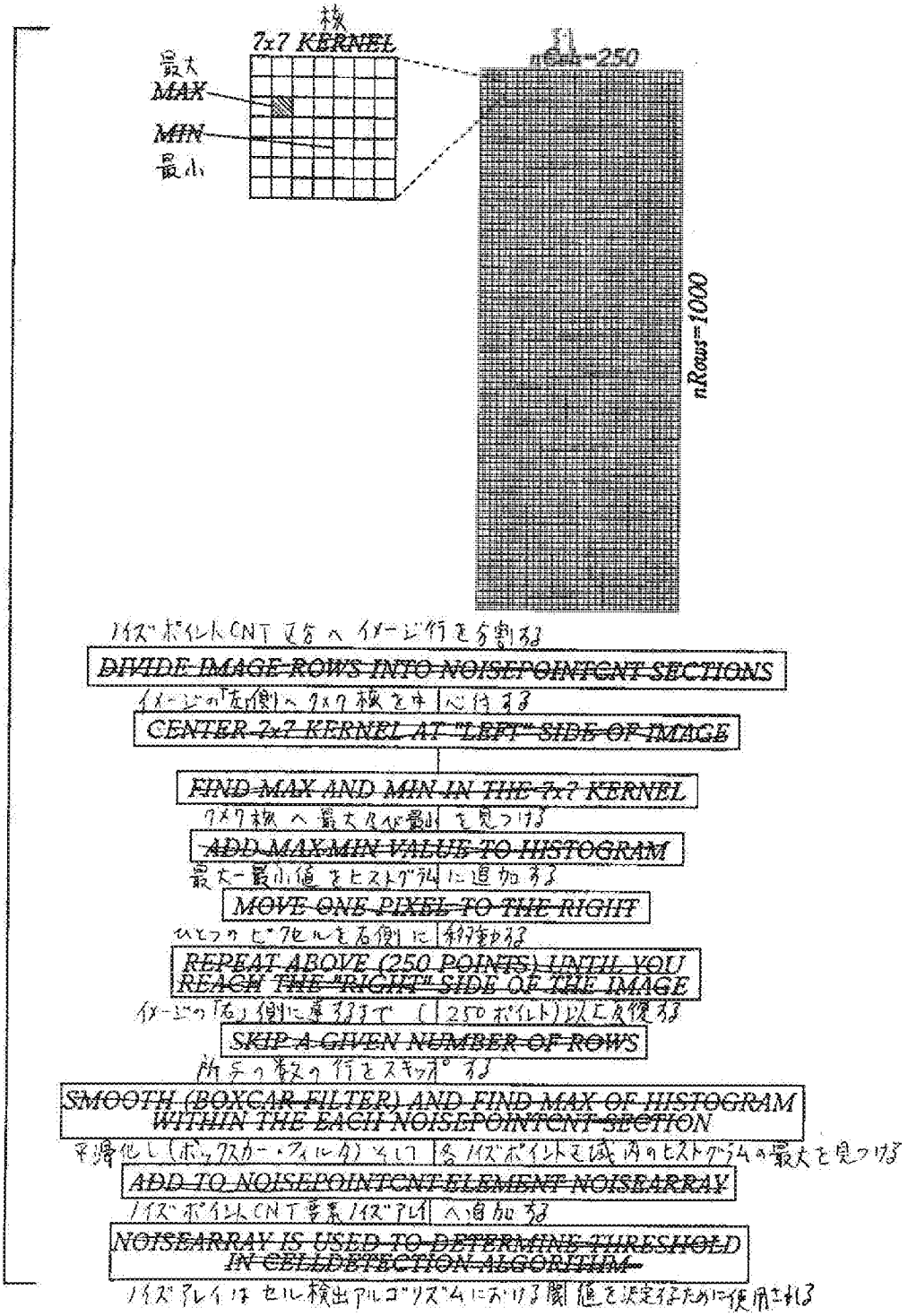
【図５】


1行を取得
GET ONE ROW
(250 POINTS, TYPICAL)
1行1行、順次的に
FIND MEDIAN OF ROW VALUES
行値のメジアンを求める
スキップ行ごとの平均を取り N=N行/スキップ値を取得
(一般に、スキップ=100)
STORE IN COLUMN ARRAY
列アルイに記憶
TAKE AVERAGE OF EVERY SKIP ROWS TO GET N=NROWS/SKIP VALUES (USUALLY, SKIP=100)
FIND MEAN AND STD. DEVIATION
平均及び標準偏差を求める
QSORT (N)
極値を削除.(最初及び最後)
DELETE EXTREME VALUES (FIRST AND LAST)
FIND MAX, MIN, MEDIAN
最大、最小、メジアンを見つける
最大－最小
MAX-MIN > 0.3* MEDIAN
メジアン
ベースライン・エラー・フラッグ・セット
BASELINE ERROR FLAG SET

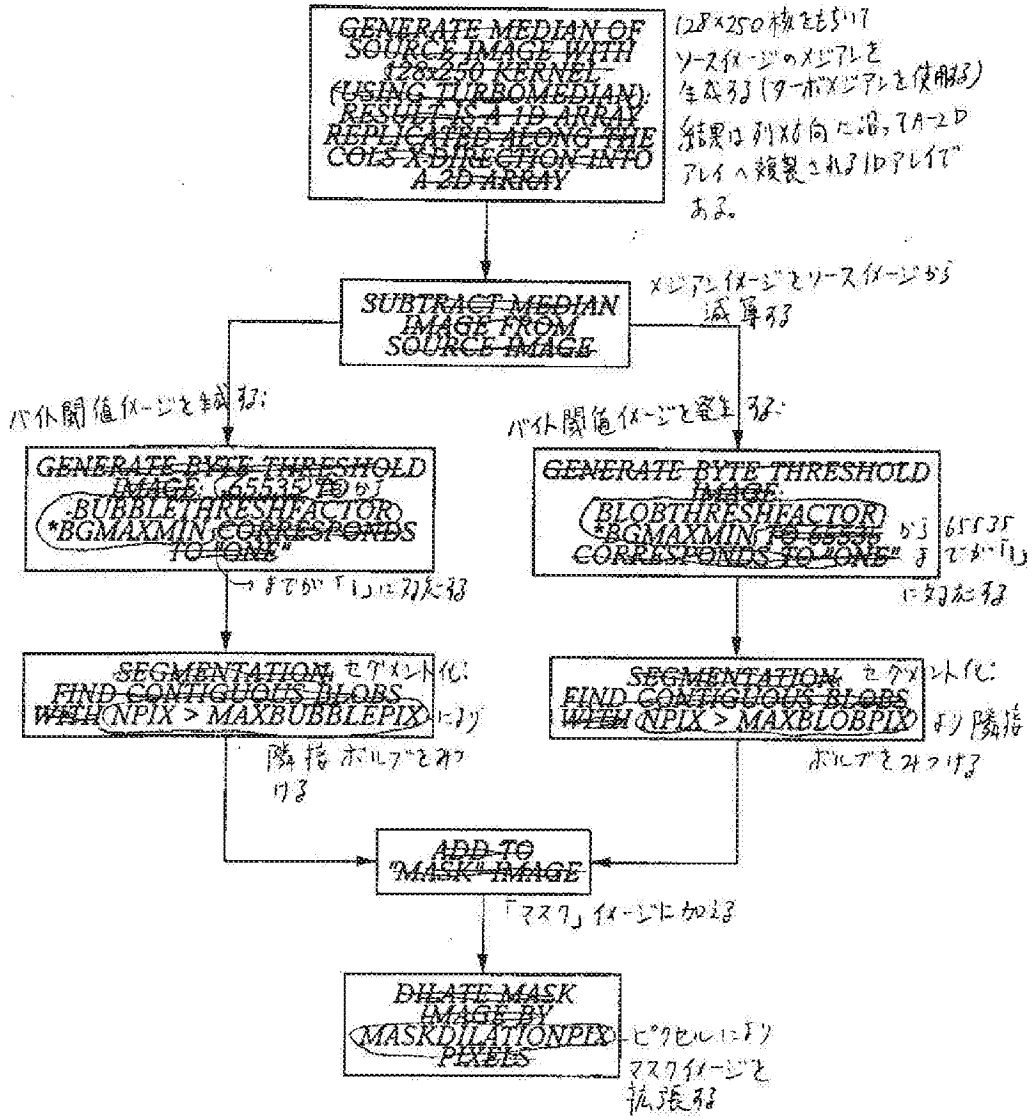
【図６】


imBlobSrc=
CONVOLVED SOURCE IMAGE はたたみ ソースイメージ
APPLY 7x7 CONVOLUTION フィルタはたたみ 積
KERNEL TO SOURCE IMAGE をソースイメージへ 適用
CONVOLUTION FILTER METHOD
はたたみフィルタ法
7x7 0 1 2 3 4 5 6
0 -3.5 -3.5 -3.5 -3.5 -3.5 -3.5 -3.5
1 -3.5 3 2 2 2 3 -3.5
2 -3.5 2 6 4 6 2 -3.5
3 -3.5 2 4 9 4 2 -3.5
4 -3.5 2 6 4 6 2 -3.5
5 -3.5 3 2 2 2 3 -3.5
6 -3.5 -3.5 -3.5 -3.5 -3.5 -3.5 -3.5
バイナリ・イメージ・セグメント化のために
閾値イメージを創作する
CREATE THRESHOLDED IMAGE FOR BINARY LEVEL SEGMENTATION
imBlobSrc=
DIFFERENTIAL SOURCE IMAGE
差分 ソースイメージ
FIND CELL () 細胞() を見出す
CREATE POTENTIAL CELL LIST 潜在細胞リストを創作
重心位置
- CENTROID POSITION (FLOAT) X,Y
フロート
- NUMBER OF PIXELS 多くのピクセル
- MAXX, MINX, MAXY, MINY 最大X, 最小X, 最大Y 最小Y
PERIMETER METHOD
ペリメータ法
5x5 様々なペリメータ値へポインターを割当る
ASSIGN POINTERS TO OUTER PERIMETER VALUES OF A 5x5 KERNEL
PLACE KERNEL AT UPPER LEFT CORNER OF SOURCE IMAGE ソースイメージの 上方左側 コーナーに 設置 置く
IF (FULL PERIMETER DETECT) もし(十分な ペリメータ検出)ならば
FIND LARGEST VALUE AMONG ALL PERIMETER PIXELS 全ペリメータ値に おける最大値を見つける
ELSE FIND LARGEST VALUE AMONG FOUR CORNER PIXELS 4つのコーナーピクセル間で 最大値をみつける
SUBSTRACT THE LARGEST VALUE FROM THE CENTER PIXEL AND STORE IN PIXDIFF 最大値を中央ピクセルから減算しピクセルに記憶する
ENSURE THAT THE CENTER PIXEL IS THE LARGEST AMONG ALL REMAINING PIXELS 中央ピクセルは全残りのピクセル 間の最大であることを確認する
REPLACE CENTER PIXEL WITH PIXDIFF AND MOVE KERNEL TO NEXT PIXEL (REPEAT FOR EVERY PIXEL)
中央ピクセルを PIXDIFF と置き換え、核を次のピクセルへ移動
(ピクセル毎に反復する)

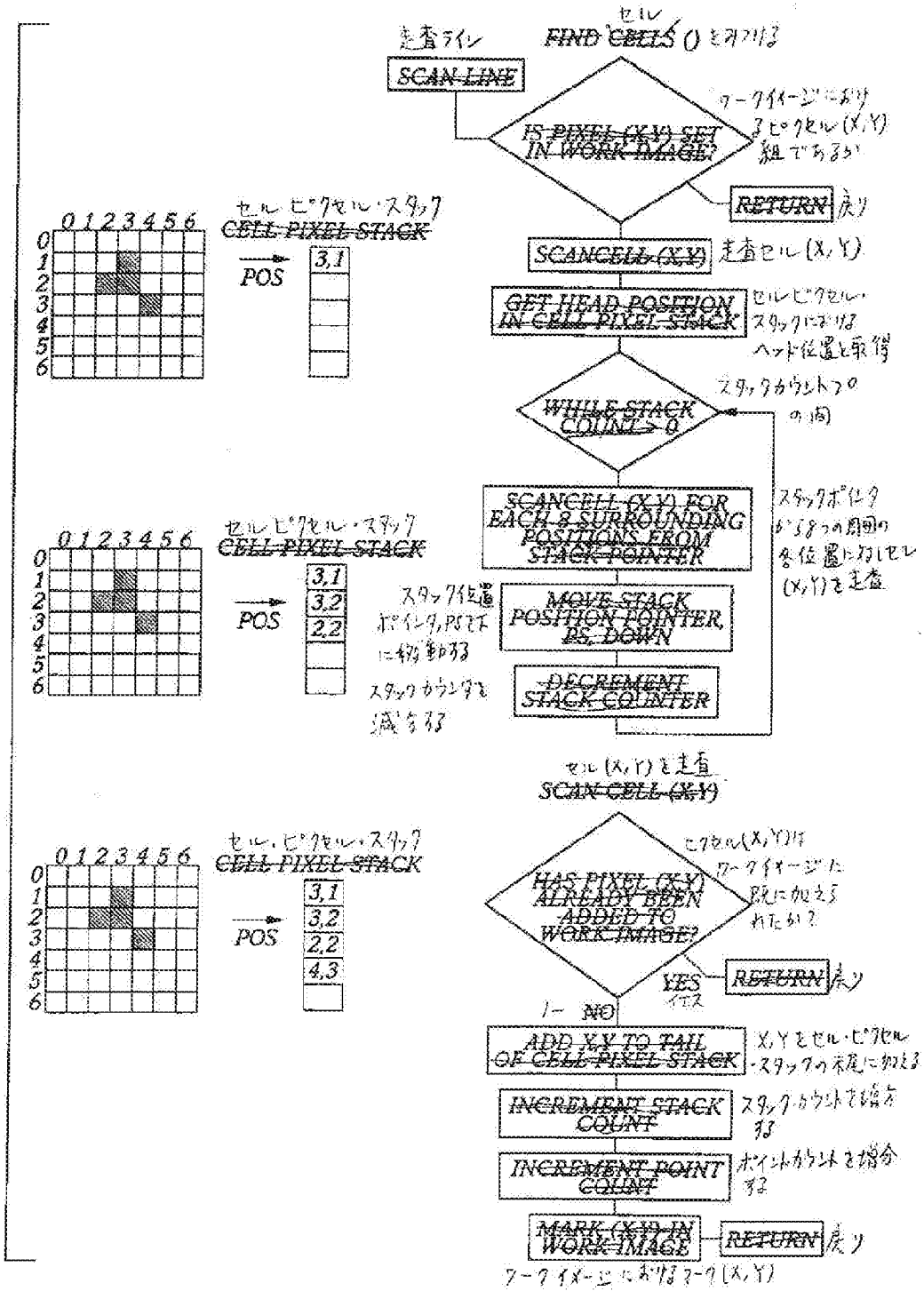
【図７】


核
7x7 KERNEL
最大
MAX
MIN
最小
nCols=250
nRows=1000
ノイズポイントCNT 毎に イメージ行を分割する
DIVIDE IMAGE ROWS INTO NOISEPOINTCNT SECTIONS
イメージの左側へ7x7 核を中心に持ってくる
CENTER 7x7 KERNEL AT "LEFT" SIDE OF IMAGE
FIND MAX AND MIN IN THE 7x7 KERNEL
7x7 核 へ 最大及び最小 を見つける
ADD MAX,MIN VALUE TO HISTOGRAM
最大－最小値 をヒストグラム に追加する
MOVE ONE PIXEL TO THE RIGHT
ひとつの ピクセル を右側に 移動する
REPEAT ABOVE (250 POINTS) UNTIL YOU REACH THE "RIGHT" SIDE OF THE IMAGE
イメージの右側に 来るまで (250 ポイント) 以上 反復する
SKIP A GIVEN NUMBER OF ROWS
所与の数の行をスキップする
SMOOTH (BOXCAR FILTER) AND FIND MAX OF HISTOGRAM WITHIN THE EACH NOISEPOINTCNT SECTION
平滑化し (ボックスカー・フィルタ) そして 各ノイズポイントセクション内のヒストグラムの最大を見つける
ADD TO NOISEPOINTCNT ELEMENT NOISEARRAY
ノイズポイントCNT 要素 ノイズアレイ へ追加する
NOISEARRAY IS USED TO DETERMINE THRESHOLD IN CELLDETECTION ALGORITHM
ノイズアレイは セル検出アルゴリズム における 閾値を決定するために使用される

【図８】


GENERATE MEDIAN OF SOURCE IMAGE WITH (128x250 KERNEL (USING TURBOMEDIAN). RESULT IS A 1D ARRAY REPLICATED ALONG THE COLS X-DIRECTION INTO A 2D ARRAY
128×250 枠をもちいて ソースイメージのメジアンを 生成する（ターボメジアンを使用） 結果は列X方向に沿って2D アレイへ複製される1Dアレイで ある。
SUBTRACT MEDIAN IMAGE FROM SOURCE IMAGE
メジアンイメージをソースイメージから 減算する
バイト閾値イメージを生成する:
バイト閾値イメージを生成する:
GENERATE BYTE THRESHOLD IMAGE. (65535 より "BUBBLETHRESHFACTOR *BGMAXMIN CORRESPONDS TO "ONE"
GENERATE BYTE THRESHOLD IMAGE. (BLOBTHRESHFACTOR *BGMAXMIN より 65535 CORRESPONDS TO "ONE"
まで が「1」に対応する
65535 まで が「1」に対応する
SEGMENTATION セグメント化: FIND CONTIGUOUS BLOBS WITH (NPIX > MAXBUBBLEPIX) により
SEGMENTATION セグメント化: FIND CONTIGUOUS BLOBS WITH (NPIX > MAXBLOBPIX) より
隣接 ボルブ をみつける
隣接 ボルブ をみつける
ADD TO "MASK" IMAGE
「マスク」イメージに加える
DILATE MASK IMAGE BY MASKDILATIONPIX PIXELS
ピクセル により マスクイメージ を拡張する

【図９】


走査ライン
SCAN LINE
セル
FIND CELLS () をコールする
IS PIXEL (X,Y) SET IN WORK IMAGE?
ワークイメージにおける(X,Y)ピクセルが組であるか
RETURN
戻り
SCANCELL (X,Y)
走査セル (X,Y)
GET HEAD POSITION IN CELL PIXEL STACK
セルピクセル・スタックにおけるヘッド位置を取得
WHILE STACK COUNT > 0
スタックカウントゼロの間
SCANCELL (X,Y) FOR EACH 8 SURROUNDING POSITIONS FROM STACK POINTER
スタックポインタから8つの周囲の各位置におけるセル(X,Y)を走査
MOVE STACK POSITION POINTER, PS DOWN
スタック位置ポインタ, PSを下に移動する
DECREMENT STACK COUNTER
スタックカウンタを減らす
セル (X,Y) を走査
SCAN CELL (X,Y)
HAS PIXEL (X,Y) ALREADY BEEN ADDED TO WORK IMAGE?
ピクセル(X,Y)はワークイメージに既に加えられたか?
YES イエス
RETURN
戻り
NO
ADD X,Y TO TAIL OF CELL PIXEL STACK
X,Yをセル・ピクセル・スタックの末尾に加える
INCREMENT STACK COUNT
スタックカウントを増分する
INCREMENT POINT COUNT
ポイントカウントを増分する
MARK (X,Y) IN WORK IMAGE
RETURN
戻り
ワークイメージにおけるマーク(X,Y)
セル・ピクセル・スタック
CELL PIXEL STACK
POS
3,1
3,2
2,2
4,3
0 1 2 3 4 5 6

【図１０】

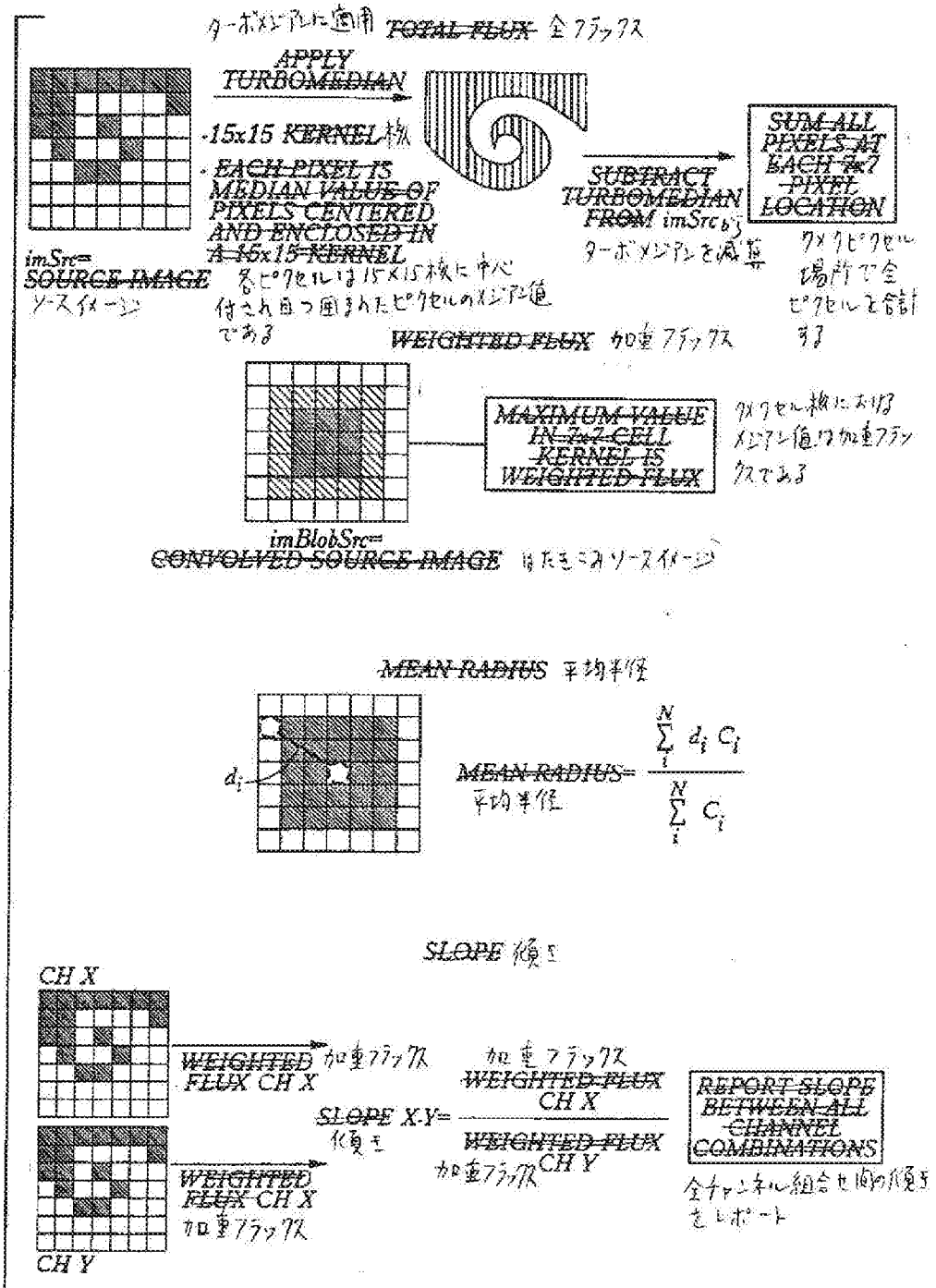


Figure 10

【図１０－１】

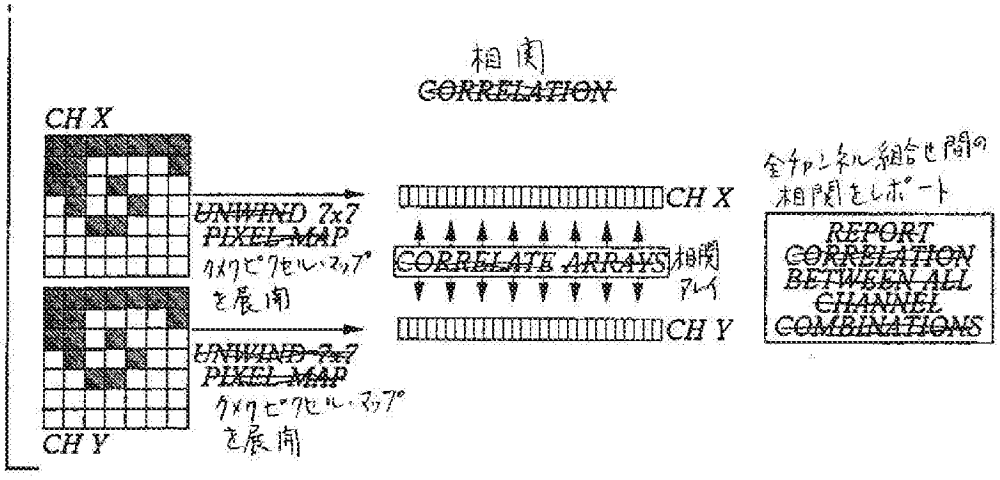


Figure 10 (cont.)

【図１１】

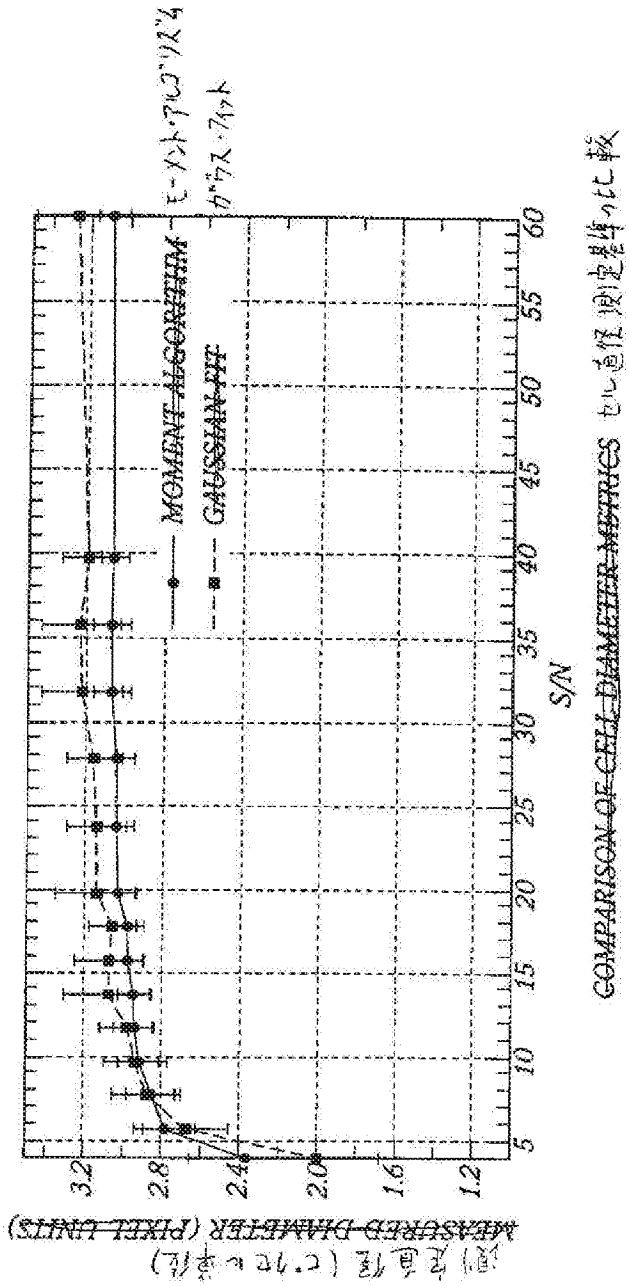


Figure 11

【図１２】

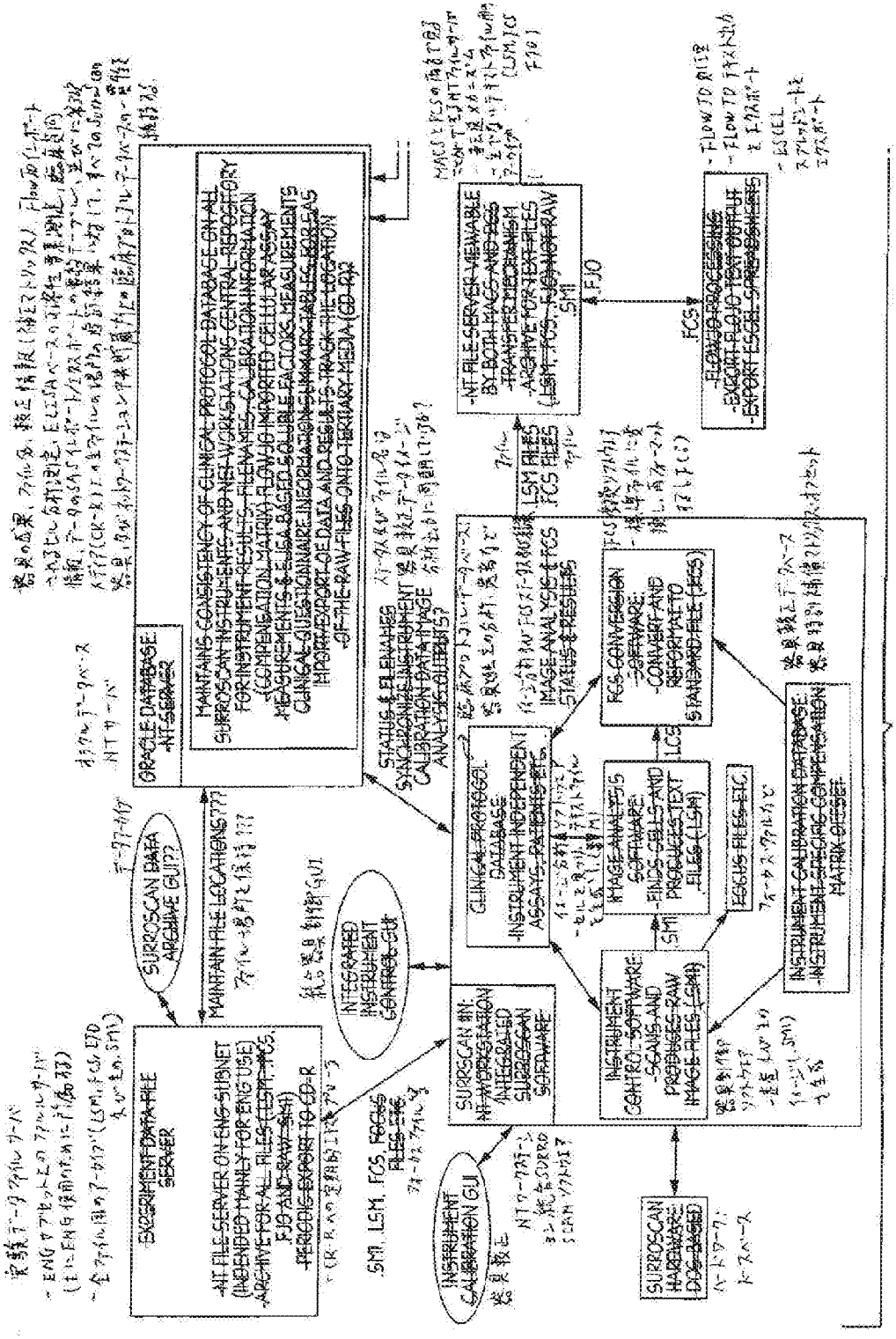


Figure 12

【図１２－１】

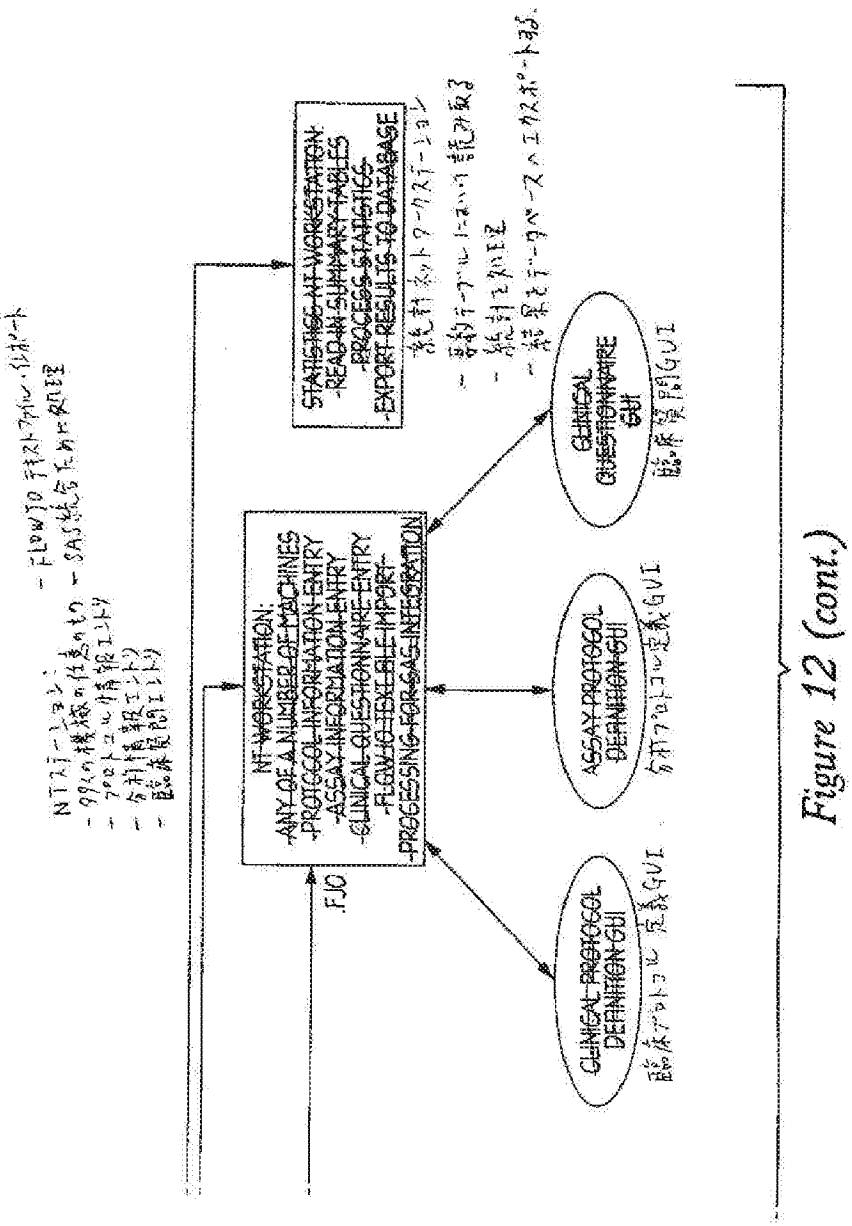


Figure 12 (cont.)

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/11133

A. CLASSIFICATION OF SUBJECT MATTER
(IPC(7) : G06K 9/00
US CL : 382/128; 250/461.1; 356/300; 435/6; 600/311, 317, 319
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)
U.S. : 382/128; 250/461.1; 356/300; 435/6; 600/311, 317, 319

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
East, IEEE
search terms: fluorescent, pixel, daignostic, dichroic, filter, wavelength, channel

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,456,252 A (VARI et al.) 10 October 1995, fig. 13, elements 50, 52; col. 5, lines 25-55; col. 7, lines 45-65,	1-6
Y	US 4,786,813 A (SVANBERG et al.) 22 November 1988, abstract, lines 1-12; col. 3, lines 5-65.	1-6
Y	US 5,127,730 A (BRELJE et al.) 07 July 1992, fig. 4, element 401; fig. 5, elements 500, 501, 507, 509; col. 2, lines 30-55; col. 3, lines 48-60.	1-6
A	US 5,377,003 A (LEWIS et al.) 27 December 1994, col. 8, lines 55-68.	1-6
Y	US 5,741,411 A (YEUNG et al.) 21 April 1998, col. 10, lines 12-65.	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"B"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search
23 AUGUST 2000

Date of mailing of the international search report
02 NOV 2000

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FB12 JA01

Electronic Acknowledgement Receipt

EFS ID:	32869076
Application Number:	15638461
International Application Number:	
Confirmation Number:	9847
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	32425
Filer:	Mark G Tison/Hannah Wimberley
Filer Authorized By:	Mark G Tison
Attorney Docket Number:	BECC.P0002US.CIP.C1
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Filing Date:	30-JUN-2017
Time Stamp:	10:27:57
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		BECCP0002USCIPC1_SIDS1.pdf	191346 305cea924fbf232ff55651cffdd811c5f48bd2fd	yes	4

	Multipart Description/PDF files in .zip description					
	Document Description		**Start**		**End**	
	Transmittal Letter		1		2	
	Information Disclosure Statement (IDS) Form (SB08)		3		4	
Warnings:						
Information:						
2	Foreign Reference	JP2004500572A.pdf	2123221 35229ac4f78f9f728f708fda12d49981a3887907		no	74
Warnings:						
Information:						
3	Foreign Reference	JP2008534960A.pdf	645204 4463356756a3e3e424293398d87a3279df95bacd		no	14
Warnings:						
Information:						
4	Foreign Reference	JP2010085194A.pdf	735591 1b74d8b010b8728146921d13e4be1b1347c32066		no	15
Warnings:						
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5	Foreign Reference	JPH03197641A.pdf	584568 cb23392fe2b1af47cb6ad036b5a8734e6496fd06		no	7
Warnings:						
Information:						
6	Foreign Reference	JPH05119035A.pdf	425420 d25489871e76e6e7d32c7718b8929bb5edd9807a		no	8
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Information:						
7	Foreign Reference	JPS55144226A.pdf	323221 46beddc30661c57bf241787792cb487ecf46be33		no	5
Warnings:						

Information:					
8	Foreign Reference	JPS61184427A.pdf	429263 970375983a2dc649ecce9718c35a62522d3452cf	no	6
Warnings:					
Information:					
9	Foreign Reference	WO2013181453A2.pdf	4603362 55a68e0c1e56fea76532861b218ae2082b147e97	no	103
Warnings:					
Information:					
10	Other Reference-Patent/App/Search documents	OA_JP.pdf	455590 15913b30b6d1324dcb7c241e9993dba96a72be7d	no	4
Warnings:					
Information:					
11	Other Reference-Patent/App/Search documents	OA_CN.pdf	2804285 25b2e4d4dc98306c39aa3a41e43a2e5abb65c7ce	no	8
Warnings:					
Information:					
12	Foreign Reference	JP2003505707.pdf	19095960 e967c1fbe18e7da538c3569fa9f958f82815e02e	no	70
Warnings:					
Information:					
13	Other Reference-Patent/App/Search documents	OA_JPD1.pdf	938642 9c4f86dd8140093eeff2c437825e06096d841359	no	20
Warnings:					
Information:					
			Total Files Size (in bytes):	33355673	

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: IRIS INTERNATIONAL, INC.	Group Art Unit: 2884
Serial No.: 15/638,461	Examiner: Mindy D. Vu
Filed: June 30, 2017	Atty. Dkt. No.: BECC.P0002US.CIP.C1
For: FLOW CYTOMETER	Confirmation No.: 9847

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 12, 2018	/Mark G. Tison/
Date	Mark G. Tison

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.C1.

Applicant respectfully requests that the listed documents be made of record in the present case.

Respectfully submitted,

/Mark G. Tison/

Mark G. Tison
Reg. No. 65,985
Attorney for Applicant

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Date: June 12, 2018

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/638,461	06/30/2017	Yong Qin Chen	BECC.P0002US.CIP.C1	9847

32425 7590 06/29/2018

NORTON ROSE FULBRIGHT US LLP
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SUITE 1100
AUSTIN, TX 78701-4255

EXAMINER	
VU, MINDY D	

ART UNIT	PAPER NUMBER
2884	

NOTIFICATION DATE	DELIVERY MODE
06/29/2018	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

Office Action Summary	Application No. 15/638,461	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 _____.

☐ 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) *364-389* 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) *364-373,376-380 and 382-389* is/are rejected.

8)☒ Claim(s) *374,375 and 381* 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 *6/30/17* 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 *8/2/17, 6/12/18*.

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.

Information Disclosure Statement

The examiner has considered information considered by the Office in the parent application(s) when examining this continuing application, and the application file reflects that fact. A list of the information need not be submitted in the continuing application unless applicant desires the information to be printed on the patent. MPEP § 609.

Claim Objections

Claim 383 is objected to because of the following informalities: the word “and" in line 2 should probably be "an". Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 368 rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the enablement requirement. The claim(s) contains

subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Claim 368 claims "**the optical relay is a concave-shaped dichroic filter**, However, in the specification of the present application, it discloses the **optical relay is a concave mirror 907** and **dichroic filter 909** may be inserted in between the relay imaging concave mirror 907 and the second image near focusing lens 908 (para. 0411 of published application). As evidence from the specification, the dichroic filter is not an optical relay nor having a concave-shaped dichroic filter.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim(s) 364-366, 370-372, 376-380, 383 and 385-389 is/are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Heffelfinger et al. (US 2003/0044967).

With respect to claim 364, Heffelfinger discloses an optical module (abstract), the optical module comprising:

(a) a light source (beam combiner 18 combines first laser 12 and second laser 14 into illumination beam 24, see fig.1);

(b) a collimating optical element arranged to receive light from the light source, the collimating optical element configured to project a collimated beam [0039-0040]; and

(c) an optical relay (relay lens 42) arranged to receive at least a portion of the collimated beam from the collimating optical element, the optical relay configured to project at least a portion of the collimated beam to a first focusing optical element (48), wherein the optical relay is one of a curved mirror and a refractive lens [0047, 0049].

With respect to claim 365, Heffelfinger discloses the light source is one of light passing through a pinhole [0061] and light emitted from a facet of a multimode optical fiber [0021, 0038].

With respect to claim 366, Heffelfinger discloses the first focusing optical element is configured to focus the collimated beam to a size smaller than 1 millimeter in diameter to a first semiconductor detector.

With respect to claim 370, Heffelfinger discloses a first optical filter arranged between the collimating optical element and the optical relay, the first optical filter configured to separate the collimated beam into a first branch and a second branch [0041].

With respect to claim 371, Heffelfinger discloses the collimated beam comprises light of a plurality of wavelengths, wherein the first optical filter is configured to reflect light of a first range of wavelengths to form the first branch of the collimated beam, wherein the first optical filter is configured to pass light of a second range of wavelengths to form the second branch of the collimated beam, wherein the first branch is directed to the optical relay (fig. 1 and its description).

With respect to claim 372, Heffelfinger discloses a second focusing optical element arranged to receive the second branch of the collimated beam, the second focusing optical element configured to focus the second branch of the collimated beam to a second semiconductor detector (fig. 1 and its description).

With respect to claims 376 and 377, Heffelfinger discloses an optical module (abstract), the optical module comprising:

(a) a light source (12, see fig.4);

(b) a collimating optical element arranged to receive light from the light source, the collimating optical element configured to project a collimated beam and a first image, wherein the collimated beam has a collimated distance [0039-0040, 0057]; and

(c) an optical relay (relay lens 42, 46) arranged to receive the collimated beam, the optical relay configured to extend the collimated distance of the collimated beam, wherein the optical relay is one of a curved mirror and a refractive lens [0047, 0049].

With respect to claim 378, Heffelfinger discloses the optical relay projects a second image, wherein the second image and the first image are substantially the same size [0059-0061].

With respect to claim 379, Heffelfinger discloses wherein the optical relay projects a second image, wherein the collimated beam from the collimating optical element to the first image has substantially the same diameter as the collimated beam from the optical relay to the second image [0061].

With respect to claim 380, Heffelfinger discloses a first focusing optical element arranged to receive the collimated beam from the optical relay at the extended

collimated distance, the first focusing optical element configured to focus the extended collimated beam to a first semiconductor detector (fig. 4 and its description).

With respect to claim 383, Heffelfinger discloses an optical module (abstract), the optical module comprising:

(a) a light source providing light that forms an object (12, see fig.4);

(b) a collimating optical element arranged to receive light from the light source, the collimating optical element configured to project a first image of the object [0039-0040]; and

(c) an optical relay (relay lens 42 and 46) arranged near the first image, the optical relay configured to receive light from the collimating optical element and to project a second image of the object; wherein the size of the first image is substantially the same as the size of the second image [0047, 0049, 0057-0061].

With respect to claim 385, Heffelfinger discloses the light source is an extended light source that forms the object, wherein the first image is a magnified image of the object [0042-0046].

With respect to claim 386, Heffelfinger discloses the light source is one of light passing through a pinhole [0057] and light emitted from a facet of a multimode optical fiber [0021, 0040].

With respect to claim 387, Heffelfinger discloses the collimating optical element has an effective size defined by its cross section, wherein the size of the first image is substantially the same as the effective size of the collimating optical element [0045, 0056].

With respect to claim 388, Heffelfinger discloses the first image is less than approximately 10 times the size of the object [0060].

With respect to claim 389, Heffelfinger discloses a first focusing optical element arranged near the second image, the first focusing optical element configured to focus the light from the optical relay to a size smaller than the object of the light source to a first semiconductor detector [0060-0061].

Claim(s) 364, 370, 376-378, 380 and 382-387 is/are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Oostman, Jr. et al. (US 2003/0044967).

With respect to claim 364, Oostman, Jr. discloses an optical module (abstract, fig. 5), the optical module comprising:

(a) a light source (optical fiber 123, see fig.5);

(b) a collimating optical element (collimating lens 145) arranged to receive light from the light source, the collimating optical element configured to project a collimated beam (col. 6 lines 40-44); and

(c) an optical relay (dichroic mirror 151) arranged to receive at least a portion of the collimated beam from the collimating optical element, the optical relay configured to project at least a portion of the collimated beam to a first focusing optical element (158), wherein the optical relay is one of a curved mirror and a refractive lens (col. 6 lines 40-50).

With respect to claim 370, Oostman, Jr. discloses a first optical filter (155) arranged between the collimating optical element (145) and the optical relay, the first

optical filter configured to separate the collimated beam into a first branch and a second branch (fig. 5).

With respect to claim 376, Oostman, Jr. discloses an optical module (abstract), the optical module comprising:

(a) a light source (123, see fig.5);

(b) a collimating optical element (145) arranged to receive light from the light source, the collimating optical element configured to project a collimated beam and a first image, wherein the collimated beam has a collimated distance (col. 6 lines 40-44); and

(c) an optical relay (dichroic mirror 151) arranged to receive the collimated beam, the optical relay configured to extend the collimated distance of the collimated beam (fig. 5 and its description).

With respect to claim 378, Oostman, Jr. discloses the optical relay projects a second image, wherein the second image and the first image are substantially the same size (fig. 5 and its description).

With respect to claim 379, Oostman, Jr. discloses wherein the optical relay projects a second image, wherein the collimated beam from the collimating optical element to the first image has substantially the same diameter as the collimated beam from the optical relay to the second image (fig. 5 and its description).

With respect to claim 380, Oostman, Jr. discloses a first focusing optical element arranged to receive the collimated beam from the optical relay at the extended

collimated distance, the first focusing optical element configured to focus the extended collimated beam to a first semiconductor detector (fig. 5 and its description).

With respect to claim 382, Oostman, Jr. discloses the optical distance from the collimating optical element to the optical relay is substantially the same as the optical distance from the optical relay to the first focusing optical element (fig. 5).

With respect to claim 383, Oostman, Jr. discloses an optical module (abstract), the optical module comprising:

(a) a light source providing light that forms an object (123, see fig.5);

(b) a collimating optical element (145) arranged to receive light from the light source, the collimating optical element configured to project a first image of the object; and

(c) an optical relay (151) arranged near the first image, the optical relay configured to receive light from the collimating optical element and to project a second image of the object; wherein the size of the first image is substantially the same as the size of the second image (fig. 5 and its description).

With respect to claim 384, Oostman, Jr. discloses the optical distance from the collimating optical element to the first image is substantially the same as the optical distance from the optical relay to the second image (fig. 5 and its description).

With respect to claim 385, Oostman, Jr. discloses the light source is an extended light source that forms the object, wherein the first image is a magnified image of the object (col. 9 lines 44-47).

With respect to claim 386, Oostman, Jr. discloses the light source is emitted from a facet of a multimode optical fiber (col. 6 lines 5-7).

With respect to claim 387, Oostman, Jr. discloses the collimating optical element has an effective size defined by its cross section, wherein the size of the first image is substantially the same as the effective size of the collimating optical element (col. 10 lines 25-47).

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, 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.

Claim 369 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Heffelfinger et al. (US 2003/0044967) in view of Johnson (US 4,385,234).

With respect to claim 369, Heffelfinger discloses collimating lenses but does not explicitly disclose the lens being achromatic doublet lens. In a similar field of endeavor, Johnson discloses using achromatic doublet lens for collimating is well-known (col. 3 lines 51-65). 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 substitute one known lens for another and the results of collimating would have been predictable.

Claim 366 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Oostman, Jr. et al. (US 2003/0044967).

With respect to claim 366, Oostman, Jr. discloses the focusing element but does not specify to what kind of diameter size the focusing element focuses light. However, it is common in an optical device that a focusing element focuses light to a spot the size of 1 mm or less, which is smaller than the diameter of an optical fiber. It is, therefore, 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 for focusing in view of the feature is merely a variation in design and the results would have been predictable.

Claim 367 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Oostman, Jr. et al. (US 2003/0044967) in view of Govorkov et al. (US 2005/0002425).

With respect to claim 367, Oostman, Jr. discloses the light source forms an object, wherein the collimating optical element is configured to project a magnified image of the object. Oostman, Jr. discloses an optical relay element is a dichroic mirror instead of a concave mirror, wherein the radius of curvature approximately equal to the distance between the collimating optical element and the magnified image projected thereby. In a similar field of endeavor, Govorkov discloses the imaging mirrors can be used to provide relay imaging are concave, spherical mirrors with a radius of curvature approximately equal to the distance between the mirrors [0043]. 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 substitute one known mirror (dichroic) with another (concave mirror) to

configure so that the optical path length between a collimating optical element and a focusing optical element is substantially the same as the optical path length between an image relay optical element and a second focusing optical system in view of obtaining the desired delay in smaller physical space and better alignment.

Claim 373 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Heffelfinger et al. (US 2003/0044967).

With respect to claim 373, Heffelfinger discloses the optical system as recited. Heffelfinger does not explicitly disclose the diameter of the collimated beam between the optical relay and the first focusing optical element is substantially the same as the diameter of the collimated beam between the collimating optical element and the second focusing optical element. 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 have the diameter of the beams to be substantially the same in view of the feature is merely a variation in design and the results would have been predictable.

Allowable Subject Matter

Claims 374, 375 and 381 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

The following is a statement of reasons for the indication of allowable subject matter: the prior art does not teach or suggest a second optical filter, the second optical

filter arranged between the optical relay and the first focusing optical element, the second optical filter configured to pass light of a third range of wavelengths in the first branch of the collimated beam to the first focusing optical element, wherein the third range of wavelengths is a subset of the first range of wavelengths; optical filter arranged between the collimating optical element and the optical relay, the optical filter configured to separate the collimated beam into a first branch and a second branch, wherein the first branch and the second branch are different colors, wherein the optical relay receives the first branch; and a second focusing optical element arranged to receive the second branch, the second focusing optical element configured to focus the second branch to a second semiconductor detector.

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.

/M. V./
Examiner, Art Unit 2884

/MARCUS TANINGCO/
Primary Examiner, Art Unit 2884

Notice of References Cited	Application/Control No. 15/638,461	Applicant(s)/Patent Under Reexamination CHEN, YONG QIN	
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	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
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	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
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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.

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SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/638,461	06/30/2017 RULE	250	2884	BECC.P0002US.CIP.C1

APPLICANTS
IRIS INTERNATIONAL, INC., Chatsworth, CA;

INVENTORS
Yong Qin Chen, San Jose, CA;

**** CONTINUING DATA ***************************
This application is a CON of 14/555,102 11/26/2014 PAT 9746412
which 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 ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****
07/18/2017

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	26	3

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Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP.C1	Serial No.: 15/638,461
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: IRIS INTERNATIONAL, INC.	
	Filing Date: June 30, 2017	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 1*	*See Page 2*

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Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP.C1	Serial No.: 15/638,461
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(Use several sheets if necessary)	Filing Date: June 30, 2017	Group: 2884

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 1*	*See Page 2*

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

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	C10	Office Action issued in Japanese Patent Application No. 2015-515208, dated May 02, 2018 (Attorney Docket No. BECC.P0002JP)
	C11	Office Action issued in Japanese Patent Application No. 2016-216891, dated December 04, 2017 (English Translation) (Attorney Docket No. BECC.P0002JP.D1)

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EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	13	(("20050134836") or ("20120211679") or ("4673289") or ("7630147") or ("7889263")).PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2018/06/19 19:16
S1	29325	(G01N15/1436 or G01N15/1459 or G01N15/1434 or G02B27/0025 or G02B21/361 or G02B21/04 or G02B6/29365 or G02B6/26 or G01N2015/144 or G01N2015/1006 or G01N2015/1452).cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 16:12
S2	57223	optical$2 with relay$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 16:14
S3	13096	S2 same (mirror or (refractive adj lens))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 16:14
S4	247890	pin$1hole or (pin adj hole)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 16:23
S5	1177	S3 and S4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 16:23
S6	645	S5 and fiber	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 16:24
S7	292	collimat$3 and filter$3 and S6	US-PGPUB;	OR	ON	2018/06/11

			USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			16:27
S8	31600	chen-yong$.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 21:17
S9	57223	optical$2 with relay$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 21:18
S10	11	S9 and S8	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 21:18
S11	928938	diameter with siz$4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 22:29
S12	57223	optical$2 with relay$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 22:29
S13	13096	S12 same (mirror or (refractive adj lens))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 22:29
S14	247890	pin$1hole or (pin adj hole)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 22:29
S15	1177	S13 and S14	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	2018/06/11 22:29

			IBM_TDB			
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S17	292	collimat$3 and filter$3 and S16	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 22:29
S18	93	S17 and S11	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/11 22:29
S19	622	("20020141902" | "20060292021" | "4073574" | "4244045" | "4727020" | "5230614" | "5583683" | "5615200" | "5788927" | "6870976" | "6975400" | "7430048" | "7450229" | "7952981" | "8009189" | "8436371" | "6159686" | "20050084402" | "20060245964" | "20130020498" | "3946239" | "3989381" | "4189236" | "4576556" | "4745285" | "4950136" | "6200101" | "6683314" | "6941047" | "7260328" | "7268953" | "7381565" | "7385682" | "8157547" | "8337096" | "8488244" | "20040165828" | "2385495" | "2764147" | "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" | "20020067895" | "5157917" | "20130010181" | "0315667" | "4482994" | "4778253" | "4997275" | "5050963" | "5245318" | "5786915" | "5971713" | "6315952" | "6647175" | "6768593" | "6813017" | "7505131" | "7580120" | "7668422" | "7768120" | "8432541" | "8436993" | "8507279" | "3199341" | "6683314" | "20060256335" |	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/13 18:47

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S20	5	(("6683314") or ("20040165828")).PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2018/06/13 19:42
S21	1071	concave with mirror with relay$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/14 17:49
S22	57	S21 with filter$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/14 18:09
S23	2417	concave with mirror with curvature with radius	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/14 19:20
S24	118	S23 same relay$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/14 19:21
S25	1028	achromat$3 near3 doublet near3 lens	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	2018/06/15 11:17

			IBM_TDB			
S26	175	S25 with collimat$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/15 11:17
S27	12	relay$3 same (curv$6 near4 mirror) same (refractive near3 lens)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/15 16:22
S28	989	relay$3 with (concave near4 mirror)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2018/06/15 19:24

EAST Search History (Interference)

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15638461	CHEN, YONG QIN
	Examiner	Art Unit
	MINDY VU	2884

CPC- SEARCHED		
Symbol	Date	Examiner
G01N 15/1436, 15/1459, 15/1434	6/15/18	MV
G02B 21/361, 27/0025, 21/804, 6/26, 6/29365	6/15/18	MV

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor name search	6/11/18	mv
Parent data considered	6/11/18	mv
EAST BRS - See search history printout	6/18/18	mv

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

Form PTO-1449 (modified)	Atty. Docket No.: BECC.P0002US.CIP.C1/11706355	Serial No.: 15/638,461
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Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A1	315667	4/14/85	Serdinko	417	475	10/20/84
	A2	2385495	9/25/45	Brian	250	487.1	8/21/43
	A3	2669709	2/16/54	Glancy et al.	348	784	10/19/60
	A4	2764147	9/25/56	Brunner	126	247	2/23/51
	A5	3199341	08/10/65	Heuer Jr. et al.	73	807	05/28/62
	A6	3411293	11/19/68	Akins	60	454	10/20/66
	A7	3542491	11/24/70	Newman	417	295	5/27/69
	A8	3661460	5/9/72	Elking et al.	356	36	8/28/70
	A9	3726613	4/10/73	von Casimir	417	477.1	10/12/70
	A10	3826593	7/30/74	von Casimir	417	53	5/11/73
	A11	3873204	3/25/75	Friedman et al.	356	39	11/29/72
	A12	3946239	3/23/76	Salzman et al.	250	461.2	1/24/75
	A13	3953727	4/27/76	d'Auria et al.	398	88	1/17/75
	A14	3989381	11/2/76	Fulwyler	356	39	5/5/75
	A15	4073574	2/14/78	Clarke et al.	359	728	10/1/76
	A16	4188543	2/12/80	Brunsting et al.	250	458.1	4/24/78
	A17	4189236	2/19/80	Hogg et al.	356	317	3/20/78
	A18	4244045	1/6/81	Nosu et al.	398	86	1/31/79
	A19	4482994	11/13/84	Ishikawa	398	86	6/7/82
	A20	4515274	5/7/85	Hollinger et al.	209	3.1	12/2/81
	A21	4564342	1/14/86	Weber et al.	417	477.6	7/24/84
	A22	4576556	3/18/86	Thompson	417	477.12	4/2/80
	A23	4623225	11/18/86	Forkner	359	669	6/29/84

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	A24	4727020	2/23/88	Recktenwald	435	6.11	2/25/85
	A25	4745285	5/17/88	Recktenwald et al.	250	458.1	8/21/86
	A26	4778253	10/18/88	Siga et al.	359	819	3/26/86
	A27	4834630	5/30/89	Godwin	417	475	2/16/88
	A28	4871249	10/3/89	Watson	356	73	7/1/88
	A29	4920275	4/24/90	Itoh	250	574	12/30/88
	A30	4950136	8/21/90	Haas et al.	417	477.7	8/14/89
	A31	4976590	12/11/90	Baldwin	417	53	6/8/88
	A32	4997275	3/5/91	Gaucher et al.	356	72	9/23/88
	A33	5050963	9/24/91	Murakami	359	808	10/9/90
	A34	5142462	8/25/92	Kashima	362	268	4/27/90
	A35	5157917	10/27/92	Liang et al.	60	2261.1	05/20/91
	A36	5177641	1/5/93	Kobayashi et al.	359	820	10/30/90
	A37	5230614	7/27/93	Zanger et al.	417	477.9	6/3/92
	A38	5245318	9/14/93	Tohge et al.	340	611	12/15/92
	A39	5251060	10/5/93	Uenishi et al.	359	328	9/30/92
	A40	5257917	11/02/93	Minarik et al.	417	475	10/02/92
	A41	5299066	3/29/94	Rombult	359	819	8/12/92
	A42	5317162	5/31/94	Pinsky et al.	250	461.2	9/9/92
	A43	5369476	11/29/94	Bowers et al.	399	49	1/28/92
	A44	5373395	12/13/94	Adachi	359	652	5/10/93
	A45	5396487	3/7/95	Abe et al.	359	819	10/31/91
	A46	5467225	11/14/95	Manabe	359	661	11/1/94

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	A47	5470211	11/28/95	Knott et al.	417	477.9	8/11/94
	A48	5475210	12/12/95	Taguchi et al.	250	205	6/8/93
	A49	5548395	8/20/96	Kosaka	356	73	8/31/92
	A50	5583683	12/10/96	Scobey	398	79	6/15/95
	A51	5615200	3/25/97	Hoshino et al.	369	112.04	4/14/95
	A52	5746585	5/5/98	McDunn et al.	417	477.11	12/31/96
	A53	5748372	5/5/98	Kitagawa	359	565	4/17/96
	A54	5777674	7/7/98	Ohmuro	348	338	4/10/95
	A55	5781351	7/14/98	Murakami et al.	359	808	5/31/96
	A56	5786915	7/28/98	Scobey	398	82	10/22/96
	A57	5788927	8/4/98	Farrell et al.	422	63	6/30/96
	A58	5805363	9/8/98	Okuda et al.	359	819	7/17/97
	A59	5850292	12/15/98	Braun	356	419	11/13/97
	A60	5915925	6/29/99	North Jr.	417	36	1/7/97
	A61	5971713	10/26/99	North Jr.	417	36	12/29/98
	A62	6008920	12/28/99	Hendrix	398	79	3/11/98
	A63	6017194	1/25/00	North Jr.	417	36	12/31/98
	A64	6102678	8/15/00	Peclat	417	477.7	4/3/98
	A65	6135734	10/24/00	Isozumi et al.	417	542	9/23/98
	A66	6159686	12/12/00	Kardos et al.	435	6	01/30/98
	A67	6200101	3/13/01	North Jr.	417	36	7/6/99
	A68	6252719	6/26/01	Eichenbaum	359	634	3/19/99
	A69	6315952	11/13/01	Sklar et al.	422	63	6/10/99

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	A70	6510007	1/21/03	Blasenheim	359	659	8/21/01
	A71	6542306	4/1/03	Goodman	359	634	3/16/01
	A72	6572255	6/3/03	Husher	366	132	4/24/01
	A73	6608682	8/19/03	Ortyn et al.	356	419	10/12/01
	A74	6618143	9/9/03	Roche et al.	356	339	10/2/01
	A75	6638481	10/28/03	Sklar et al.	422	63	9/19/01
	A76	6647175	11/11/03	LoRegio et al.	385	24	10/8/01
	A77	6683314	1/27/04	Oostman Jr. et al.	250	461.2	8/28/01
	A78	6713019	3/30/04	Ozasa et al.	422	82.09	3/27/02
	A79	6748133	6/8/04	Liu et al.	385	24	11/26/01
	A80	6767188	7/27/04	Vrane et al.	417	40	8/15/02
	A81	6768593	7/27/04	Jutamulia	359	641	7/24/03
	A82	6788409	9/7/04	Goodwin	356	339	9/3/02
	A83	6794671	9/21/04	Nicoli et al.	250	574	7/17/02
	A84	6813017	11/2/04	Hoffman et al.	356	317	10/19/00
	A85	6839367	1/4/05	Nagamatsu et al.	372	34	3/16/01
	A86	6870679	3/22/05	Randall et al.	359	580	6/10/02
	A87	6870976	3/22/05	Chen et al.	385	14	3/13/01
	A88	6897954	5/24/05	Bishop et al.	356	317	12/20/02
	A89	6941047	9/6/05	Capewell et al.	385	47	3/1/04
	A90	6954722	10/11/05	Parks et al.	702	194	10/17/03
	A91	6975400	12/13/05	Ortyn et al.	356	419	7/28/03
	A92	7038778	5/2/06	Yamauchi	356	419	8/13/02

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	A93	7072540	7/4/06	Szapiel et al.	385	24	4/22/03
	A94	7110192	9/19/06	Sauter et al.	359	754	1/12/05
	A95	7113266	9/26/06	Wells	356	73	3/30/05
	A96	7127356	10/24/06	Nicoli et al.	702	26	5/18/04
	A97	7129505	10/31/06	Oostman Jr. et al.	250	458.1	10/17/03
	A98	7212343	5/1/07	He et al.	359	618	7/11/03
	A99	7260328	8/21/07	Kropp	398	82	2/24/03
	A100	7262838	8/28/07	Fritz	356	73	1/16/04
	A101	7268953	9/11/07	Matthae et al.	359	656	6/7/06
	A102	7305018	12/4/07	Otoma	372	50.1	12/29/05
	A103	7381565	6/3/08	Kurabayashi et al.	436	63	3/20/06
	A104	7385682	6/10/08	Chu et al.	356	73	9/7/06
	A105	7430048	9/30/08	Reel et al.	356	432	2/16/06
	A106	7450229	11/11/08	Ortyn et al.	356	326	5/4/05
	A107	7453915	11/18/08	Imai	372	50.1	12/5/06
	A108	7456960	11/25/08	Cerni et al.	356	336	11/15/05
	A109	7496463	2/24/09	Nicoli et al.	702	104	9/12/06
	A110	7505131	3/17/09	Roth	356	317	3/6/08
	A111	7507588	3/24/09	Mehrpouyan et al.	436	518	4/14/06
	A112	7561267	7/14/09	Luo et al.	356	336	12/22/06
	A113	7580120	8/25/09	Hamada et al.	356	73	4/6/06
	A114	7623243	11/24/09	Kato	356	419	9/13/04
	A115	7645127	1/12/10	Hagen et al.	417	477.12	6/29/06

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	A116	7668422	2/23/10	Kropp	385	47	3/12/04
	A117	7758324	6/20/10	Baumann et al.	417	477.2	5/12/05
	A118	7768120	8/3/10	Takashima	257	706	12/27/07
	A119	7781227	8/24/10	Mehrpouyan et al.	436	523	3/4/09
	A120	7787197	8/31/10	Chen	359	793	9/4/08
	A121	7835000	11/16/10	Graves et al.	356	338	11/3/06
	A122	7876436	1/25/11	Chu	356	338	10/31/07
	A123	7894047	2/22/11	Hamada et al.	356	39	7/24/09
	A124	7952981	3/31/11	Suzuki	369	121	10/23/08
	A125	7981661	7/19/11	Rich	435	286.5	4/17/07
	A126	8009189	8/30/11	Ortyn et al.	348	80	6/3/08
	A127	8049185	11/1/11	Hayashi et al.	250	459.1	2/4/09
	A128	8149525	4/3/12	Do	359	797	3/28/07
	A129	8157547	4/17/12	Vrielink	417	477.3	4/23/07
	A130	8187888	5/29/12	Rich	436	63	7/8/11
	A131	8213472	7/3/12	Hirai et al.	372	36	1/23/08
	A132	8229707	7/24/12	Olson et al.	702	189	7/4/11
	A133	8233146	7/31/12	Chen	356	246	1/5/10
	A134	8253938	8/28/12	Vacca et al.	356	337	5/26/11
	A135	8270098	9/18/12	Fukuta et al.	359	784	2/19/09
	A136	8284402	10/9/12	Frazier et al.	356	419	2/26/10
	A137	8337096	12/25/12	Shen et al.	385	89	11/30/09
	A138	8345237	1/1/13	Tsukii et al.	356	338	12/23/11

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	A139	8405048	3/26/13	Hayashi	250	458.1	2/4/09
	A140	8432541	4/30/13	Rich	356	246	11/4/10
	A141	8436371	5/7/13	Medendorp Jr., et al.	257	88	5/24/07
	A142	8436993	5/7/13	Kaduchak et al.	356	337	2/3/12
	A143	8488244	7/16/13	Li et al.	359	618	2/17/11
	A144	8507279	8/13/13	Ball et al.	436	10	6/2/10
	A145	2004165828	08/26/04	Capewell et al.	385	47	03/01/04
	A146	20020067895	06/06/02	Flanders	385	88	12/01/00
	A147	20020141902	10/3/02	Ozasa et al.	422	82.09	3/27/02
	A148	20030142720	7/31/03	Bradburn et al.	372	108	5/24/02
	A149	20040218184	11/4/04	Joregenson et al.	356	419	2/27/04
	A150	20050084402	4/21/05	Vanek	417	477.3	10/27/04
	A151	20060221325	10/5/06	Wells	356	73	3/30/05
	A152	20060245964	11/2/06	Koslov	417	477.1	6/29/06
	A153	20060256335	11/16/06	Chen	356	369	5/3/06
	A154	20060292021	12/28/06	Tu	417	477.1	11/7/02
	A155	20070124947	6/7/07	Munroe et al.	33	286	10/25/06
	A156	20080024758	1/31/08	Tabata	356	39	7/26/07
	A157	20080241911	10/2/08	Ueno et al.	435	287.1	3/28/08
	A158	20090091746	4/9/09	Fukuda et al.	356	73	10/1/08
	A159	20090190128	7/30/09	Cerni et al.	356	336	11/24/08
	A160	20110014075	1/20/11	Reif et al.	417	477.12	7/1/10
	A161	20110032522	2/10/11	Graves et al.	356	338	10/12/10

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	A162	20120156074	6/21/12	Kusch et al.	417	477.3	2/4/10
	A163	20120274925	11/1/12	Chen et al.	356	73	11/1/12
	A164	20120281204	11/8/12	Hoshishima et al.	356	72	1/13/11
	A165	20120282126	11/8/12	Brandt et al.	417	477.9	11/4/10
	A166	20120287419	11/15/12	Sharpe et al.	356	51	5/11/12
	A167	20130010181	1/10/13	Baba	348	360	3/14/11
	A168	20130020498	1/24/13	Ebi et al.	250	435	7/18/12
	A169	20130050782	2/28/13	Heng et al.	358	494	2/17/12
	A170	20130070243	3/21/13	Goehde	356	338	11/9/12
	A171	20130094102	4/18/13	Baba	359	784	7/13/10
	A172	20130204538	8/8/13	Rich	702	21	10/25/11

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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)

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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.C1/11706355	Serial No.: 15/638,461
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant: IRIS INTERNATIONAL, INC	
	Filing Date: June 30, 2017	Group: Not Yet Assigned

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1-8	*See Page 8-11*	*See Page 10-11*

Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	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 2004-341204	12/02/04	Japan	Japanese (English Abstract)
	B19	JP 2012-026837	02/09/12	Japan	Japanese (English Abstract)
	B20	JP 829726	2/2/96	Japan	Japanese (English translation)
	B21	JP H04-184241	07/01/92	Japan	Japanese (English Abstract)
	B22	JP H09-502794	03/18/97	Japan	Japanese (English Abstract)
	B23	JP Hl0-73528	03/17/98	Japan	Japanese (English Abstract)
	B24	WO 0057173	9/28/00	WIPO	English
	B25	WO 0127590	4/19/01	WIPO	English
	B26	WO 0140764	6/7/01	WIPO	English
	B27	WO 03012403	2/13/03	WIPO	French (English abstract)
	B28	WO 2005033654	4/14/05	WIPO	English
	B29	WO 2006013316	2/9/06	WIPO	English
	B30	WO 2009095358	8/6/09	WIPO	German (English abstract)
	B31	WO 2009098867	8/13/09	WIPO	Japanese (English abstract)

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U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1-8	*See Page 8-11*	*See Page 10-11*

Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Language
	B32	WO 2011026942	3/10/11	WIPO	English
	B33	WO 2012056217	5/3/12	WIPO	English
	B34	WO 2012177367	12/27/12	WIPO	English
	B35	WO 2013013229	1/24/13	WIPO	English
	B36	WO 2013093035	6/27/13	WIPO	English
	B37	WO 9307471	4/15/93	WIPO	English
	B38	WO 9316368	8/16/93	WIPO	English
	B39	WO 9429695	12/22/94	WIPO	English
	B40	WO 9714066A2	4/17/97	WIPO	English

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C1	Brogioli et al., "Heterodyne near-field scattering," *Applied Physics Letters* 81(22):4109-4111, 2002.
	C2	Extended European Search Report for EP 13796734.5, mailed March 18, 2016
	C3	Goda et al., "Serial time-encoded amplified imaging for observation of fast dynamic phenomena," *Nature* 458:1145-1149. 2009
	C4	International Search Report and Written Opinion for PCT/US2013/043453 mailed December 20, 2013.
	C5	International Search Report and Written Opinion for PCT/US2014/067682 mailed April 9, 2015.
	C6	Office Action issued in Japanese Patent Application No. 2015-515208, issued May 29, 2017 (English Translation Provided) (Attorney Docket No. BECC.P0002JP)
	C7	Schmidt, B., "Ein lichtstarkes komafreies Spiegelsystem," *Mitteilungen der Haburger Sternwarte in Bergedorf* 7(36):15-17, 1932.

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	Filing Date: June 30, 2017	Group: Not Yet Assigned

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1-8	*See Page 8-11*	*See Page 10-11*

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

	C8	Smiley, C. H., "The Schmidt Camera," *Popular Astronomy* 44:415-421, 1936.

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EXAMINER: /MINDY D VU/	DATE CONSIDERED: 06/13/2018

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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15638461	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	06/18/2018								
	364	✓								
	365	✓								
	366	✓								
	367	✓								
	368	✓								
	369	✓								
	370	✓								
	371	✓								
	372	✓								
	373	✓								
	374	O								
	375	O								
	376	✓								
	377	✓								
	378	✓								
	379	✓								
	380	✓								
	381	O								
	382	✓								
	383	✓								
	384	✓								
	385	✓								
	386	✓								
	387	✓								
	388	✓								
	389	✓								

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: Yong Qin CHEN

App. No.:	15/638,461	Group Art Unit:	2884
Filed:	June 30, 2017	Examiner:	VU, Mindy D.
Customer No.:	129381	Atty. Dkt. No.:	BECC.P0002US.CIP.C1/1000195036
Title: FLOW CYTOMETER		Confirmation No.:	9847

M/S: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 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:

November 29, 2018
Date

/Mark G. Tison/
Mark G. Tison

RESPONSE TO NON-FINAL OFFICE ACTION

Dear Commissioner:

In response to the Non-Final Office Action mailed June 29, 2018 (the "Action"), please reconsider the above-identified application in light of the following remarks. A petition for a two-month extension of time, with appropriate fee, is filed concurrently via EFS. Accordingly, with the two-month extension of time, this response is properly filed on or before November 29, 2018. No additional fees are believed due in connection with this response; however, should Applicant's payment information be missing, or any other fees be due, Applicant authorizes the Commissioner to debit the deficiency or fee from Norton Rose Fulbright US LLP's Deposit Account No. 50-1212/1000195036.

Amendments to the Claims are reflected in the listing of claims, which begins on page 2 of this paper.

Remarks begin on page 8 of this paper.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1-363. (Canceled).

364. (Currently Amended) An optical ~~module~~ subsystem for a flow cytometer, the optical ~~module~~ subsystem comprising:

~~(a) a light source;~~

~~(b)~~ a collimating optical element arranged to receive light from ~~the~~ a light source, the collimating optical element configured to project a collimated beam; ~~and~~

~~(c)~~ an optical relay element arranged to receive at least a portion of the collimated beam from the collimating optical element, the optical relay element comprising a curved mirror configured to ~~project at least a~~ reflect the portion of the collimated beam received from the collimating optical element to produce a first image;

a first focusing optical element~~, wherein the optical relay is one of a curved mirror and a refractive lens~~ arranged to receive at least a portion of the collimated beam reflected by the optical relay element; and

a first semiconductor detector,

wherein the first focusing optical element is configured to focus the portion of the collimated beam received from the optical relay element onto the first semiconductor detector.

365. (Currently Amended) The optical ~~module~~ subsystem of claim 364, further comprising the light source, wherein the light source ~~is one of~~ comprises light passing through a pinhole ~~and~~ or light emitted from a facet of a multimode optical fiber.

366. (Currently Amended) The optical ~~module~~ subsystem of claim 365, wherein the first focusing optical element is configured to focus the portion of the collimated beam received from the optical relay element to a size smaller than 1 millimeter in diameter ~~to a first semiconductor detector~~.

367. (Currently Amended) The optical ~~module~~ subsystem of ~~claim 364~~ claim 365, wherein the light source forms an object, wherein the collimating optical element is configured to project a magnified image of the object, wherein the optical ~~relay is~~ relay element comprises a concave mirror having a radius of curvature approximately equal to the distance between the collimating optical element and the magnified image projected thereby.

368. (Canceled).

369. (Currently Amended) The optical ~~module~~ subsystem of claim 364, wherein the collimating optical element ~~is~~ comprises an achromatic doublet lens.

370. (Currently Amended) The optical ~~module~~ subsystem of claim 364, further comprising a first optical filter ~~arranged~~ disposed along an optical path between the collimating optical element and the optical relay element, the first optical filter configured to separate the collimated beam into a first branch and a second branch.

371. (Currently Amended) The optical ~~module~~ subsystem of claim 370, wherein the collimated beam comprises light of a plurality of wavelengths, wherein the first optical filter is configured to reflect light of a first range of wavelengths of the plurality of wavelengths to form the first branch of the collimated beam, wherein the first optical filter is configured to pass light of a second range of wavelengths of the plurality of wavelengths to form the second branch of the collimated beam, wherein the first branch ~~is directed to~~ includes the portion of the collimated beam projected from the collimating optical element and received by the optical relay element.

372. (Currently Amended) The optical ~~module~~ subsystem of claim 371, further comprising a second focusing optical element and a second semiconductor detector, the second focusing optical element arranged to receive the second branch of the collimated beam~~, the second focusing optical element~~ and configured to focus the second branch of the collimated beam ~~to a~~ onto the second semiconductor detector.

373. (Currently Amended) The optical ~~module~~ subsystem of claim 372, wherein the diameter of the portion of the collimated beam ~~between~~ reflected by the optical relay element and

received by the first focusing optical element is substantially the same as the diameter of the second branch of the collimated beam ~~between the collimating optical element and the second focusing optical element~~.

374. (Currently Amended) The optical ~~module~~ subsystem of claim 372, further comprising a second optical filter, the second optical filter arranged along an optical path between the optical relay element and the first focusing optical element, the second optical filter configured to pass light of a third range of wavelengths in the first branch of the collimated beam to the first focusing optical element, wherein the third range of wavelengths is a subset of the first range of wavelengths.

375. (Currently Amended) The optical ~~module~~ subsystem of claim 374, ~~wherein~~ further comprising successive combinations of optical relay element, optical filter, and focusing optical element ~~are~~ cascaded to produce focused spots having a diameter of less than 1 millimeter for multiple ranges of wavelengths of light of the collimated beam.

376. (Currently Amended) An optical ~~module~~ subsystem for a flow cytometer, the optical ~~module~~ subsystem comprising:

~~(a) a light source;~~

~~(b)~~ a collimating optical element arranged to receive light from ~~the~~ a light source, the collimating optical element configured to project a collimated beam ~~and~~ including a first image, wherein the collimated beam has a collimated distance; and

~~(c)~~ an optical relay element arranged to receive the collimated beam, the optical relay element configured to extend the collimated distance of the collimated beam,

wherein the optical relay element comprises a curved mirror or a concave-shaped dichroic filter configured to produce a second image.

377. (Canceled).

378. (Currently Amended) The optical ~~module~~ subsystem of claim 376, ~~wherein the optical relay projects a second image,~~ wherein the second image and the first image are substantially the same size.

379. (Currently Amended) The optical ~~module~~ subsystem of claim 376, ~~wherein the optical relay projects a second image,~~ wherein the collimated beam from the collimating optical element to the first image has substantially the same diameter as the collimated beam from the optical relay element to the second image.

380. (Currently Amended) The optical ~~module~~ subsystem of claim 376, further comprising a first focusing optical element arranged to receive the collimated beam from the optical relay element at the extended collimated distance, the first focusing optical element configured to focus the ~~extended~~ collimated beam onto a first semiconductor detector.

381. (Currently Amended) The optical ~~module~~ subsystem of claim 380, further comprising:

an optical filter arranged along an optical path between the collimating optical element and the optical relay element, the optical filter configured to separate the collimated beam into a first branch and a second branch, wherein the first branch and the second branch ~~are~~ comprise light of different colors, wherein the optical relay ~~receives~~ element is arranged to receive the first branch; and

a second focusing optical element arranged to receive the second branch, the second focusing optical element configured to focus the second branch to a second semiconductor detector.

382. (Currently Amended) The optical ~~module~~ subsystem of claim 380, wherein the length of an optical ~~distance~~ path of the collimated beam from the collimating optical element to the optical relay element is substantially the same as the length of an optical ~~distance~~ path of the collimated beam from the optical relay element to the first focusing optical element.

383. (Currently Amended) An optical ~~module~~ subsystem for a flow cytometer, the optical ~~module~~ subsystem comprising:

~~(a) a light source providing light that forms and object;~~

~~(b)~~ a collimating optical element arranged to receive light from ~~the~~ a light source, the collimating optical element configured to project a first image ~~of the object~~; and

~~(c)~~ an optical relay element arranged near the first image, the optical relay element configured to receive light from the collimating optical element and to project a second image ~~of the object~~;

wherein the size of the first image is substantially the same as the size of the second image, and

wherein the optical relay element comprises a curved mirror or a concave-shaped dichroic filter.

384. (Currently Amended) The optical ~~module~~ subsystem of claim 383, wherein the optical distance from the collimating optical element to the first image is substantially the same as the optical distance from the optical relay element to the second image.

385. (Currently Amended) The optical ~~module~~ subsystem of claim 383, further comprising the light source, wherein the light source ~~is~~ comprises an extended light source that forms ~~the~~ an object, wherein the first image is a magnified image of the object.

386. (Currently Amended) The optical ~~module~~ subsystem of claim 385, wherein the light source ~~is one of~~ comprises light passing through a pinhole ~~and~~ or light emitted from a facet of a multimode optical fiber.

387. (Currently Amended) The optical ~~module~~ subsystem of claim 385, wherein the collimating optical element has an effective size defined by its cross section in a plane parallel to the first image, wherein the size of the first image is substantially the same as the effective size of the collimating optical element.

388. (Currently Amended) The optical ~~module~~ subsystem of claim 385, wherein the first image is less than approximately 10 times the size of the object.

389. (Currently Amended) The optical ~~module~~ subsystem of claim 385 further comprising a first focusing optical element arranged near the second image, the first focusing optical element configured to focus the light from the optical relay element to a size smaller than the object of the light source to a first semiconductor detector.

390. (New) The optical subsystem of claim 364, wherein the collimating optical element is further configured to produce a second image, wherein the first image is a reimage of the second image.

391. (New) The optical subsystem of claim 364, wherein the first image is an image of the collimating optical element.

REMARKS

Status of the Claims

Claims 1-363 were previously canceled without prejudice or disclaimer prior to this response. With this response, claims 368 and 377 have been canceled without prejudice or disclaimer, claims 364-367, 369-376, and 378-389 have been amended, and new claims 390 and 391 have been added. No new matter has been added. Support for the claim amendments and the new claims can be found in the published Application at least at ¶¶ [0282], [0401]-[0423], [0445], [0464], and FIGS. 25, 27, and 28.

Incompleteness Of The Office Action

In rejecting one or more claims, the Office identified U.S. Pat. App. Pub. No. 2003/0044967 to Oostman, Jr. et al. However, U.S. Pat. App. Pub. No. 2003/0044967 corresponds to Heffelfinger et al. and does not correspond to Oostman, Jr. et al. Applicant is unable to readily identify with certainty which reference the Office intended to apply as Oostman, Jr. et. al. Accordingly, the Office Action is incomplete with respect to all matters and Applicant has not been given a fair opportunity to respond to each of the rejections presented by the Office. Applicant respectfully submits that at least because the current Office Action is incomplete, that a next Office Action in this case cannot be made final.

Allowable Subject Matter

Applicant thanks the Examiner for indicating that claim 374, 375, and 381 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Claim Objections

The Office objected to claim 383 because of an informality. Claim 383 has been amended to correct the informality rendering the objection moot. Withdrawal of the objection is requested.

35 U.S.C. § 112(a)

The Office rejected claim 368, under 35 U.S.C. § 112(a), as allegedly failing to comply with the enablement requirement. Claim 368 has been canceled without prejudice or disclaimer, rendering the rejection moot. Withdrawal of the 35 U.S.C. § 112(a) rejection is requested.

Claims 364-366, 370-372, 376, 378-380, 383, and 385-389

The Office rejected claims 364-366, 370-372, 376-380, 383, and 385-389, under 35 U.S.C. § 102(a), as allegedly being anticipated by U.S. Pat. App. Pub. No. 2003/0044967 ("Heffelfinger"). Claim 377 has been canceled without prejudice or disclaimer. Without agreeing with the Office, and in an effort to advance prosecution, each of claims 364, 376, and 383 has been amended.

Claims 364-366 and 370-372

The cited portions of Heffelfinger fail to disclose the specific combination of claim 364. For example, Heffelfinger fails to disclose "an optical relay element arranged to receive at least a portion of the collimated beam from the collimating optical element, the optical relay element comprising a curved mirror configured to reflect the portion of the collimated beam received from the collimating optical element to produce a first image", as in claim 364.

Heffelfinger does not disclose an optical relay element that includes a curved mirror (and/or a concave-shaped dichroic filter). In rejecting claim 364, the Office identified relay lens 42 and ¶¶ [0047] and [0049] of Heffelfinger as allegedly corresponding to and describing an optical relay. Heffelfinger describes an apparatus and method in which illumination light and collected emitted light share a pathway and subsequently are physically separated. *See* Heffelfinger at Title and Abstract. Referring to FIG. 1 (reproduced below) of Heffelfinger, Heffelfinger describes that a first laser 12 emits laser light 20 that is directed by steering mirror 16 through beam combiner (e.g. dichroic mirror) 18, and a second laser 14 directs a second laser light 22 to beam combiner 18 that combines first laser light 20 and second laser light 22 into illumination beam 24. *See* Heffelfinger at ¶ [0036].

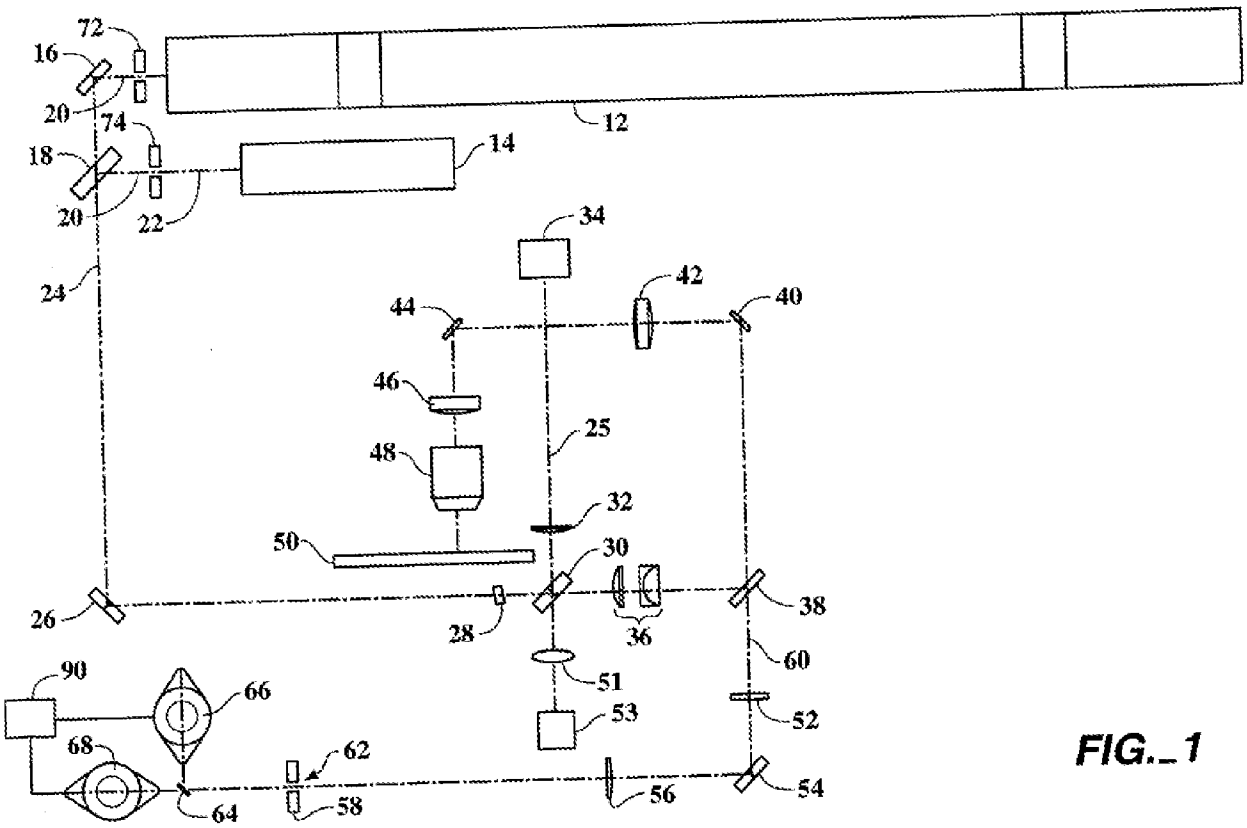


FIG._1

In Heffelfinger, illumination beam 24 is directed by steering mirror 26 through laser line filter 28 (e.g., band pass interference filters and absorption filters) and is impinged upon beam splitter 30 which directs a portion of the beam through focus detection lens 32 and onto output detector 34. *See* Heffelfinger at ¶¶ [0043] and [0044]. Additionally, beam splitter 30 may split diverted beam 25 into two component beams with each beam composed of a subset of the wavelengths of beam 25. *Id.* The light from excitation beam 24 that is not diverted to output detector 34 passes through zoom contractor/expander optics 36 and onto beam splitter optic 38. *See* Heffelfinger at ¶¶ [0045] and [0046]. Beam splitter 38 directs the excitation wavelengths onto beam scanner 40 (e.g., a galvanometer mirror, a rotating polygonal mirror, a resonant scanner, or an acousto-optical scanner, such as a Bragg cell) which produces a line scan of the focused beam. *See* Heffelfinger at ¶ [0046]. The beam is directed to relay lens 42 and is then directed by steering mirror 44 through objective relay lens 46 and objective 48 onto target substrate 50. *See* Heffelfinger at ¶¶ [0047] and [0049].

Additionally, with reference to FIG. 1B (reproduced below) of Heffelfinger, Heffelfinger

further states:

> As illustrated in FIG. 1B, a Bragg cell may be used to effect the beam spot line scan. In this embodiment, fixed mirrors 61, 62, 63 direct excitation beam 80 to Bragg cell 65 at an angle θ relative to the optical axis. RF drive 67 introduces an acoustic sound field within the Bragg cell. This acoustic field causes the excitation beam to be deflected through angle θ′. This deflection produces a back and forth movement of the excitation beam along one axis. The optical scanner directs the beam to relay lenses 42 and 46 that keeps the focused laser light in register.
>
> . . .
>
> The light passes through relay lens 42 and is directed by steering mirror 44 through objective relay lens 46 and objective 48 and onto target substrate 50.

Heffelfinger at ¶¶ [0047] and [0049].

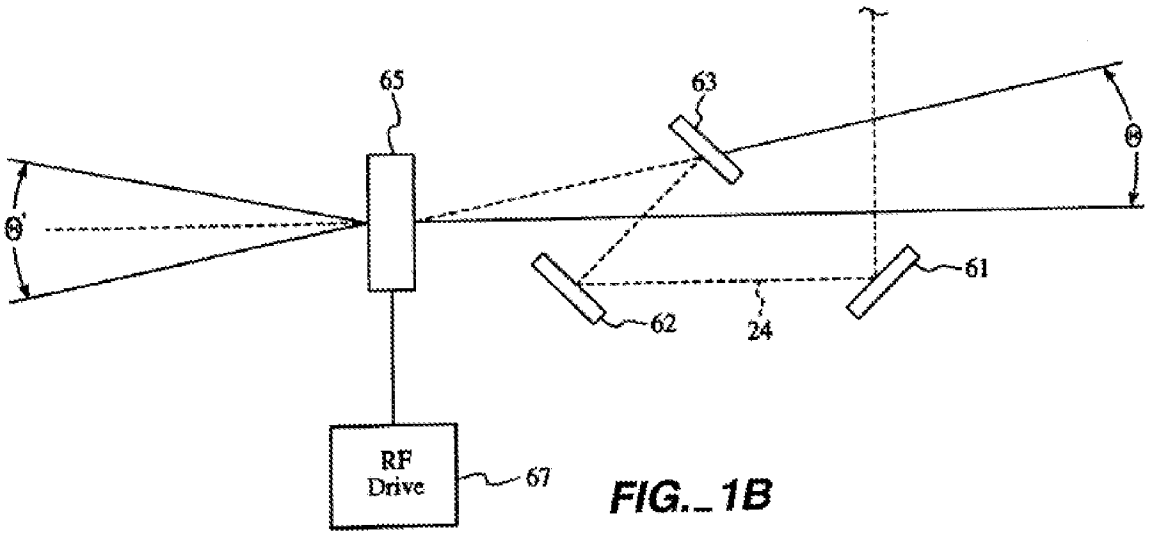


FIG._1B

The cited portions of Heffelfinger are silent with respect to the relay lens 42 being a curved mirror and/or a concave-shaped dichroic filter. Therefore, Heffelfinger fails to disclose "an optical relay element arranged to receive at least a portion of the collimated beam from the collimating optical element, the optical relay element comprising a curved mirror configured to reflect the portion of the collimated beam received from the collimating optical element to produce a first image", as in claim 364.

Further, Heffelfinger does not disclose an optical relay element (that includes a curved mirror) configured to reflect a portion of a collimated beam received from a collimating optical

element. In rejecting claim 364, the Office identified relay lens 42 and ¶¶ [0047] and [0049] of Heffelfinger as allegedly corresponding to and describing an optical relay. As explained above, the beam splitter 38 directs the excitation wavelengths onto beam scanner 40 which produces a line scan of the focused beam that is directed to relay lens 42 and is then directed by steering mirror 44 through objective relay lens 46 and objective 48 onto target substrate 50. *See* Heffelfinger at ¶¶ [0046], [0047], and [0049]. Thus, Heffelfinger describes that the focused beam passes through relay lens 42. Heffelfinger does not describe that the relay lens reflects a portion of a collimated beam (received form a collimated optical element. For at least this additional reason, Heffelfinger fails to disclose the specific combination of claim 364.

Consequently, Heffelfinger fails to disclose each and every element of claim 364. Hence, claim 364 is patentable. Claims 365, 366, and 370-372 are patentable, at least by virtue of depending from a patentable claim.

Claims 376 and 378-380

The cited portions of Heffelfinger fail to disclose the specific combination of claim 364. For example, Heffelfinger fails to disclose "an optical relay element arranged to receive the collimated beam, the optical relay element configured to extend the collimated distance of the collimated beam, wherein the optical relay element comprises a curved mirror or a concave-shaped dichroic filter configured to produce a second image", as in claim 376.

As explained above, Heffelfinger describes an apparatus and method in which illumination light and collected emitted light share a pathway and subsequently are physically separated. *See* Heffelfinger at Title and Abstract. The cited portions of Heffelfinger are silent with respect to the relay lens 42 being a curved mirror and/or a concave-shaped dichroic filter. Therefore, Heffelfinger fails to disclose "an optical relay element arranged to receive the collimated beam, the optical relay element configured to extend the collimated distance of the collimated beam, wherein the optical relay element comprises a curved mirror or a concave-shaped dichroic filter configured to produce a second image", as in claim 376.

Consequently, Heffelfinger fails to disclose each and every element of claim 376. Hence, claim 376 is patentable. Claims 378-380 are patentable, at least by virtue of depending from a patentable claim.

Claims 383 and 385-389

As explained above, Heffelfinger does not disclose an optical relay element arranged to receive a collimated beam, the optical relay element configured to extend the collimated distance of the collimated beam, where the optical relay element includes a curved mirror or a concave-shaped dichroic filter configured to produce a second image. Therefore, Heffelfinger does not disclose "an optical relay element arranged near the first image, the optical relay element configured to receive light from the collimating optical element and to project a second image . . . wherein the optical relay element comprises a curved mirror or a concave-shaped dichroic filter", as in claim 383. Hence, claim 383 is patentable. Claims 385-389 are patentable, at least by virtue of depending from a patentable claim.

Claims 364, 370, 376, 378, 380, and 382-387

The Office rejected claims 364, 370, 376-378, 380, and 382-387, under 35 U.S.C. § 102(a), as allegedly being anticipated by U.S. Pat. App. Pub. No. 2003/0044967 ("Oostman"). As explained above, U.S. Pat. App. Pub. No. 2003/0044967 corresponds to Heffelfinger et al. and does not correspond to Oostman, Jr. et al. Applicant believes that the Office intended to identify U.S. Pat. No. 6,683,314 ("Oostman '314) or U.S. Pat. No. 7,129,505 ("Oostman '505), which is a continuation of Oostman '314. Accordingly, in an effort to advance prosecution, Applicant provides remarks based on Oostman '314. Claim 377 has been canceled without prejudice or disclaimer. Without agreeing with the Office, and in an effort to advance prosecution, each of claims 364, 376, and 383 has been amended.

Without agreeing with the Office, and in an effort to advance prosecution, claim 364 has been amended to recite:

Claims 364 and 370

The cited portions of Oostman '314 fail to disclose the specific combination of claim 364. For example, Oostman '314 fails to disclose "an optical relay element arranged to receive at least a portion of the collimated beam from the collimating optical element, the optical relay element comprising a curved mirror configured to reflect the portion of the collimated beam received from the collimating optical element to produce a first image", as in claim 364.

Oostman '314 does not disclose an optical relay element that includes a curved mirror

(and/or a concave-shaped dichroic filter). In rejecting claim 364, the Office identified element 151 and col. 6, lines 40-50 of Oostman '314 as allegedly corresponding to an optical relay. Oostman '314 describes a fluorescence detection instrument with reflective transfer legs for color decimation. *See* Oostman '314 at Title and Abstract. Referring to FIG. 5 (reproduced below) of Oostman '314, Oostman '314 describes a top planar polygonal detector arrangement showing decimation of an incoming beam with reflective transfer leg beam splitters, where the incoming beam is received from a fiber bundle. *See* Oostman '314 at 4:60-64 and 6:40-50.

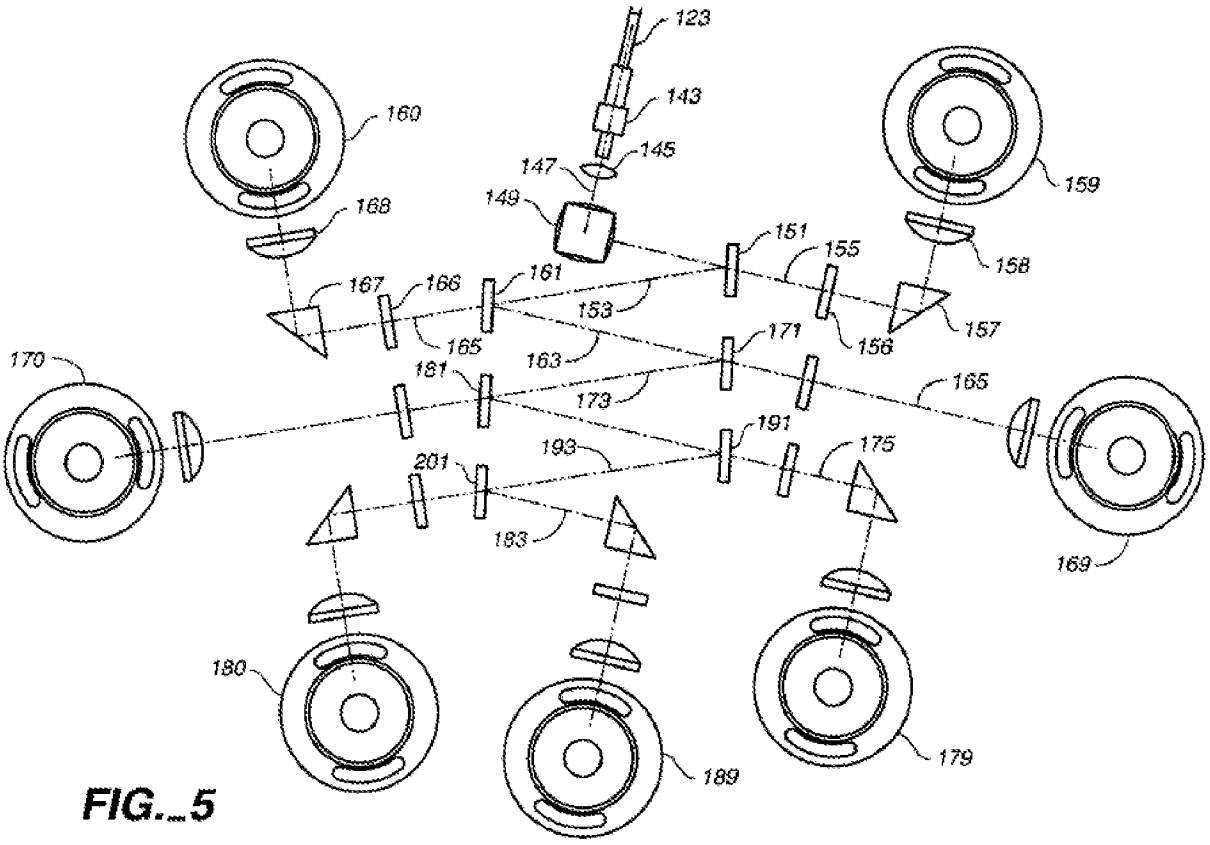


FIG._5

In Oostman '314, the fluorescence detection instrument includes an optical fiber 122 having a fiber terminal 143, a collimating lens 145, beam splitting elements 151 (i.e., dichroic mirrors), and focusing elements 158, 168 to form a beam 155 emitted from a light source (e.g., 123), and a photomultiplier tube 159 to detect the beam 155. *See* Oostman '314 at 6:40-49. Referring to FIG. 5, it is noted that the beam splitting elements 151 are depicted as planar optical elements and are **not** curved. It is further noted that the only optical elements that are curved include the collimating lens 145 and the focusing elements 158, 168.

Oostman '314 further describes FIG. 5 and states:

> The optical fiber 123 has a fiber terminal 143, which allow emergence of light and formation of a beam by means of a collimating lens 145. The output beam 147 is directed toward a beam-turning mirror 149 directing light at a first beam splitting element 151 which is a dichroic mirror transmitting light in beam 155 in a transmitted detector leg toward a filter 156, a beam-turning element 157, and a focusing element 158 which directs the beam onto a light sensitive element of a photomultiplier tube 159. The tube is adjusted so that its most sensitive area is exposed to the incoming beam. This beam is known as the transmitted detector leg because it is transmitted through the beam splitting element 151. The beam splitting element has a coating which allows transmission of one band of optical signals, while reflecting light in another band in the form of beam 153 which forms a reflective transfer leg for the light which was originally in beam 147. The reflective transfer leg 153 is seen to fall upon another beam splitting element 161 which is a dichroic mirror having different optical characteristics from the beam splitter 151. The colors removed by beam splitter 161, as well as the other beam splitters, are different, each beam splitter removing a selected band of colored light in the same manner, but different wavelengths . . .

Oostman '314 at 6:40-64. The cited portions of Oostman '314 are silent with respect to the beam splitting elements 151 being a curved mirror and/or a concave-shaped dichroic filter. Therefore, Oostman '314 fails to disclose "an optical relay element arranged to receive at least a portion of the collimated beam from the collimating optical element, the optical relay element comprising a curved mirror configured to reflect the portion of the collimated beam received from the collimating optical element to produce a first image", as in claim 364.

Consequently, Oostman '314 fails to disclose each and every element of claim 364. Hence, claim 364 is patentable. Claim 370 is patentable, at least by virtue of depending from a patentable claim.

Claims 376, 378, 380, and 382

The cited portions of Oostman '314 fail to disclose the specific combination of claim 364. For example, Oostman '314 fails to disclose "an optical relay element arranged to receive the collimated beam, the optical relay element configured to extend the collimated distance of the collimated beam, wherein the optical relay element comprises a curved mirror or a concave-shaped dichroic filter configured to produce a second image", as in claim 376.

As explained above, Oostman '314 describes a fluorescence detection instrument with

reflective transfer legs for color decimation. *See* Oostman '314 at Title and Abstract. The cited portions of Oostman '314 are silent with respect to the beam splitting elements 151 being a curved mirror and/or a concave-shaped dichroic filter. Therefore, Oostman '314 fails to disclose "an optical relay element arranged to receive the collimated beam, the optical relay element configured to extend the collimated distance of the collimated beam, wherein the optical relay element comprises a curved mirror or a concave-shaped dichroic filter configured to produce a second image", as in claim 376.

Consequently, Oostman '314 fails to disclose each and every element of claim 376. Hence, claim 376 is patentable. Claims 378, 380, and 382 are patentable, at least by virtue of depending from a patentable claim.

Claims 383-387

As explained above, Oostman '314 does not disclose an optical relay element arranged to receive a collimated beam, the optical relay element configured to extend the collimated distance of the collimated beam, where the optical relay element includes a curved mirror or a concave-shaped dichroic filter configured to produce a second image. Therefore, Oostman '314 does not disclose "an optical relay element arranged near the first image, the optical relay element configured to receive light from the collimating optical element and to project a second image . . . wherein the optical relay element comprises a curved mirror or a concave-shaped dichroic filter", as in claim 383. Hence, claim 383 is patentable. Claims 384-387 are patentable, at least by virtue of depending from a patentable claim.

Claim 369

The Office rejected claim 369, under 35 U.S.C. § 103(a), as allegedly being unpatentable over Heffelfinger in view of U.S. Pat. No. 4,385,234 ("Johnson"). Applicant disagrees with the rejection.

Claim 369 depends from claim 364. As explained above, claim 364 is patentable over Heffelfinger. The Office has not shown that Johnson would supply the deficiencies of Heffelfinger, and dependent claim 369 is therefore patentable over Heffelfinger and Johnson for at least the same reasons. Reconsideration and withdrawal of the rejection is requested.

Claim 366

The Office rejected claim 366, under 35 U.S.C. § 103(a), as allegedly being unpatentable over U.S. Pat. App. Pub. No. 2003/0044967 ("Oostman"). As explained above, U.S. Pat. App. Pub. No. 2003/0044967 corresponds to Heffelfinger et al. and does not correspond to Oostman, Jr. et al. Applicant believes that the Office intended to identify Oostman '314 or Oostman '505, which is a continuation of Oostman '314. Accordingly, in an effort to advance prosecution, Applicant provides remarks based on Heffelfinger and, alternatively, Oostman '314. Applicant disagrees with the rejection.

Claim 366 depends from claim 364. As explained above, claim 364 is patentable over each of Heffelfinger and Oostman '314. Therefore, claim 366 is patentable, at least by virtue of depending from a patentable claim. Reconsideration and withdrawal of the rejection is requested.

Claim 367

The Office rejected claim 367, under 35 U.S.C. § 103(a), as allegedly being unpatentable over U.S. Pat. App. Pub. No. 2003/0044967 ("Oostman") in view of U.S. Pat. App. Pub. No. 2005/0002425 ("Govorkov"). As explained above, U.S. Pat. App. Pub. No. 2003/0044967 corresponds to Heffelfinger et al. and does not correspond to Oostman, Jr. et al. Applicant believes that the Office intended to identify Oostman '314 or Oostman '505, which is a continuation of Oostman '314. Accordingly, in an effort to advance prosecution, Applicant provides remarks based on Heffelfinger and, alternatively, Oostman '314. Applicant disagrees with the rejection.

Claim 367 depends from claim 364. As explained above, claim 364 is patentable over each of Heffelfinger and Oostman '314. The Office has not shown that Govorkov would supply the deficiencies of Heffelfinger or Oostman '314, and dependent claim 367 is therefore patentable over Heffelfinger and Govorkov, or Oostman '314 and Govorkov, for at least the same reasons. Reconsideration and withdrawal of the rejection is requested.

Claim 373

The Office rejected claim 373, under 35 U.S.C. § 103(a), as allegedly being unpatentable over Heffelfinger. Applicant disagrees with the rejection.

Claim 373 depends from claim 364. As explained above, claim 364 is patentable over

Heffelfinger. Therefore, claim 373 is patentable, at least by virtue of depending from a patentable claim. Reconsideration and withdrawal of the rejection is requested.

Claims 374, 375, and 381

Applicant thanks the Examiner for indicating that claim 374, 375, and 381 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Claims 374 and 375 depend from claim 364, which Applicant has shown to be patentable. Hence, claims 374 and 375 are patentable, at least by virtue of depending from a patentable claim. Claim 381 depends from claim 376, which Applicant has shown to be patentable. Hence, claim 381 is patentable, at least by virtue of depending from a patentable claim.

New Claims 390 and 391

New claims 390 and 391 have been added and are supported by the Specification. No new matter has been added. Claims 390 and 391 depend from claim 364, which Applicant has shown to be patentable. Hence, claims 390 and 391 are patentable, at least by virtue of depending from a patentable claim.

* * *

CONCLUSION

The pending claims are in condition for allowance for at least the reasons set forth above. Applicant does not acquiesce to the Office's characterizations of the claim language or the cited reference(s), and reserves the right to challenge any such characterizations in the future. Any changes to the claims in this response, which have not been specifically noted to overcome a rejection based upon the cited portions of the above-cited reference(s), should be considered to have been made for a purpose unrelated to patentability, and no estoppel should be deemed to attach thereto. The Examiner is invited to contact the undersigned attorney at the telephone number listed below if such a call would in any way facilitate allowance of this application.

Respectfully submitted,

Date: November 29, 2018

/Mark G. Tison/
Mark G. Tison, Reg. No. 65,985
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98 San Jacinto Blvd., Suite 1100
Austin, TX 78701
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Facsimile: (512) 536-4598

PTO/AIA/22 (03-13)
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)

Docket Number (Optional): BECC.P0002UC.CIP.C1/1000195036

Application Number: 15/638,461

Filed: June 30, 2017

For: Flow Cytometer

Art Unit: 2884

Examiner: Mindy 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	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☑ Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$ 600
☐ 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.

☑ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☑ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-1212/1000195036.

☑ 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 65,985.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/Mark G. Tison/ — Signature

November 29, 2018 — Date

Mark G. Tison — Typed or printed name

(512) 536-3033 — 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 _____ forms are submitted.

This collection of information is required by 37 CFR 1.136(a). 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 6 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: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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.

Electronic Patent Application Fee Transmittal

Application Number:	15638461
Filing Date:	30-Jun-2017
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Filer:	Mark G Tison/Julia Hohle
Attorney Docket Number:	BECC.P0002US.CIP.C1

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 - 2 months with $0 paid	1252	1	600	600
Miscellaneous:				
			Total in USD ($)	**600**

Electronic Acknowledgement Receipt

EFS ID:	34436903
Application Number:	15638461
International Application Number:	
Confirmation Number:	9847
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	32425
Filer:	Mark G Tison/Julia Hohle
Filer Authorized By:	Mark G Tison
Attorney Docket Number:	BECC.P0002US.CIP.C1
Receipt Date:	29-NOV-2018
Filing Date:	30-JUN-2017
Time Stamp:	15:09:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$600
RAM confirmation Number	113018INTEFSW15105400
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		BECCP0002USCIPC1_ROA_filing.pdf	285852 697f5f60cbb0988fe415eb2c69d299c24738349f	yes	19
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
	Claims		2	7	
	Applicant Arguments/Remarks Made in an Amendment		8	19	
Warnings:					
Information:					
2	Extension of Time	BECCP0002USCIPC1_EOT.pdf	153313 236f1693a357f01e485ef5fbb8f5fa518f599b26	no	2
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30818 477f962ae73baea0de3a8fdda90ec53690448352	no	2
Warnings:					
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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.

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

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PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/638,461	Filing Date 06/30/2017	☐ 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 $80 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $420 =	
☐ 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)			
AMENDMENT	11/29/2018	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 26	Minus	** 26	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 3	Minus	*** 3	= 0		x $460 =	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)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ 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.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/ZURIASHWORK ZENEBE/
*** 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.	

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

32425 7590 02/07/2019

NORTON ROSE FULBRIGHT US LLP
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SUITE 1100
AUSTIN, TX 78701-4255

EXAMINER	
BOOSALIS, FANI POLYZOS	
ART UNIT	PAPER NUMBER
2884	

DATE MAILED: 02/07/2019

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/638,461	06/30/2017	Yong Qin Chen	BECC.P0002US.CIP.C1	9847

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	$1000	$0.00	$0.00	$1000	05/07/2019

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: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

Page 504 of 880

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (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)

32425 7590 02/07/2019
NORTON ROSE FULBRIGHT US LLP
98 SAN JACINTO BOULEVARD
SUITE 1100
AUSTIN, TX 78701-4255

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 transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/638,461	06/30/2017	Yong Qin Chen	BECC.P0002US.CIP.C1	9847

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	$1000	$0.00	$0.00	$1000	05/07/2019

EXAMINER	ART UNIT	CLASS-SUBCLASS
BOOSALIS, FANI POLYZOS	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 03-09 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 must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). 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. Fees submitted: ❑ Issue Fee ❑ Publication Fee (if required) ❑ Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

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.
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.
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. _____

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.
15/638,461	06/30/2017	Yong Qin Chen	BECC.P0002US.CIP.C1	9847

32425 7590 02/07/2019

NORTON ROSE FULBRIGHT US LLP
98 SAN JACINTO BOULEVARD
SUITE 1100
AUSTIN, TX 78701-4255

EXAMINER	
BOOSALIS, FANI POLYZOS	
ART UNIT	**PAPER NUMBER**
2884	

DATE MAILED: 02/07/2019

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.

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 30 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. 15/638,461	Applicant(s) Chen, Yong Qin	
	Examiner FANI P BOOSALIS	Art Unit 2884	AIA 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 submission of November 29, 2018.
   ☐ 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 364-367,369-376 and 378-391 . 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 areply 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 _____.

/DAVID P PORTA/ Supervisory Patent Examiner, Art Unit 2884	/FaniBoosalis/

Notice of Pre-AIA or AIA Status

1. The present application is being examined under the pre-AIA first to invent provisions.

EXAMINER'S STATEMENT OF REASONS FOR ALLOWANCE

Response to Arguments

2. Applicant's arguments, pages 8-19, filed November 29, 2018, with respect to claim(s) 364-373, 376-380, 382-389, have been fully considered and are persuasive. The rejection of claim(s) 364-373, 376-380, 382-389 has been withdrawn.

Response to Amendment

3. The amendment submitted November 29, 208 has been accepted and entered. Claims 364-367, 369-376, 378-389 are amended. Claims 368, 377 are cancelled. New claims 390, 391 are added. Thus, claims 364-367, 369-376, 378-391 are examined.

Allowable Subject Matter

4. Claims 364-367, 369-376, 378-391 are allowable over the prior art.

Independent claims 364 is allowable for reasons of record as presented in applicant's remarks; filed on November 29, 2018, especially pages 11-12, regarding an optical relay element, including a curved mirror, configured to reflect a portion of a collimated beam received from a collimating optical element, as clamed in combination with the rest of the claim limitations, so as to improve structure an operation of a flow cytometer together with individual subassemblies.

Independent claims 376, 383 are allowable for reasons of record as presented in applicant's remarks; filed on November 29, 2018, especially pages 11-12, regarding an optical relay element arranged to receive the collimated beam, the optical relay element configured to extend the collimated distance of the collimated beam, wherein the optical relay element comprise a curved mirror or a concave-shaped dichroic filter configured to produce a second image, as clamed in combination with the rest of the claim limitations, without significantly expanding the beam diameter.

5. Claims 365-367, 369-375, 378-382, 384-391 are allowable based on their dependency.

Conclusion

6. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Wells et al (US 7,113,266 B1) discloses a flow cytometer for differentiating e.g. blood cell, has optical component positioned in laser beam between laser diode and optical flow cell and rotating plane of polarization of laser beam before beam irradiates particles

Swalwell et al (US 8,928,881 B2) discloses a system and method for performing flow cytometry is disclosed wherein the alignment of the light source with respect to the sensing region of the fluid stream is continuously controlled using a feedback control loop. An imaging apparatus is provided that images the system along the optical axis, between the field stop and the sensing region. In an embodiment, the cytometer comprises a field stop having an H-shaped aperture and light diverters over the ends of the aperture that divert a portion of the light to peripheral detectors.

Osamu et al (JP 55144226 A) discloses a concave mirror and fluorescent screen fixed to a holding and fixing means. An aberration correction lens is designed in the position suitable for the intermediate curvature of two kinds of screens 1, 2 fixed to a moving means and movable on the main beam axis Z-Z'.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to FANI POLYZOS BOOSALIS whose telephone number is (571)272-2447. The examiner can normally be reached on 7:30-3:30 PM.

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, David 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.

8. 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 P PORTA/
Supervisory Patent Examiner, Art Unit 2884

/FB/

Notice of References Cited	Application/Control No. 15/638,461	Applicant(s)/Patent Under Reexamination Chen, Yong Qin	
	Examiner FANI P BOOSALIS	Art Unit 2884	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20050274890-A1	12-2005	Anderton, Rupert Nicholas	G01V8/005	250/334
*	B	US-8928881-B2	01-2015	Swalwell; Jarred E.	G01N15/1434	356/336
*	C	US-20110216401-A1	09-2011	Curry; Douglas N.	G02B7/16	359/384
*	D	US-7113266-B1	09-2006	Wells; Mark A.	G01N15/1404	356/336
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*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.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/638,461	Chen, Yong Qin
	Examiner	Art Unit
	FANI P BOOSALIS	2884

CPC - Searched*		
Symbol	Date	Examiner
G01N 15/1436, 15/1459, 15/1434	6/15/18	MV
G02B 21/361, 27/0025, 21/804, 6/26, 6/29365	6/15/18	MV
Search Updated	01/23/2019	/FB/
G01N2015/1006 G01N15/1434 G01N15/1436 G01N15/1459 G01N2015/1452 G01N2015/144 G02B21/04 G02B27/0025 G02B6/26	01/23/2019	/FB/

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Inventor name search	6/11/18	mv
Parent data considered	6/11/18	mv
EAST BRS - See search history printout	6/18/18	mv
EAST - Text Search	01/23/2019	/FB/
EAST - Inventor Name Search	01/23/2019	/FB/
EAST - Interference Search History Printout	01/23/2019	/FB/

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/638,461	Chen, Yong Qin
	Examiner	Art Unit
	FANI P BOOSALIS	2884

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
PGPub	Text Search	01/23/2019	/FB/

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/638,461	Chen, Yong Qin
	Examiner	Art Unit
	FANI P BOOSALIS	2884

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/18/2018	01/23/2019							
1	364	✓	=							
2	365	✓	=							
3	366	✓	=							
4	367	✓	=							
	368	✓	-							
5	369	✓	=							
6	370	✓	=							
7	371	✓	=							
8	372	✓	=							
9	373	✓	=							
10	374	O	=							
11	375	O	=							
14	376	✓	=							
	377	✓	-							
15	378	✓	=							
16	379	✓	=							
17	380	✓	=							
18	381	O	=							
19	382	✓	=							
20	383	✓	=							
21	384	✓	=							
22	385	✓	=							
23	386	✓	=							
24	387	✓	=							
25	388	✓	=							
26	389	✓	=							
12	390		=							
13	391		=							

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/638,461	Chen, Yong Qin
	Examiner	**Art Unit**
	FANI P BOOSALIS	2884

CPC						
Symbol					**Type**	**Version**
G01N	/	15	/	1436	F	2013-01-01
G01N	/	15	/	1434	I	2013-01-01
G02B	/	6	/	29365	I	2013-01-01
G02B	/	6	/	26	I	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
G01N	/	2015	/	1452	A	2013-01-01
G01N	/	2015	/	144	A	2013-01-01
G01N	/	2015	/	1006	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

/FaniBoosalis/	23 January 2019	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	26	
/DAVID P PORTA/ SPE, Art Unit 2884	24 January 2019	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/638,461	Chen, Yong Qin
	Examiner	**Art Unit**
	FANI P BOOSALIS	2884

INTERNATIONAL CLASSIFICATION				
CLAIMED				
G01N	/	15	/	1436
G01N	/	15	/	434
G02B	/	6	/	26

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

/FaniBoosalis/ (Assistant Examiner)	23 January 2019 (Date)	**Total Claims Allowed:** 26	
/DAVID P PORTA/ SPE, Art Unit 2884 (Primary Examiner)	24 January 2019 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/638,461	Chen, Yong Qin
	Examiner	**Art Unit**
	FANI P BOOSALIS	2884

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
	1		50		99		148		197		246		295		344
	2		51		100		149		198		247		296		345
	3		52		101		150		199		248		297		346
	4		53		102		151		200		249		298		347
	5		54		103		152		201		250		299		348
	6		55		104		153		202		251		300		349
	7		56		105		154		203		252		301		350
	8		57		106		155		204		253		302		351
	9		58		107		156		205		254		303		352
	10		59		108		157		206		255		304		353
	11		60		109		158		207		256		305		354
	12		61		110		159		208		257		306		355
	13		62		111		160		209		258		307		356
	14		63		112		161		210		259		308		357
	15		64		113		162		211		260		309		358
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/FaniBoosalis/	23 January 2019	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	26	
/DAVID P PORTA/ SPE, Art Unit 2884	24 January 2019	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/638,461	Chen, Yong Qin
	Examiner	**Art Unit**
	FANI P BOOSALIS	2884

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
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/FaniBoosalis/	23 January 2019	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	26	
/DAVID P PORTA/ SPE, Art Unit 2884	24 January 2019	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L7	3	(flow cytomet$4 ((concav$4 or curv$4) (mirror or reflect$4)) relay beam collimat$4 focus).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:33
L10	52393	(optic$4 relay) (mirror or (refract$4 lens))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:35
L11	8377	(collimat$4 filter) and l10	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:36
L12	1118	(pin$1 adj hole) and l10	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:36
L13	1118	l12 and l10	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:36
L14	622	(diamet$4 siz$4) and l13	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:36
L15	442	l14 and filter	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:36
L16	96	l15 and (relay concav$4 mirror)	US-PGPUB;	AND	OFF	2019/01/23

			USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			13:37
L17	1	l16 AND ((G01N2015/1006 OR G01N15/1434 OR G01N15/1436 OR G01N15/1459 OR G01N2015/1452 OR G01N2015/144 OR G02B21/04 OR G02B27/0025 OR G02B6/26).CPC.)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:37
L18	4139548	chen.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:37
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L21	21	l12 AND ((G01N2015/1006 OR G01N15/1434 OR G01N15/1436 OR G01N15/1459 OR G01N2015/1452 OR G01N2015/144 OR G02B21/04 OR G02B27/0025 OR G02B6/26).CPC.)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 13:38
L22	25	("2003/0143391").URPN.	USPAT	OR	OFF	2019/01/23 13:38
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L27	13	("2008/0268469").URPN.	USPAT	OR	OFF	2019/01/23 13:39
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S29	286	S28 and (focus$4 detect$4 semiconduct$4)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 11:32
S30	116	S29 AND ((G01N2015/1006 OR G01N15/1434 OR G01N15/1436 OR G01N15/1459 OR G01N2015/1452 OR G01N2015/144 OR G02B21/04 OR G02B27/0025 OR G02B6/26).CPC.)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 11:34
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S34	12	(flow cytometer) and collimat$4 optic$4 source beam image and (mirror curv$4)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	SAME	OFF	2019/01/23 11:42
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S38	1	S37 and cytometer	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 12:03
S43	1	S35 and cytomet$4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	AND	OFF	2019/01/23 12:05

1/ 23/ 2019 1:39:26 PM
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Document Description: Issue Fee Payment (PTO-85B)

Issue Fee Transmittal Form

Application Number	Filing Date	First Named Inventor	Atty. Docket No.	Confirmation No.
15638461	30-Jun-2017	Yong Chen	BECC.P0002US.CIP.C1	9847

TITLE OF INVENTION :

FLOW CYTOMETER

Entity Status	Application Type	Art Unit	Class - Subclass	EXAMINER
Regular Undiscounted	Utility under 35 USC 111(a)	2884	458100	FANI BOOSALIS

Issue Fee Due	Publication Due	Total Fee(s) Due	Date Due	Prev. Paid Fee
$1000	$0	$1000	07-May-2019	$0

1.Change of Correspondence Address and/or Indication Of Fee Address (37 CFR 1.33 & 1.363)

Current Correspondence Address:	Current Indicated Fee Address :
32425 NORTON ROSE FULBRIGHT US LLP 98 SAN JACINTO BOULEVARD SUITE 1100 AUSTIN TX 78701-4255 UNITED STATES 512-474-5201 aoipdocket@nortonrosefulbright.com	
☐ Change of correspondence address requested, system generated AIA/122-EFS form attached	☐ Fee Address indication requested, system generated SB/47-EFS form attached

2.Entity Status

Change in Entity Status

○ Applicant certifying micro entity status; system generated Micro Entity certification form attached. See 37 CFR 1.29.
Note: Absent a valid certification of micro entity status, issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment. If this box is checked, you will be prompted to choose a micro entity status on the gross income basis (37 CFR 1.29(a)) or the institution of higher education basis (37 CFR 1.29(d)), and make the applicable certification online.

○ 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.

Document Description: Issue Fee Payment (PTO-85B)

3.The Following Fee(s) Are Submitted:

☒ Issue Fee	☐ I authorize USPTO to apply my previously paid issue fee to the current fees due
☐ Publication Fee	☐ The Director is hereby authorized to apply my previously paid issue fee to the current fee due and to charge deficient fees to Deposit Account Number _____
☐ Advance Order - # of copies _____	☐ If **in addition to** the payment of the issue fee amount submitted with this form, there are any discrepancies in any amount(s) due, the Director is authorized to charge any deficiency, or credit any overpayment, to Deposit Account Number _____ . The **issue fee must be submitted** with this form. **If payment of the issue fee does not accompany this form, checking this box and providing a deposit account number will NOT be effective to satisfy full payment of the fee(s) due.**

4.Firm and/or Attorney Names To Be Printed

NOTE: If no name is listed, no name will be printed
For printing on the patent front page, list to be displayed as entered

1. NORTON ROSE FULBRIGHT US LLP
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3.

5.Assignee Name(s) and Residence Data To Be Printed

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Name	City	State	Country	Category
IRIS INTERNATIONAL, INC.	CHATSWORTH	CALIFORNIA	united states	corporation

6.Signature

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 filed and has been granted power of attorney in this application. I also certify that this Fee(s) Transmittal form is being transmitted to the USPTO via EFS-WEB on the date indicated below.

Signature	/Gina N. Shishima/	**Date**	05-06-2019
Name	Gina N. Shishima	**Registration Number**	45104

Electronic Patent Application Fee Transmittal

Application Number:	15638461
Filing Date:	30-Jun-2017
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Filer:	Gina N. Shishima/Pam Gerik
Attorney Docket Number:	BECC.P0002US.CIP.C1

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY APPL ISSUE FEE	1501	1	1000	1000
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1000**

Electronic Acknowledgement Receipt

EFS ID:	35923671
Application Number:	15638461
International Application Number:	
Confirmation Number:	9847
Title of Invention:	FLOW CYTOMETER
First Named Inventor/Applicant Name:	Yong Qin Chen
Customer Number:	32425
Filer:	Gina N. Shishima/Pam Gerik
Filer Authorized By:	Gina N. Shishima
Attorney Docket Number:	BECC.P0002US.CIP.C1
Receipt Date:	06-MAY-2019
Filing Date:	30-JUN-2017
Time Stamp:	13:08:01
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1000
RAM confirmation Number	050619INTEFSW13075900
Deposit Account	
Authorized User	

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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	Web85b.pdf	46034 0ba42ac90fa8f57afe4d207a36937c5ccf8ca340	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	31944 cd549aa99bb2081642bd8d9e538adeac1b3995a5	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	77978		

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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.

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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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/638,461	06/25/2019	10330582	BECC.P0002US.CIP.C1	9847

32425 7590 06/05/2019

NORTON ROSE FULBRIGHT US LLP
98 SAN JACINTO BOULEVARD
SUITE 1100
AUSTIN, TX 78701-4255

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 0 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):

IRIS INTERNATIONAL, INC., Chatsworth, CA;
Yong Qin Chen, San Jose, CA;

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PTO/AIA/122 (06-15)
Approved for use through 09/30/2025. OMB 0651-0035
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Application

Address to:
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P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	15/638,461
Filing Date	June 30, 2017
First Named Inventor	Yong Chen
Art Unit	2884
Examiner Name	Fani Polyzos BOOSALIS
Attorney Docket Number	BECC.P0002US.CIP.C1

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I am the:

☐ Applicant

☒ Attorney or agent of record. Registration Number 65,985.

☐ Registered practitioner named in the application transmittal papers who acts in a representative capacity under 37 CFR 1.34. See 37 CFR 1.33(a)(1). Registration Number _____.

Signature /Mark G. Tison/

Typed or Printed Name Mark G. Tison

Date	Telephone
March 30, 2023	512 536-3033

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

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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. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013). https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf

Routine uses of the information in this record may include disclosure to: 1) law enforcement, in the event that the system of records indicates a violation or potential violation of law; 2) a Federal, state, local, or international agency, in response to its request; 3) a contractor of the USPTO having need for the information in order to perform a contract; 4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record; 5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record; 6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations; 7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals; 8) 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)); 9) the Office of Personnel Management (OPM) for personnel research purposes; and 9) the Office of Management and Budget (OMB) for legislative coordination and clearance.

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Additional USPTO uses of the information in this record may include disclosure to: 1) the International Bureau of the World Intellectual Property Organization, if the record is related to an international application filed under the Patent Cooperation Treaty; 2) the public i) after publication of the application pursuant to 35 U.S.C. 122(b), ii) after issuance of a patent pursuant to 35 U.S.C. 151, iii) 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 inspections, or an issued patent, or iv) without publication of the application or patent under the specific circumstances provided for by 37 CFR 1.14(a)(1)(v)-(vii); and/or 3) the National Archives and Records Administration, for inspection of records.

PTO/AIA/82A (07-13)
Approved for use through 03/31/2021. OMB 0651-0035
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TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	15/638,461
Filing Date	June 30, 2017
First Named Inventor	Yong Chen
Title	FLOW CYTOMETER
Art Unit	2884
Examiner Name	Fani Polyzos BOOSALIS
Attorney Docket Number	BECC.P0002US.CIP.C1

SIGNATURE of Applicant or Patent Practitioner

Signature	/Mark G. Tison/	Date (Optional)	March 30, 2023
Name	Mark G. Tison	Registration Number	65,985
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☐ *Total of _____ 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.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
Approved for use through 11/30/2014. OMB 0651-0051
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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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:

☑ The address associated with the above-mentioned Customer Number

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.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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 the Freedom of Information Act requires disclosure of these records.
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 inspections 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.

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
15/638,461	**03/30/2023 03:51:30 PM ET**	**BECC.P0002US.CIP.C1**

Title of Invention

FLOW CYTOMETER

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	10330582
CONFIRMATION #	9847	FILED BY	Grace Adame-Delgado
PATENT CENTER #	61842733	FILING DATE	06/30/2017
CUSTOMER #	32425	FIRST NAMED INVENTOR	Yong Qin Chen
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Mark Tison

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
BECC.P0002US.CIP.C1 - POA.pdf	3	Power of Attorney	328 KB
BECC.P0002US.CIP.C1 Change of Correspondence Address.pdf	2	Change of Address	262 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
BECC.P0002US.CIP.C1 -	738D39B940402F1C714DBB2017CD7B866949420A7E06AD23E

POA.pdf	D5110D9FC6CB38E0CEEDB7385CD648938B0936B36CE6A3BA D0718AF6C8E29B0B20065B43F65B6ED
BECC.P0002US.CIP.C1 Change of Correspondence Address.pdf	00EEEF256652D55DC60585E88C88FC32378AC9715292B4F021 AE56711CC87E1A92946DF5F1A2373B69BEAB00E5B74AEFE6E 81728850B6E653C5D5A3E76AB9C0A

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 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.

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 NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/638,461	06/30/2017	Yong Qin Chen	BECC.P0002US.CIP.C1

CONFIRMATION NO. 9847

129381
NORTON ROSE FULBRIGHT US LLP - BCI
98 San Jacinto Blvd., Suite 1100
Austin, TX 78701

POA ACCEPTANCE LETTER

OC000000137685112

Date Mailed: 04/03/2023

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 03/30/2023.

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.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/vostuart/

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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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
15/638,461	06/30/2017	Yong Qin Chen	BECC.P0002US.CIP.C1

CONFIRMATION NO. 9847

POWER OF ATTORNEY NOTICE

32425
NORTON ROSE FULBRIGHT US LLP
98 SAN JACINTO BOULEVARD
SUITE 1100
AUSTIN, TX 78701-4255

OC000000137685110

Date Mailed: 04/03/2023

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 03/30/2023.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/vostuart/