8225608

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

April 7, 2022

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF THE FILE WRAPPER AND CONTENTS OF:

APPLICATION NUMBER: *13/222,688*
FILING DATE: *August 31, 2011*
PATENT NUMBER: *8,901,516*
ISSUE DATE: *December 2, 2014*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

Wanda Montgomery
Certifying Officer

PTO/SB/14 (11-08)
Approved for use through 09/30/2010. 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 contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SPEC US-4 (027560-0014)
	Application Number	
Title of Invention	EXCITATION LIGHT SOURCE ASSEMBLY	

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.)

Applicant Information:

Applicant 1

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Michael	B.	Nelson	

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

City	Tucson	State/Province	AZ	Country of Residence	US

Citizenship under 37 CFR 1.41(b)	US

Mailing Address of Applicant:

Address 1	420 North Bonita Avenue
Address 2	

City	Tucson	State/Province	AZ
Postal Code	85745	Country	US

Applicant 2

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Michael	D.	Cable	

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

City	Tucson	State/Province	AZ	Country of Residence	US

Citizenship under 37 CFR 1.41(b)	US

Mailing Address of Applicant:

Address 1	420 North Bonita Avenue
Address 2	

City	Tucson	State/Province	AZ
Postal Code	85745	Country	US

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

Correspondence Information:

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

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

PTO/SB/14 (11-08)
Approved for use through 09/30/2010. 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 contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SPEC US-4 (027560-0014)
	Application Number	
Title of Invention	EXCITATION LIGHT SOURCE ASSEMBLY	

Customer Number	27104		
Email Address	ip@fclaw.com	Add Email	Remove Email

Application Information:

Title of the Invention	EXCITATION LIGHT SOURCE ASSEMBLY		
Attorney Docket Number	SPEC US-4 (027560-0014)	Small Entity Status Claimed	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		Sub Class (if any)	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)	12	Suggested Figure for Publication (if any)	1

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). Enter either Customer Number or complete the Representative Name section below. If both sections are completed the Customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	27104		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	non provisional of	61379282	2010-09-01

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

PTO/SB/14 (11-08)
Approved for use through 09/30/2010. 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 contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SPEC US-4 (027560-0014)
	Application Number	
Title of Invention	EXCITATION LIGHT SOURCE ASSEMBLY	

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. 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(a).

Remove

Application Number	Country[i]	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
			○ Yes ◉ No

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Assignee Information:

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

Assignee 1

If the Assignee is an Organization check here. ☒

Organization Name	SPECTRAL INSTRUMENTS IMAGING, LLC		
Mailing Address Information:			
Address 1	420 North Bonita Avenue		
Address 2			
City	Tucson	**State/Province**	AZ
Country[i]	US	Postal Code	85745
Phone Number		Fax Number	
Email Address			

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

Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/Bruce E. Dahl/			Date (YYYY-MM-DD)	2011-08-31
First Name	Bruce	Last Name	Dahl	Registration Number	33670

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.**

EXCITATION LIGHT SOURCE ASSEMBLY

Cross-Reference to Related Application

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/379,282, filed on September 1, 2010, which is hereby incorporated herein by reference for all that it discloses.

Technical Field

[0002] This invention relates in general to systems for exciting fluorescent materials and more particularly to systems for exciting fluorescent materials in molecular imaging applications.

Background

[0003] Molecular imaging systems are known in the art and are commonly used to capture various types or modes of images from an object or specimen being analyzed. The objects or specimens that are imaged may comprise any of a wide range of compositions and objects, as is well-known. Primarily, such imaging systems are configured to extremely low levels of light emitted by the specimen or object under study. The light emitted by the object or specimen may be generated by a bio-luminescence process, a fluorescence process, or by a combination thereof. Such imaging systems may also be capable of capturing reflected light images, in which light reflected by the object is captured by the imaging system camera. Such a reflected light image is often combined with one or more emitted light images to form a single, composite image. Such a composite image allows a user to more easily correlate features and attributes of the emitted light image(s) with physical locations on the specimen or other characteristics that are contained in the reflected light image.

[0004] As is known, light emission by fluorescence results from the prior or simultaneous exposure of the fluorescent material to excitation light of suitable wavelength. However,

not all fluorescent materials fluoresce or emit light in response to excitation light of the same wavelength. Consequently, the wavelength of the particular excitation light must be selected so that it will excite the particular fluorescent material involved.

[0005] Because most molecular imaging systems seek to detect fluorescence from a wide range of fluorescent materials, most such imaging systems are provided with excitation light sources that can be operated to illuminate the fluorescent material with excitation light of the appropriate wavelength. Unfortunately, however, most excitation light sources tend to be expensive and/or difficult to implement in use, and systems are constantly being sought that improve on existing systems.

Summary of the Invention

[0006] An excitation light source assembly according to one embodiment of the invention may include a housing defining a central opening therein and a plurality of lamp receptacles surrounding the central opening. The housing is mountable to a support structure having a camera mounted thereto so that a field of view of the camera is substantially unobstructed by the housing. A light source is positioned within each of the plurality of lamp receptacles. A diffuser is positioned adjacent the light source in each of the plurality of lamp receptacles so that each of the diffusers diffuses light produced by each of the light sources. A control system operatively connected to each of the light sources operates selected ones of the light sources to provide a desired excitation illumination to an object within the field of view of the camera.

[0007] Also disclosed is an excitation light source assembly that includes a housing and a first connector portion mounted to the housing. A lamp receptacle produces light in a first defined wavelength band. A second connector portion is mounted to the lamp receptacle so that the lamp receptacle can be removably engaged with the first connector portion mounted to the housing.

The housing is mountable to a support structure so that a field of view of a camera also mounted to support structure is substantially unobstructed by the housing and by the lamp receptacle.

[0008] Another excitation light source assembly includes a base member that defines a central opening therein. A plurality of first connector portions are mounted to the base member at positions located around the central opening. The light source assembly also includes a plurality of lamp receptacles, at least some of which produce light in different wavelength bands. A second connector portion mounted to each of the plurality of lamp receptacles is releasably engagable with a corresponding one of the plurality of first connector portions mounted to the base member. The base member is mountable to a support structure so that the central opening is aligned with a camera mounted to the support structure and so that a field of view of the camera is substantially unobstructed by the base member and the lamp receptacles.

[0009] Also disclosed is an assembly that includes a support structure and a camera mounted to the support structure. An excitation light source assembly mounted to the support structure includes a housing that defines a central opening therein and a plurality of lamp receptacles that surround the central opening. The housing is mounted to the support structure so that the central opening is aligned with the camera and so that a field of view of the camera is substantially unobstructed by the housing. At least one narrow-band light source is positioned within at least one of the plurality of lamp receptacles. A diffuser is positioned adjacent the narrow-band light source. A control system operatively connected to each of the narrow-band light sources operates selected ones of the narrow-band light sources to provide a desired excitation illumination to a fluorescent material within the field of view of said camera.

Brief Description of the Drawings

[0010] Illustrative and presently preferred exemplary embodiments of the invention are shown in the drawings in which:

[0011] Figure 1 is a perspective view of an excitation light source assembly according to one embodiment of the present invention;

[0012] Figure 2 is a sectional view in perspective of a molecular imaging system having the excitation light source assembly of Figure 1 provided therein;

[0013] Figure 3 is a plan view of the support structure of the molecular imaging system of Figure 2 showing the relative positions of the excitation light source assembly and the lens assembly of the camera;

[0014] Figure 4 is an external view in perspective of the molecular imaging system of Figure 2;

[0015] Figure 5 is a sectional view in perspective of the excitation light source assembly taken along the plane 5-5 of Figure 1;

[0016] Figure 6 is a sectional view in perspective of the excitation light source taken along the plane 6-6 of Figure 1;

[0017] Figure 7 is fluorescent image of desert flora obtained with excitation light having a wavelength of about 465 nm and observed at a wavelength of about 550 nm;

[0018] Figure 8 is a fluorescent of the desert flora of Figure 7 but observed at a wavelength of about 735 nm;

[0019] Figure 9 is a perspective view of a second embodiment of an excitation light source assembly having a plurality of removable lamp modules;

[0020] Figure 10 is a sectional view in elevation of one of the plurality of removable lamp modules of the second embodiment of the excitation light source assembly of Figure 9;

[0021] Figure 11 is an exploded perspective view of one of the removable lamp modules of the second embodiment of the excitation light source assembly of Figure 9;

[0022] Figure 12 is a perspective view of a third embodiment of an excitation light source assembly; and

[0023] Figure 13 is a sectional view in elevation of the excitation light source assembly of Figure 12.

Detailed Description of the Preferred Embodiments

[0024] One embodiment of an excitation light source assembly 10 is shown in Figures 1-3 as it could be used in, or comprise a portion of, a molecular imaging system 12 (also shown in Figure 4) of the type commonly used in molecular imaging applications. Briefly, the excitation light source assembly 10 may comprise a housing or main body member 14 that defines a central opening 16 therein. The housing or main body member 14 may also define a plurality of lamp receptacles 18 that are arranged around the central opening 16, as best seen in Figures 1 and 3. The various lamp receptacles 18 are configured to receive various light sources or lamps 20. More specifically, and as will be described in greater detail below, the light sources or lamps 20 may comprise one or more narrow band light sources 22 and/or one or more broad-band light sources 24, as best seen in Figures 5 and 6. Generally speaking, the narrow band light sources 22 are used in fluorescent imaging applications, whereas the broad-band light sources 24 are used in reflected light applications (e.g., to capture a visible light image), although this need not necessarily be the case.

[0025] Some of the lamp receptacles 18 may be provided with one or more diffusers 26 and/or one or more filters 28, as best seen in Figure 5. The diffusers 26 are used to diffuse or spread light produced by the lamps 20, whereas the filters 28 are used to filter or remove light of unwanted or undesirable wavelengths. In a typical application, those lamp receptacles 18 that are provided with narrow band light sources 22 may also be provided with diffusers 26 and filters 28, whereas those receptacles 18 that are provided with broad-band light sources 24 will not be

provided with diffusers 26 and filters 28. Alternatively, other arrangements are possible, as will be described herein.

[0026] In an embodiment where in the excitation light source assembly 10 is to be used in conjunction with a molecular imaging system 12, it will be generally desirable to provide the excitation light source assembly 10 with the capability to generate or produce excitation light or illumination in a variety of individual bands or colors that extend over a comparatively large wavelength range (e.g., from about 430 nanometers (nm) to about 745 nm). However, other embodiments may extend this range to 800 nm or even 900 nm. In the particular embodiment shown and described herein, the excitation light source assembly 10 is capable of producing or generating ten (10) individual illumination bands or colors having comparatively narrow bandwidths (e.g., in the range of about 30-35 nm), although bandwidths having other ranges may also be used. The ten (10) individual illumination bands or colors (i.e., having relatively narrow bandwidths of about 30-35 nm) thus may cover the large wavelength range of from about 430 nm to about 745 nm. In another embodiment, the wavelength range may be extended to about 900 nm by providing one or more additional illumination bands or colors having wavelengths that extend to 900 nm.

[0027] In addition to the ability to provide illumination via one or more narrow illumination bands, the excitation light source assembly 10 may also be used to provide broad-band (e.g., white light) illumination, which may be desirable in certain imaging applications.

[0028] In the particular embodiment shown and described herein, each individual illumination band or color is generated or produced by light sources 22 provided in lamp receptacles 18 that are located on opposite sides of the central opening 16. As will be described in greater detail below, this arrangement provides for more even illumination than would otherwise be the case if the individual illumination band or color were provided by only a single lamp receptacle 18.

[0029] In an application wherein the excitation light source assembly 10 is used in conjunction with a molecular imaging system 12, the housing or main body member 14 of the excitation light source assembly 10 may be mounted to a base member 30 that, in turn, may be mounted to a suitable support structure 32 associated with the imaging system 12. See Figures 2 and 3. More particularly, the excitation light source assembly 10 is mounted to the support structure 32 so that the central opening 16 of main body 14 is substantially aligned with (i.e., concentric to) a lens assembly 34 of a camera 36 associated with the imaging system 12. The arrangement is such that a field of view (represented schematically by lines 38) of camera 36 is substantially unobstructed by the housing 14 and/or central opening 16 of housing 14 of excitation light source assembly 10.

[0030] The excitation light source assembly 10 may be operatively connected to a control system 40 (Figure 2). Control system 40 may be used to operate selected ones of the various light sources 20 (e.g., narrow-band light sources 22 and broad-band light sources 24) provided in the excitation light source assembly 10 to provide a desired illumination to an object or specimen 42 located within the field of view 38 of camera 36. Camera 36 may then capture an image of fluorescing material in the object or specimen 42. It should be noted that different fluorescent materials in the specimen 42 may be excited or activated by illuminating the specimen 42 with light of various colors or wavelength bands. In addition, different fluorescent materials in the specimen 42 may fluoresce or emit light of different wavelengths even when illuminated with light of the same color or wavelength band. Images of the specimen 42 produced by light emitted in such different wavelengths may be captured by using an appropriate filter in conjunction with the camera 36.

[0031] For example, and with reference now to Figures 7 and 8, a first fluorescent image 44 of a specimen 42, in this case a cactus pad and flowers, may be obtained by using the control

system 40 to activate those light sources 20 that emit light in an individual wavelength band or color that will excite the particular fluorescent material of interest in the specimen 42. In this particular example, the individual wavelength band or color comprised a middle or "center" wavelength of about 465 nm, with a bandwidth of about 30-35 nm. A 550 nm filter was used to capture the image 44 shown in Figure 7, which was then combined with a visible light image of the specimen 42. The emission at 550 nm reveals the fluorescence of the individual spines of the cactus. However, no fluorescence of the flower appears at this wavelength. In contrast, a second fluorescent image 46 of the specimen 42, depicted in Figure 8, but captured with a 735 nm filter, reveals emission from the leaves of the flowers as well as from the entire surface of the cactus pad.

[0032] A significant advantage of the excitation light source assembly of the present invention is that it may be used to illuminate an object or specimen with light in multiple narrow bands or colors, thereby allowing a single excitation light source assembly to be used in imaging applications involving a wide range of fluorescent materials that are excited over a wide range of wavelengths. Moreover, the excitation light of each individual wavelength band or color may be readily increased and decreased in brightness by controlling the number of light sources in each lamp receptacle that are illuminated or activated at any one time. Still further, the ability of the excitation light source assembly to also provide broad-band illumination allows for the ready capture of conventional reflected light images of the object or specimen, which are typically desired in most imaging applications.

[0033] Still other advantages are associated with the paring of the lamp receptacles on opposite sides of the central opening, in that such a paring allows for the more even illumination of the object or specimen than would otherwise be the case if the illumination were provided by only a single lamp receptacle.

[0034] Still yet other advantages are associated with embodiments having removable lamp receptacles. In such embodiments, the lamp receptacles can be readily removed and replaced in the field, thereby allowing users to conveniently and rapidly tailor the wavelength bands or colors that may be provided by the excitation light source assembly. The present invention also dispenses with the need to provide the illumination via fiber optic bundles, which is expensive and cumbersome. Moreover, such fiber optic bundles must also be carefully selected so that they do not contain materials or elements that themselves would fluoresce in use.

[0035] Having briefly described one embodiment of the excitation light source assembly of the present invention, as well as some of its more significant features and advantages, various exemplary embodiments of the excitation light source assembly will now be described in detail. However, before proceeding with the description, it should be noted that while the particular embodiments are shown and described herein as they could be used to provide illumination over certain wavelength ranges and in certain narrow illumination bands or colors having certain bandwidths, the particular wavelength ranges, numbers of illumination bands, as well as the bandwidths of the illumination bands may be varied depending on the any of a wide range of factors, including the requirements of the particular application. Consequently, the present invention should not be regarded as limited to the particular examples, ranges, wavelength bands, and applications shown and described herein.

[0036] Referring back now to Figures 1-4, an excitation light source assembly 10 according to one embodiment of the present invention is shown and described herein as it may be used in conjunction with a molecular imaging system 12 of the type commonly used in the molecular imaging field. In the particular embodiment shown and described herein, the imaging system 12 may comprise a generally rectangularly-shaped chassis or main enclosure 48 configured to house and support the various

components and subsystems required to perform various types of molecular imaging processes. In addition, the main enclosure 48 also may be provided with various external finish panels 50 that cover or overlay the underlying chassis or main enclosure structure 48. In the particular embodiment shown and described herein, the imaging system 12 is designed or configured to be connected to separate computer system (not shown) to allow a user to operate the imaging system 12 and view images produced by the imaging system 12 on a suitable display system (also not shown).

[0037] The main enclosure 48 of imaging system 12 may be provided with an access door 52 that can be moved vertically between a closed position (shown in Figure 4) and an opened position (not specifically illustrated in the drawing figures) to allow the user to access an imaging compartment or chamber 54 defined by the main enclosure 48, as best seen in Figure 2. The imaging compartment 54 is sized to receive one or more objects or specimens 42 to be imaged. Objects or specimens 42 suitable for use with the imaging system 12 include samples that may be provided in a well plate 56, as well as living organisms (not shown). Other types of objects or specimens 42 may be imaged for other purposes as well, as would become apparent to persons having ordinary skill in the art after having become familiar with the teachings provided herein.

[0038] Referring now primarily to Figure 2, the imaging system 12 also may include an imaging system sub-assembly 58 having an object platform or stage 60, as well as a mounting or support structure 32. The object platform 60 is moveably mounted to the sub-assembly 58 so that the object platform 60 can be moved vertically toward and away from the support structure 32, i.e., generally in the direction indicated by arrows 62. The support structure 32 is configured to receive camera 36, control system 40, as well as various other components and systems required for operation of the imaging system 12. However, because such various other components and systems that may be required or desired for the operation of the imaging system 12 are not

required to understand or practice the present invention, such additional components and systems will not be described in further detail herein.

[0039] The excitation light source assembly 10 may comprise a housing or main body 14 that defines a central opening 16 therein, as best seen in Figure 1. The housing or main body member 14 may be mounted to a base member 30 to allow the excitation light source assembly 10 to be readily mounted to the support structure 32 of the imaging system 12, as best seen in Figures 2 and 3. As mentioned above, the excitation light source assembly 10 is mounted to the support structure 32 so that the central opening 16 of main body 14 is substantially aligned with the lens assembly 34 of camera 36. The arrangement is such that the field of view 38 of camera 36 is substantially unobstructed by the housing 14 and/or central opening 16 of housing 14.

[0040] Referring now primarily to Figures 3, 5, and 6, the main body member 14 of excitation light source assembly 10 also defines a plurality of lamp receptacles 18 therein that are arranged around the central opening 16. Each of the lamp receptacles 18 is sized or configured to receive one or more light sources or lamps 20 (Figures 5 and 6). In particular, the large diameter lamp receptacles 18 may be sized to receive one or more narrow-band light sources 22, as best seen in Figure 5, whereas the small diameter lamp receptacles 18 may be sized to receive a single broad-band light source 24, as best seen in Figure 6.

[0041] The various lamp receptacles 18 may be provided at locations on the main body member 14 so as to provide substantially uniform illumination to the object or specimen 42 provided on the imaging platform or stage 60. In the particular embodiment shown and described herein, the main body member 14 is provided with twenty (20) large diameter lamp receptacles 18, each of which is provided with a plurality of narrow-band light sources 22 and, optionally, diffusers 26, and filters 28. Moreover, and as will be described in further detail below, a

given large diameter lamp receptacle 18 (such as receptacle 64), may be matched with a counterpart large diameter receptacle 18 (such as receptacle 64') on the opposite side of central opening 16. See Figure 1. Both the receptacle 64 and its counterpart receptacle 64' may be configured to emit light of the same color or wavelength range. Thus, when energized, each such receptacle 64 and counterpart receptacle 64' will emit light of the same color or wavelength range. Because each such receptacle 64 and counterpart receptacle 64' are located on opposite sides of the central opening 16, the specimen 42 will be more evenly illuminated with this arrangement than would otherwise be the case if the light were emitted only by a single receptacle 18.

[0042] As briefly described above, each of the small diameter receptacles 18 may be provided with a single broad-band light source 24. The various small diameter receptacles 18 also may be provided in spaced-apart relation around the central opening 16 of body member 14 in order to provide substantially uniform illumination of the object 42 provided on the stage 60 when the broad-band light sources 24 are energized.

[0043] The housing or main body member 14 may be fabricated from any of a wide range of materials, such as metals or plastics, that would be suitable for the intended application. Generally speaking, it will be desirable to use a material that will not fluoresce in response to the illumination provided by the various light sources 20 provided therein, as such fluorescence of the material comprising the main body member 14 will degrade the performance of the imaging system 12. By way of example, in one embodiment, the housing or main body member 14 is fabricated from a polyoxymethylene thermoplastic material, such as Delrin®. Alternatively, other materials may also be used, as would become apparent to persons having ordinary skill in the art after having become familiar with the teachings provided herein. Consequently, the present invention should not be regarded as limited to a main body member 14 that is fabricated from any particular material.

[0044] With reference now primarily to Figure 5, each of the large diameter lamp receptacles 18 defined by the housing 14 of the excitation light source assembly 10 is sized or configured to receive at least one, and preferably three (3) narrow-band light sources 22 (only two of which are shown in Figure 5). As will be described in greater detail below, the individual narrow-band light sources 22 may be individually activated by control system 40 to provide illumination of three (3) different intensity levels by activating various ones of the three narrow-band sources 22 provided to each lamp receptacle. In contrast, each of the small diameter lamp receptacles 18 may be sized to receive a single broad-band light source 24. However, other arrangements are possible, as would become apparent to persons having ordinary skill in the art after having become familiar with the teachings provided herein. Consequently, the present invention should not be regarded as limited to any particular configuration with respect to the number of light sources 20 that may be provided to each lamp receptacle 18.

[0045] The various light sources 20, e.g., comprising narrow-band light sources 22 and broad-band light sources 24, may be mounted to a circuit board 64 which, in turn, may be mounted or affixed to the main body 14, as best seen in Figures 1, 5, and 6. Circuit board 64 provides a means for physically supporting the various light sources 20 within the lamp receptacles 18 and for electrically connecting the light sources 20 to the control system 40. Circuit board 64 may also be provided with any of a wide range of ancillary systems and devices, such as light source drive circuits and connectors, to allow the light sources 20 to be activated by control system 40. However, because such devices and systems are well-known in the art and could be readily provided by persons having ordinary skill in the art after having become familiar with the teachings provided herein, the circuit board 64 that may be utilized in one embodiment of the present invention will not be described in further detail herein.

[0046] Each narrow-band light source 22 is suitable for producing excitation light having a wavelength band or color that comprises at least the wavelength or wavelengths required to excite or activate fluorescence in the particular material or specimen 42 to be imaged or studied. In addition, because it will be desirable to use the imaging system 12 to image a wide range of fluorescent materials, various ones of which may require excitation light having different colors or wavelength bands, in one embodiment, each of the larger diameter lamp receptacles 18 defined by the housing 14 will hold at least one, and preferably three (in one embodiment), narrow-band light source 22 that emits light having wavelengths (i.e., a wavelength band) that is slightly different from the wavelengths produced by the other narrow-band light sources 22 provided in others of the larger diameter lamp receptacles 18. Stated another way, the various lamp receptacles 18 may emit light of different colors. In this manner, then, the single housing 14 of excitation light source assembly 10 can be used to produce excitation light having wavelengths across any desired wavelength range.

[0047] In addition, and as was also mentioned above, each of the larger diameter lamp receptacles 18 (e.g., lamp receptacle 64, Figure 1) may be paired with a counterpart receptacle (e.g., lamp receptacle 64') on the opposite side of the central opening 16 in order to provide more even illumination of the specimen 42 provided on the imaging platform or stage 60. For example, if a particular lamp receptacle 64 is provided with a narrow-band light source 22 that is capable of emitting light having middle or center wavelength of about 465 nm, then its counterpart receptacle 64' should also be provided with a narrow-band light source 22 having a middle or center wavelength of about 465 nm.

[0048] In the particular embodiment shown and described herein, the excitation light source assembly 10 is capable of emitting excitation light in a plurality of individual bands or colors over a wavelength range from about 430 nm to about 745 nm. Therefore, it will be generally desirable to use for each light

source 20 a comparatively narrow-band light source 22 that emits light having wavelengths over a fairly narrow-band. By way of example, narrow-band light sources 22 having bandwidths in the range of about 30-35 nm can be used to advantage in the present invention. If such narrow-band light sources 22 are used, ten (10) such narrow-band light sources 22, properly selected, will be sufficient to cover the exemplary wavelength range. In accordance with this objective, then, the main body member 14 is provided with twenty (20) large diameter receptacles 18 that are arranged in ten pairs of two on opposite sides of the central opening 16. The corresponding receptacle pairs (e.g., 64 and 64', Figure 1) are then provided with narrow-band light sources 22 of the same wavelengths so that the excitation light source assembly 10 is capable of providing illumination in ten different narrow-band wavelength ranges or colors.

[0049] Alternatively, another embodiment of the invention may produce light having a wavelength of 800 nm by providing one or more light sources 22 capable of producing light having wavelengths of around 800 nm. In still another embodiment, the range could be extended to wavelengths of 900 nm or even longer by providing the excitation light source assembly 10 with light sources 22 capable of producing light having wavelengths of around 900 nm.

[0050] Each narrow-band light source 22 may comprise a light emitting diode (LED) that emits light in the desired wavelength band, and ideally, with the desired bandwidth (e.g., of about 30-35 nm). Light emitting diodes having such narrow bandwidths (e.g., about 30-35 nm) along the desired wavelength range (e.g., 430 nm to about 745 nm) are readily commercially available and can be used as the narrow-band sources 22. However, it should be noted that suitable LEDs that emit or produce light in each of the desired wavelength bands may not be available. If so, it may be necessary to use broader-band LEDs, i.e., that emit light having wavelengths outside the desired wavelength range. If so, such LEDs or broad-band light sources may be used in combination

with a filter element 28 to filter or remove the undesired wavelengths, as will be described in greater detail below.

[0051] Regardless of the particular light source 22 that may be used, it is also generally preferred, but not required, to provide a diffuser 26 within each lamp receptacle 18. the diffuser 26 may be provided at any convenient position within lamp receptacle 18. In one embodiment, diffuser 26 is located at a position immediately adjacent the light source 20, as best seen in Figure 5. A sleeve 66 provided in the lamp receptacle 18 may be used to support diffuser 26, although other arrangements are possible. As its name implies, the diffuser 26 diffuses or spreads light produced by the light source 20, thereby generally providing for more even illumination than would be provided by the light source 22 alone.

[0052] The diffuser 26 may comprise any of a wide range of optical diffusers that are now known in the art or that may be developed in the future that are or would be suitable for the particular application. However, because optical diffusers are well-known in the art and could be readily provided by persons having ordinary skill in the art after having become familiar with the teachings provided herein, the particular diffusers that may be used in one embodiment of the invention will not be described in further detail herein.

[0053] As mentioned above, the light output characteristics of the various light sources 20 (e.g., LEDs) that may be utilized in the excitation light source assembly 10 may be such that it may be required, or at least desirable, to further limit the wavelength range of excitation light produced by each light source 20. This may be the case regardless of whether the particular light source 20 involved is a narrow-band light source 22. Accordingly, in one embodiment, each of the large diameter lamp receptacles 18 (e.g., containing or housing the narrow-band light sources 22) may also be provided with a filter element 28. Filter element 28 may remove or filter undesired wavelengths produced by the light source 20, regardless of whether the light

source 20 comprises a narrow-band light source 22. In the particular embodiment shown and described herein, each such filter may be positioned adjacent the diffuser 26, as best seen in Figure 5, although other arrangements are possible.

[0054] In the particular embodiment shown and described herein, each filter element 28 may comprise a "notch" type filter having a relatively narrow wavelength bandpass range of about 30 nm around the desired center wavelength or color. Such notch type filters are available over a wide range of wavelengths or colors. In one embodiment of the invention, a separate notch filter 28 is provided for each light source 22, and the notch filter wavelength is selected as appropriate for the particular light source 22 with which it is paired. Thus, when individually activated, each light source/filter combination will emit excitation light having a bandwidth of about 30 nm at a desired color or wavelength band within the desired overall wavelength range (e.g., from about 430 nm to about 745 nm).

[0055] Referring now primarily to Figure 6, the excitation light source assembly 10 may also be provided with a plurality of small diameter lamp receptacles 18 therein that are sized to receive a broad-band light source 24. In the particular embodiment shown and described herein, each small diameter lamp receptacle 18 receives a single broad-band light source 24, although other embodiments may be provided with a plurality of broad-band light sources 24 for each receptacle 18. Broad-band light source 24 may comprise any of a wide range of broad-band light sources now known in the art or that may be developed in the future that are, or would be, suitable for the intended application. By way of example, in one embodiment, each broad-band light source 24 may comprise a "white" LED of the type that are readily commercially available. In another embodiment, one or more of the broad-band light sources 24 may be mounted at other locations within the imaging system 12.

[0056] Each of the various light sources 20, i.e., comprising narrow-band sources 22 and broad-band sources 24, provided in the

various lamp receptacles 18 defined by the housing 14 may be operatively connected to a control system 40 (Figure 2) via the circuit board 64. Control system 40 may be used to operate or energize the various light sources 20 to turn them on and off. In one embodiment, each of the various light sources 20 (i.e., comprising narrow-band sources 22 and broad-band sources 24) may be individually controlled so that a single light source 20, emitting excitation light in a desired wavelength band or color, may be used to excite the desired fluorescent material contained in the object or specimen 42 being studied. Alternatively, other arrangements are possible. For example, in another embodiment, the control system 40 may operate two or more light sources 20 simultaneously.

[0057] The excitation light source 10 may be operated as follows to capture a fluorescent image of a specimen 42, for example a cactus pad and flowers. Once the specimen 42 has been properly positioned on the platform or stage 60 and the imaging system 12 otherwise prepared for operation,, the control system 40 may operate selected ones of the various light sources 20 (e.g., narrow-band light sources 22 and broad-band light sources 24) to illuminate the specimen 42 with a desired wavelength band or color. Camera 36 may then capture an image of fluorescing material in the object or specimen 42. As described earlier, different fluorescent materials in the specimen 42 may be excited or activated by illuminating the specimen 42 with light of various colors or wavelength bands. In addition, different fluorescent materials in the specimen 42 may fluoresce or emit light of different wavelengths even when illuminated with light of the same color or wavelength band. Images of the specimen 42 produced by light emitted in such different wavelengths may be captured by using an appropriate filter in conjunction with the camera 36.

[0058] For example, and with reference now to Figures 7 and 8, a first fluorescent image 44 of specimen 42 may be obtained by using the control system 40 to activate those light sources

20 that emit light having a middle or center wavelength of about 465 nm (and with a bandwidth of about 30-35 nm). Certain materials contained within the specimen 42 will fluoresce in response to this excitation illumination. However, the materials may not all fluoresce or emit light of the same wavelength. Different materials will fluoresce or emit light of different wavelengths, as is known. The particular fluorescent image 44 depicted in Figure 7 was captured with a 550 nm filter adjacent the camera 36 and is shown combined with a visible light image of the specimen 42. The emission at 550 nm reveals the fluorescence of the individual spines of the cactus. However, no fluorescence of the flower appears at this wavelength. In contrast, the second fluorescent image 46 depicted in Figure 8, but captured with a 735 nm filter, reveals emission from the leaves of the flowers as well as from the entire surface of the cactus pad.

[0059] Other variations and configurations are possible for the excitation light source according to the present invention. For example, a second embodiment 110 of an excitation light source assembly according to the teachings of the present invention is illustrated in Figures 9-11. The second embodiment 110 differs from the first embodiment 10 in that the second embodiment 110 comprises a plurality of lamp receptacles 118 that are removable from corresponding main bodies 114. The removable lamp receptacles 118 allow the excitation light source 110 to be readily configured (and reconfigured) to produce light of any desired wavelength band or color by simply removing one or more of the lamp receptacles 118 and replacing it or them with another lamp receptacle 118 that configured to produce light in the desired wavelength band or color.

[0060] The second embodiment 110 may comprise a base member 130 to which are mounted a plurality of main bodies 114. In the particular embodiment illustrated in Figures 9-11, the base member 130 comprises a C-shaped member having a central opening 116. Alternatively, the base member 130 could comprise other

shapes and configurations, such as the circular shape of the first embodiment 110. The overall configuration of the C-shaped base member 130 is such that the central opening 116 thereof will be substantially aligned with the lens assembly 34 of camera 36 when mounted to the support structure 32 of imaging system 12. See Figures 2 and 3. As was the case for the first embodiment 10, the arrangement of the second embodiment 110 is such that the field of view 38 of camera 36 is substantially unobstructed by the central opening 116 of base member 130, as well as the various light receptacles 118 associated with excitation light source assembly 110.

[0061] With reference now primarily to Figure 10, each main body 114 may be substantially identical to the others and may comprise an opening 115 therein sized to receive the corresponding removable lamp receptacle 118. Main body 114 may also be configured to receive a connector assembly 117 that is sized to removably receive a mating portion 119 provided in the removable lamp receptacle 118. The connector assembly 117 and mating portion 119 provide physical and electrical engagement with the removable lamp receptacle 118. Connector assembly 117 may be electrically connected to an electrical connector assembly 165 provided in the main body 114. See also Figure 9.

[0062] In the particular embodiment shown and described herein, each main body 114 has an angled base portion 121 so that the each lamp receptacle 118 is angled inward, toward the central axis 151 of the housing 114, by an angle θ, as best seen in Figure 10 (central axis 151 is shown in a displaced position in Figure 10 for clarity). Such an arrangement allows the excitation light produced by the various lamp receptacles 118 to roughly converge at a point on the image platform or stage 60 where the specimen 42 is to be located during the imaging operation. Alternatively, other arrangements for aiming or directing the lamp receptacles 118, thus excitation light emitted thereby, could also be used, as would become apparent to persons

having ordinary skill in the art after having become familiar with the teachings provided herein.

[0063] The base member 130 and each of the main bodies 114 may be fabricated from any of a wide range of materials (e.g., metals and plastics) that would be suitable for the intended application. By way of example, in one embodiment the base member 130 may comprise aluminum. Each of the main bodies 114 may be fabricated from a polyoxymethylene thermoplastic material, such as Delrin®. Alternatively, other materials may also be used.

[0064] Connector assembly 117 and its mating portion 119 may comprise any of a wide range of connector assemblies that are now known in the art or that may be developed in the future that are, or would be, suitable for the particular application. However, by way of example, in one embodiment, connector assembly 117 and its mating portion 119 may comprise a type "FGG" connector assembly available from LEMO USA, Inc. of Rohnert Park, CA. Alternatively, other types of connectors may be used.

[0065] Referring now to Figures 10 and 11 simultaneously, each lamp receptacle 118 may be substantially identical to the others and may comprise first and second barrel sections 123 and 125 that may be connected or joined together by a threaded nipple or sleeve section 127. Sleeve section 127 is also configured to receive mating connector portion 119, as best seen in Figure 10. The upper barrel section 125 may be configured to receive one or more narrow-band light sources 122 (only a single narrow-band light source 122 is illustrated in Figures 10 and 11), which may be mounted to a circuit board 164 that is electrically connected to mating connector portion 119 via wires (not shown) or other suitable means. Barrel section 125 may also be configured to receive a diffuser 126 and/or a filter 128, as best seen in Figure 11. A sleeve 166 may be used to position the diffuser 126 and/or filter 128 with respect to the light source 122. A foam spacer 131 or other elastomeric material may be provided between circuit board 164 and sleeve 127, as best seen in Figure 10.

[0066] The upper and lower barrel sections 123 and 125 may be fabricated from any of a wide range of materials, such as metals or plastics, that would be suitable for the intended application. Consequently, the present invention should not be regarded as limited to any particular material. However, by way of example, in one embodiment, the upper and lower barrel sections 123 and 125 are fabricated from aluminum. The aluminum may be provided with a suitable non-reflective coating.

[0067] Still other variations are possible. For example, and with reference now to Figures 12 and 13, a third embodiment 210 of the excitation light source assembly may comprise a "C"-shaped housing or main body 214 that defines a central opening 216. The housing 214 also defines a plurality of lamp receptacles 218 that are arranged around the central opening, as best seen in Figure 12. The housing 214 of the excitation light source assembly 210 is also configured to be mounted to the support structure 32 of imaging system 12, so that the lens assembly 34 of camera 36 is generally concentric with the central opening 216 defined by the housing 214. See Figures 2 and 3. As was the case for the other embodiments, the arrangement is such that the field of view 38 of the camera 36 is substantially unobstructed by the housing 214 and/or central opening 216 of housing 214 of the excitation light source assembly 214.

[0068] Referring now to Figure 13, each of the lamp receptacles 218 may be provided narrow-band light source 222 that, in one embodiment, may be mounted to a circuit board 266. A diffuser 226 may also be positioned within each of the lamp receptacles 218, generally at a position adjacent the light source 222. Each diffuser 226 diffuses light produced by the corresponding light source 222 and may be held in position by a sleeve 266. As was the case for the other embodiments, each lamp receptacle 218 may also be provided with a filter 228, if desired or required, to remove or filter any undesired light wavelengths that may be produced by the light source 222.

[0069] The C-shaped housing 214 of the excitation light source assembly 210 allows each of the lamp receptacles 218 provided therein to be conveniently angled (i.e., by an angle θ with respect to the central axis 251 of housing 214), so that light from the light sources 222 may be directed to a desired area within the field of view 38 of camera 36.

[0070] The housing 214 for the excitation light source assembly 210 may be fabricated from any of a wide range of materials, such as metals or plastics, that would be suitable for the intended application. Consequently, the present invention should not be regarded as limited to housings made from any particular materials. However, by way of example, in one embodiment, the housing is fabricated as a single piece from any of a polyoxymethylene thermoplastic material, e.g., Delrin®.

[0071] Having herein set forth preferred embodiments of the present invention, it is anticipated that suitable modifications can be made thereto which will nonetheless remain within the scope of the invention. The invention shall therefore only be construed in accordance with the following claims:

CLAIMS:

1. An excitation light source assembly, comprising:

a housing defining a central opening therein and a plurality of lamp receptacles surrounding the central opening, said housing being mountable to a support structure so that the central opening is aligned with a camera also mounted to the support structure so that a field of view of the camera is unobstructed by said housing;

a light source positioned within each of the plurality of lamp receptacles; and

a diffuser positioned adjacent said light source positioned within each of the plurality of lamp receptacles, each of said diffusers diffusing light produced by each of said light sources; and

a control system operatively connected to each of said light sources positioned within said plurality of lamp receptacles, said control system operating selected ones of said light sources to provide a desired excitation illumination to an object within the field of view of said camera.

2. The excitation light source assembly of claim 1 wherein at least one of said light sources comprises a narrow-band light source that emits light having a bandwidth of about 35 nm.

3. The excitation light source assembly of claim 2 wherein said at least one narrow-band light source comprises a light emitting diode.

4. The excitation light source assembly of claim 1, further comprising at least one filter positioned adjacent at least one diffuser in at least one of the lamp receptacles defined by said housing.

5. The excitation light source assembly of claim 4, wherein said at least one filter comprises a notch filter having a wavelength bandpass range of about 30 nm.

6. The excitation light source assembly of claim 1, wherein at least one of the light sources comprises a broad-band light source.

7. The excitation light source assembly of claim 1, wherein at least some of said plurality of lamp receptacles comprise opposed pairs of lamp receptacles on opposite sides of said central opening, each opposed pair of lamp receptacles producing light of the same color.

8. The excitation light source assembly of claim 7, further comprising ten opposed pairs of lamp receptacles on opposite sides of said central opening.

9. An excitation light source assembly, comprising:

a housing;

a first connector portion mounted to said housing;

a lamp receptacle for producing light in a first defined wavelength band; and

a second connector portion mounted to said lamp receptacle so that said lamp receptacle can be removably engaged with said first connector portion mounted to said housing, said housing being mountable to a support structure so that a field of view of a camera also mounted to support structure is substantially unobstructed by said housing and by said lamp receptacle.

10. The excitation light source assembly of claim 9, wherein said lamp receptacle further comprises:

a first barrel section adapted to receive said second connector portion; and

a second barrel section; and

a light source mounted within said second barrel section.

11. The excitation light source assembly of claim 10, wherein said first and second connector portions comprise respective mating electrical connector sections, and wherein said light source is electrically connected to the electrical connector section of said second connector portion.

12. The excitation light source assembly of claim 10, further comprising a diffuser mounted within said second barrel section at a position adjacent said light source.

13. The excitation light source assembly of claim 10, further comprising a filter mounted within said second barrel section at a position adjacent said light source.

14. The excitation light source assembly of claim 10, wherein said housing comprises a main body portion and a base member portion.

15. The excitation light source assembly of claim 10, wherein said housing comprises a C-shaped member having a central aperture therein, said central aperture of said C-shaped member being substantially aligned with a lens assembly of the camera, so that the field of view of the camera is substantially unobstructed by said housing and said lamp receptacle.

16. An excitation light source assembly, comprising:

a base member defining a central opening therein;

a plurality of first connector portions mounted to said base member at positions around the central opening defined by said base member;

a plurality of lamp receptacles, at least some of said plurality of lamp receptacles producing light in different wavelength bands;

a second connector portion mounted to each of said plurality of lamp receptacles, said second connector portion being releasably engagable with a corresponding one of said plurality of first connector portions mounted to said base member, said base member being mountable to a support structure so that the central opening is aligned with a camera that is also mounted to the support structure, wherein a field of view of the camera is substantially unobstructed by said base member and said lamp receptacles engaged with said base member.

17. The excitation light source assembly of claim 16, further comprising a plurality of main body members, each of said main body members being mounted to said base member, each main body member being adapted to mount a corresponding one of said plurality of first connector portions, each main body member having an opening therein sized to receive a corresponding one of said plurality of lamp receptacles.

18. The excitation light source assembly of claim 16, wherein said base member comprises a C-shaped configuration.

19. An assembly, comprising:

a support structure;

a camera mounted to said support structure;

an excitation light source assembly mounted to said support structure, said excitation light source assembly comprising:

a housing defining a central opening therein and a plurality of lamp receptacles surrounding the central opening, said housing being mounted to said support structure so that the central opening is

aligned with said camera and so that a field of view of said camera is substantially unobstructed by said housing;

at least one narrow-band light source positioned within at least one of the plurality of lamp receptacles; and

a diffuser positioned adjacent said narrow-band light source positioned within each of the plurality of lamp receptacles, each of said diffusers diffusing light produced by each of said narrow-band light sources; and

a control system operatively connected to each of said narrow-band light sources positioned within said plurality of lamp receptacles, said control system operating selected ones of said narrow-band light sources to provide a desired excitation illumination to a fluorescent material within the field of view of said camera.

20. The assembly of claim 19, further comprising a light filter positioned adjacent each of the diffusers.

21. The assembly of claim 19, wherein each of the lamp receptacles defined by said housing are angled with respect to a central axis of said housing so that light emitted from each of said narrow-band light sources is directed to a desired area within the field of view of said camera.

22. The assembly of claim 19, wherein each of said narrow-band light sources is selected to provide desired number of illumination bands over a desired spectral range.

23. The assembly of claim 22, wherein the desired spectral range is from about 430 nm to about 745 nm.

24. The assembly of claim 22, wherein the desired number of illumination bands is 10.

25. The assembly of claim 22, wherein each desired illumination band has a bandwidth ranging from about 30 nm to about 35 nm.

26. The assembly of claim 19, wherein each of said narrow-band light sources comprises a light emitting diode.

27. The assembly of claim 19, wherein said housing comprises a polyoxymethylene thermoplastic material.

28. The assembly of claim 22, wherein the desired spectral range is from about 430 nm to about 900 nm.

29. The assembly of claim 22, wherein the desired spectral range is from about 430 nm to about 800 nm.

ABSTRACT OF THE DISCLOSURE

An excitation light source assembly includes a housing defining a central opening therein and a plurality of lamp receptacles surrounding the central opening. The housing is mountable to a support structure having a camera mounted thereto so that a field of view of the camera is substantially unobstructed by the housing. A light source is positioned within each of the plurality of lamp receptacles. A diffuser is positioned adjacent the light source in each of the plurality of lamp receptacles so that each of the diffusers diffuses light produced by each of the light sources. A control system operatively connected to each of the light sources operates selected ones of the light sources to provide a desired excitation illumination to an object within the field of view of the camera.

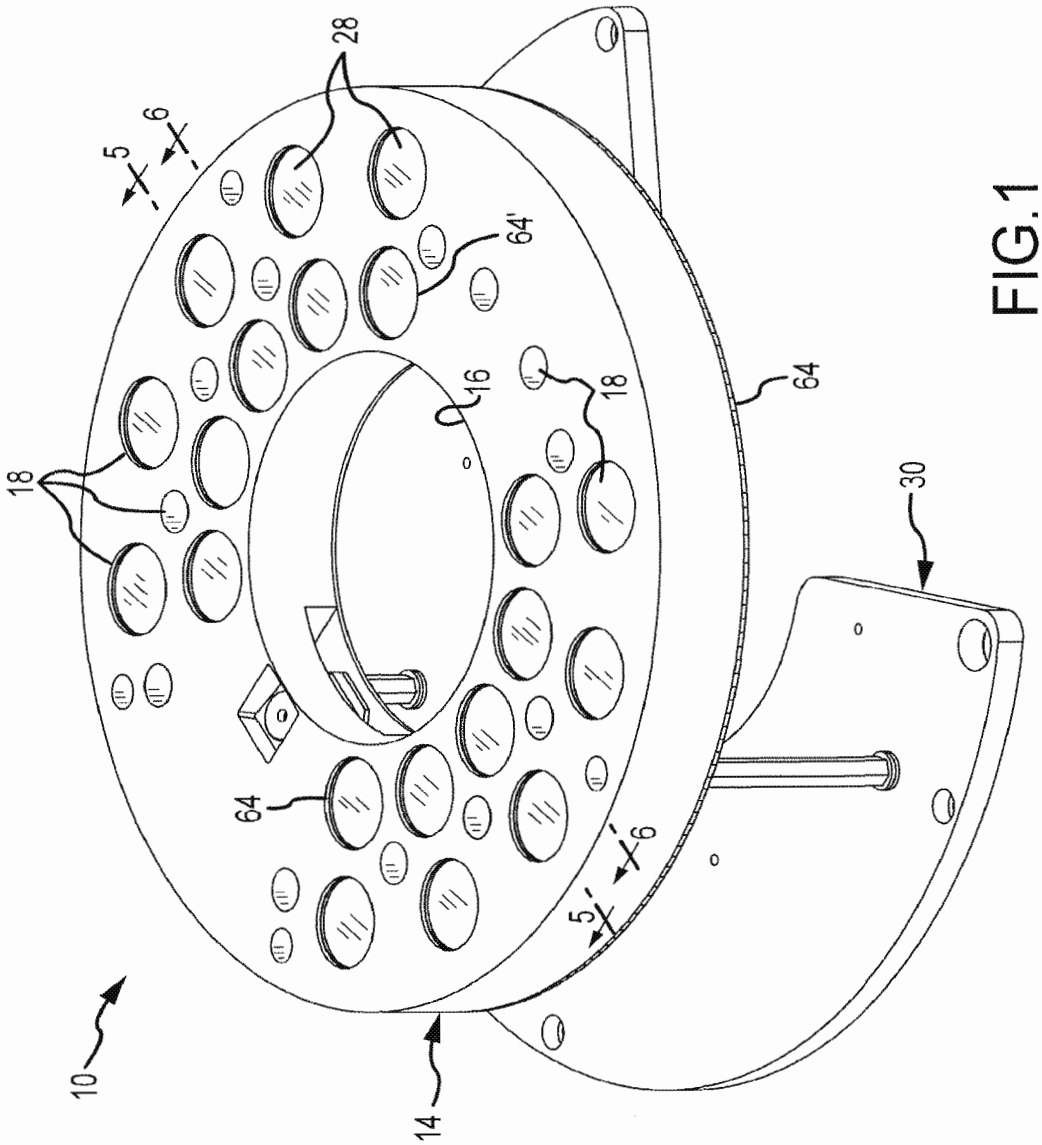


FIG.1

SPECTRAL_0000056

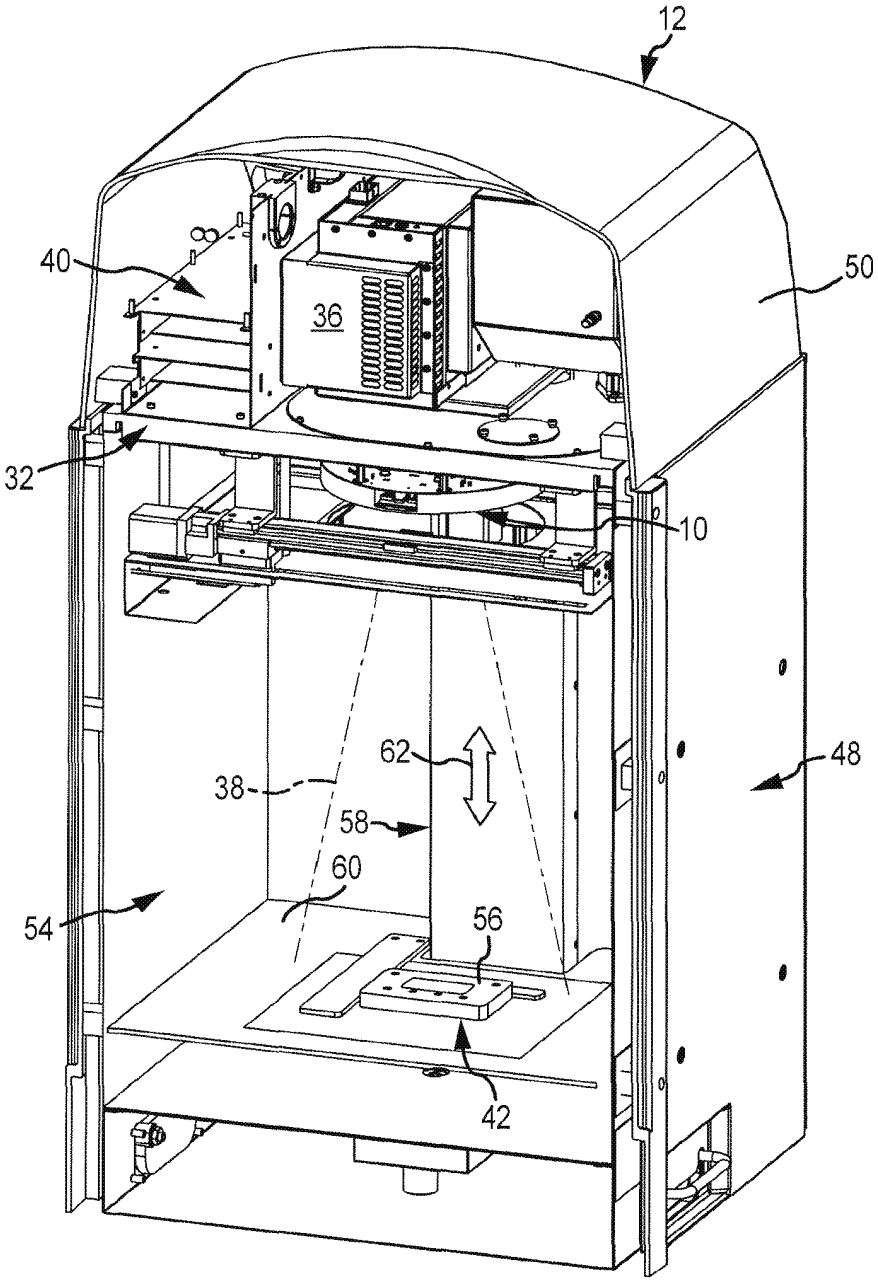


FIG.2

SPECTRAL_0000057

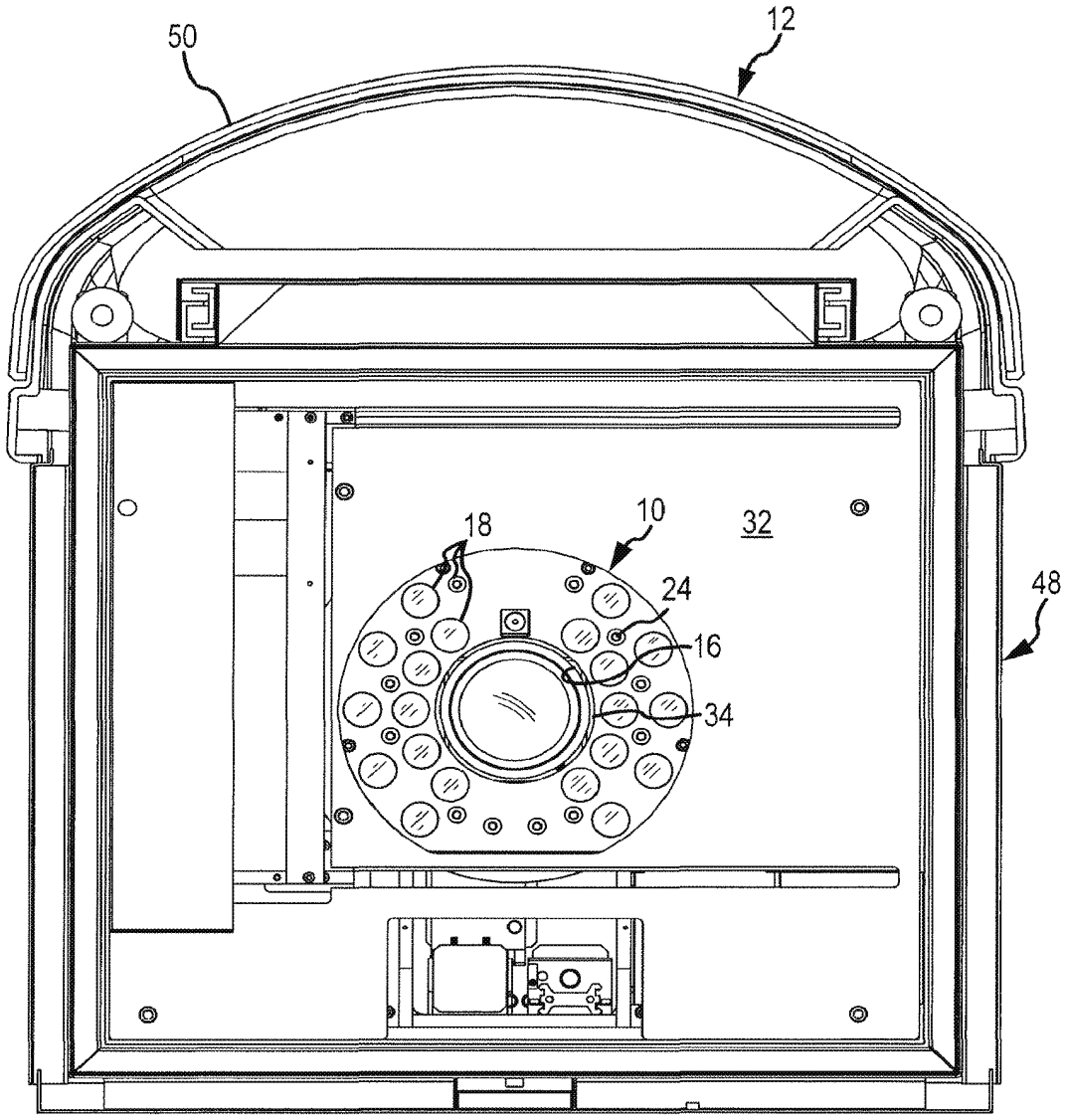


FIG.3

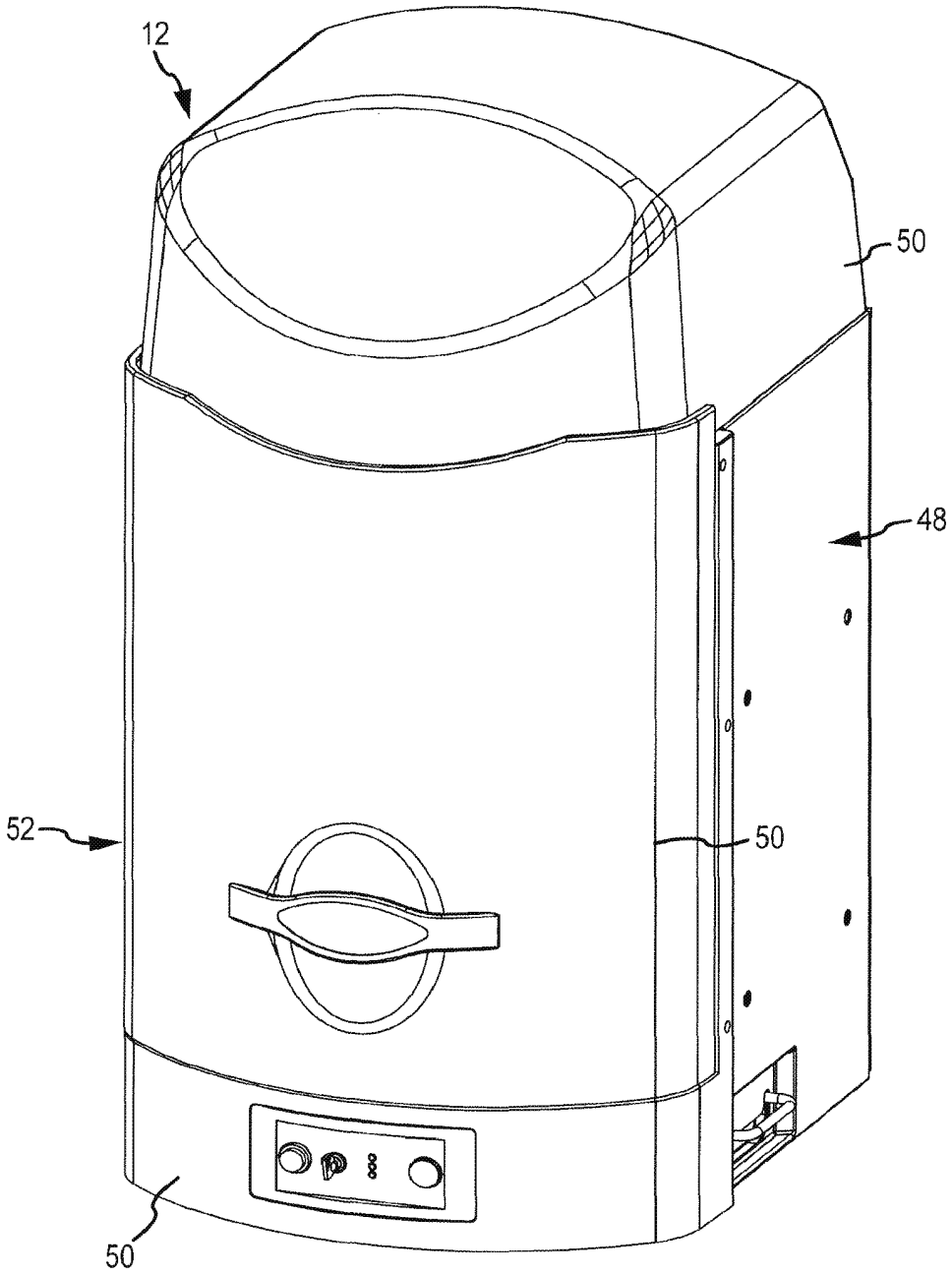


FIG.4

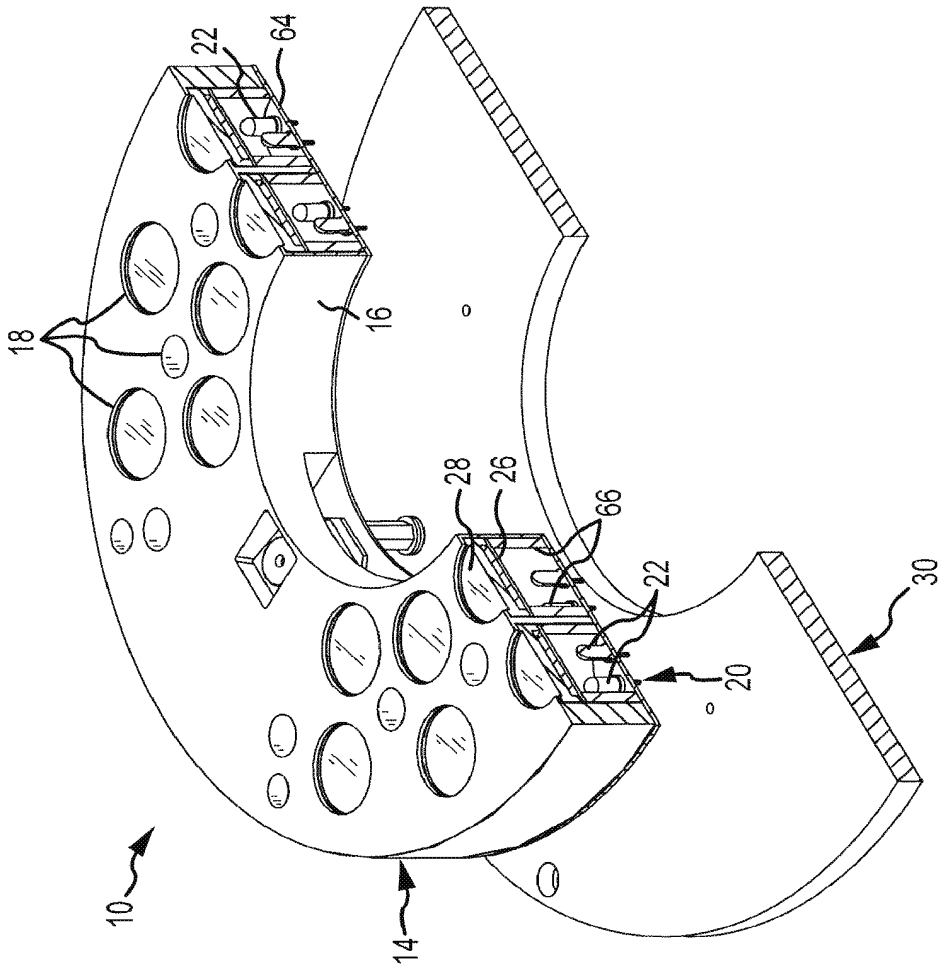


FIG.5

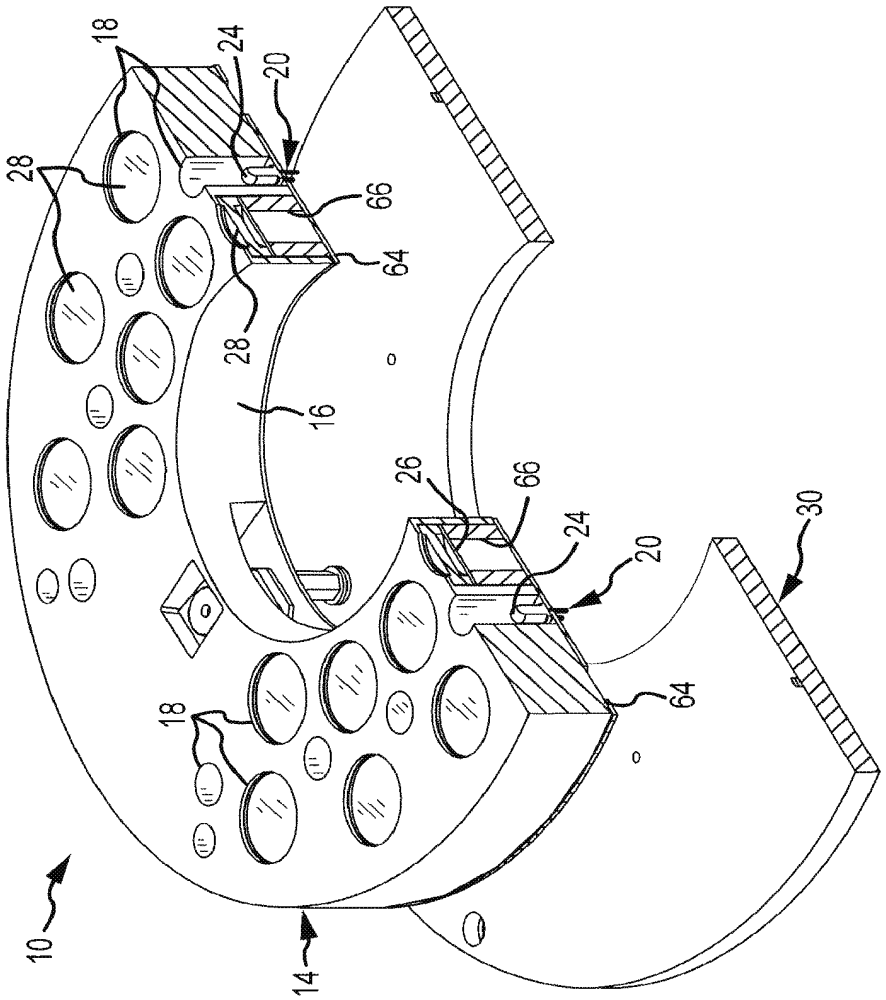


FIG.6

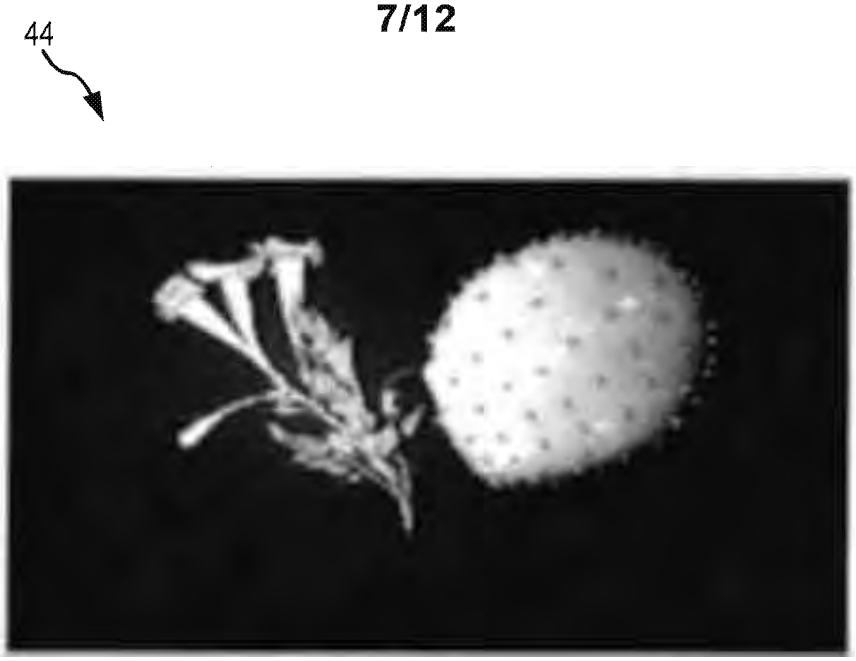


FIG.7

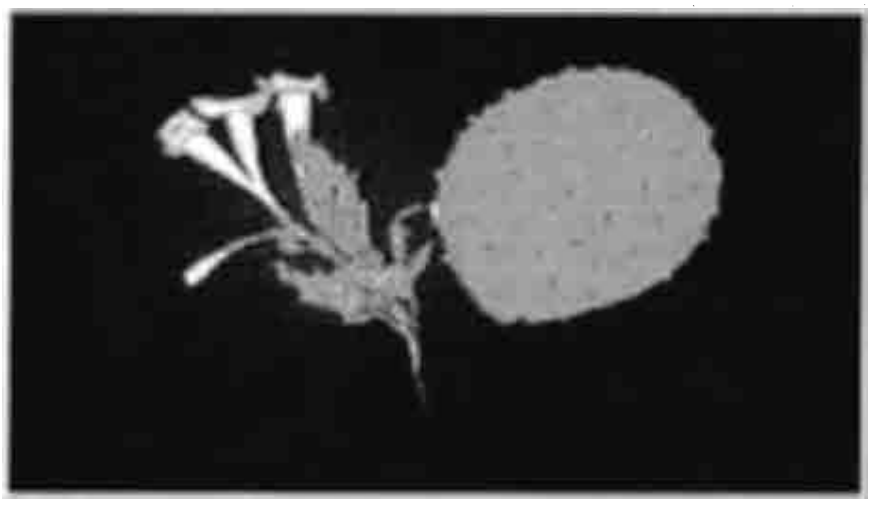


FIG.8

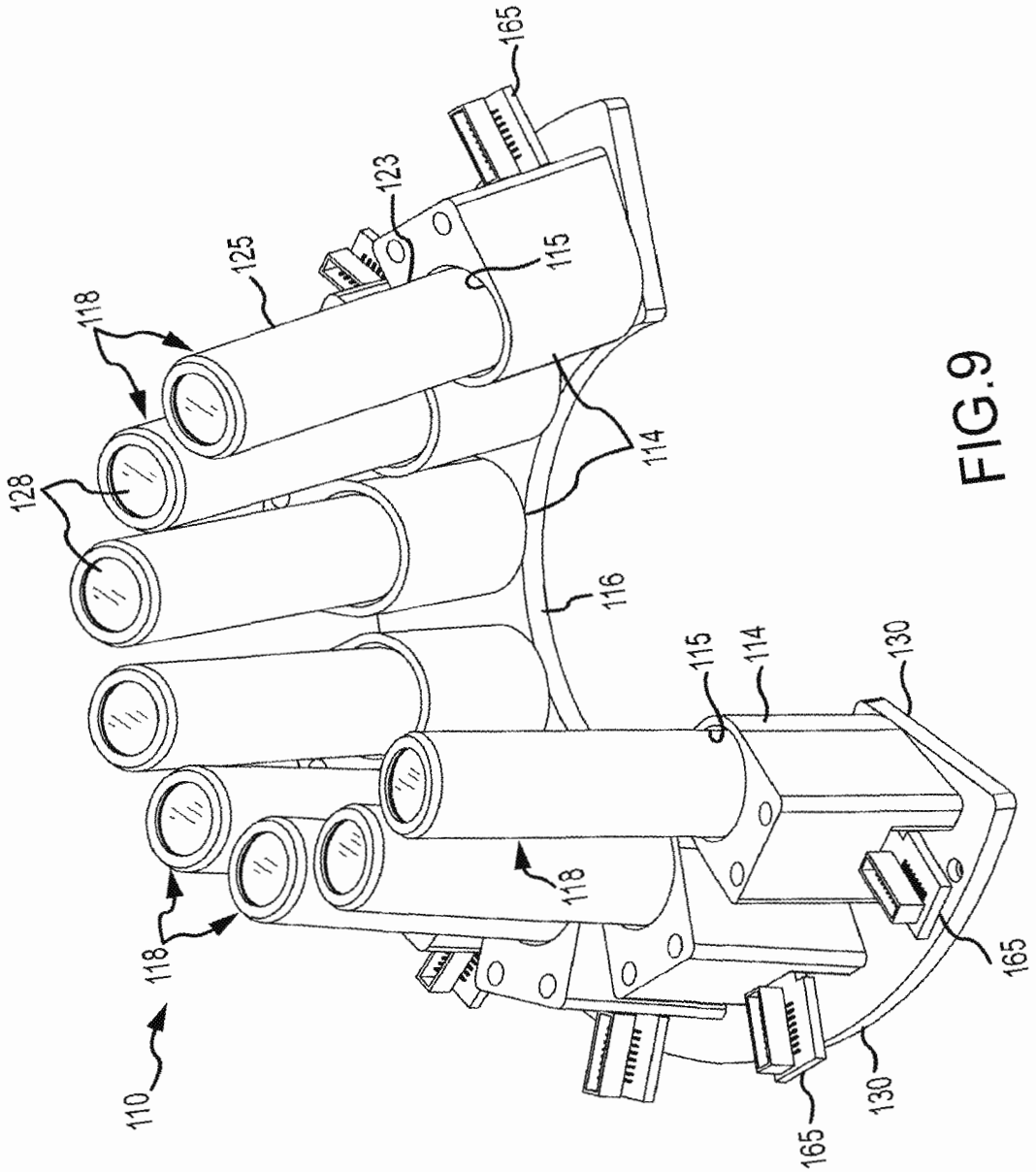


FIG.9

SPECTRAL_0

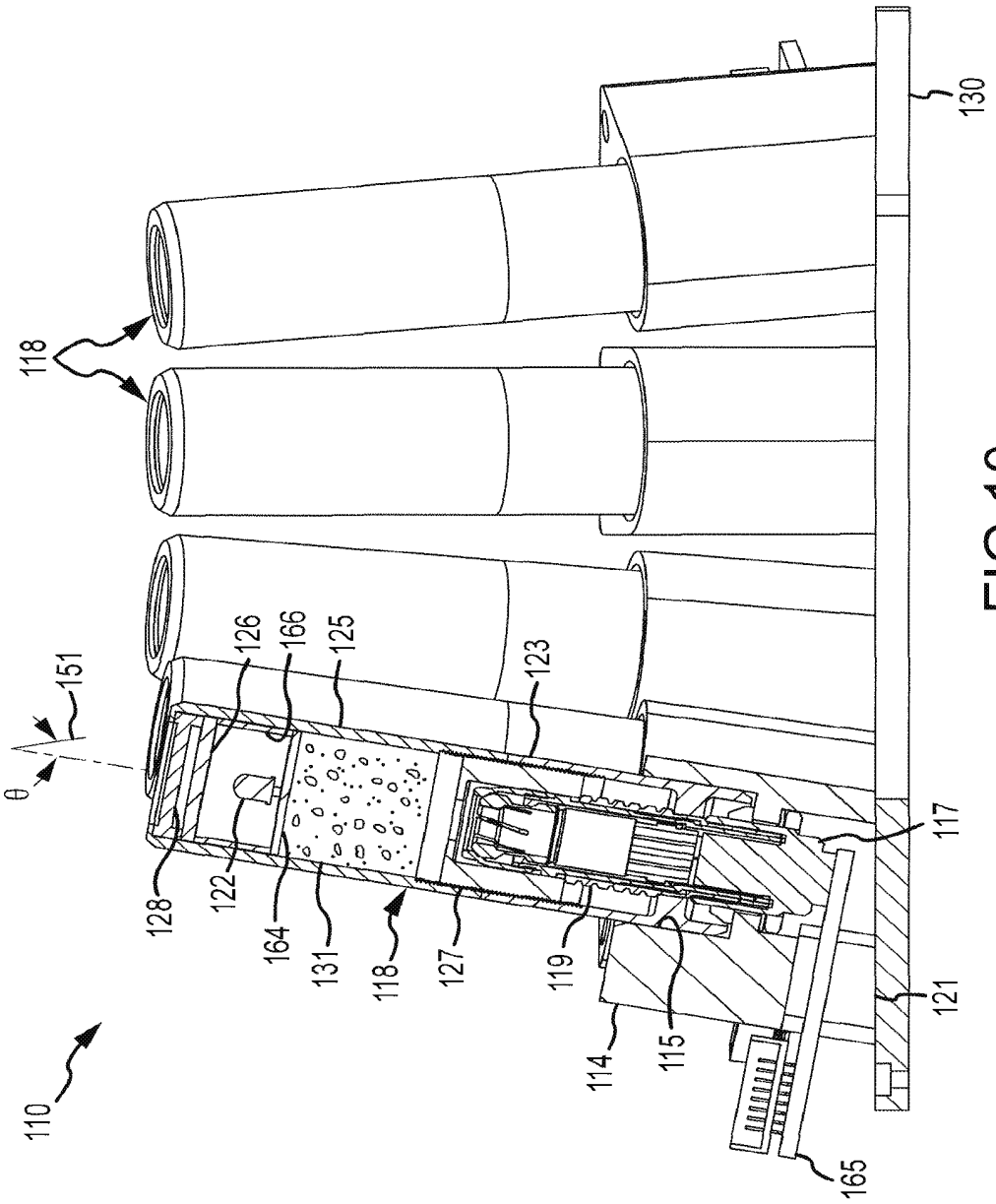

110
θ
151
118
130
126
166
125
123
128
122
164
131
118
127
119
114
115
117
121
165

FIG.10

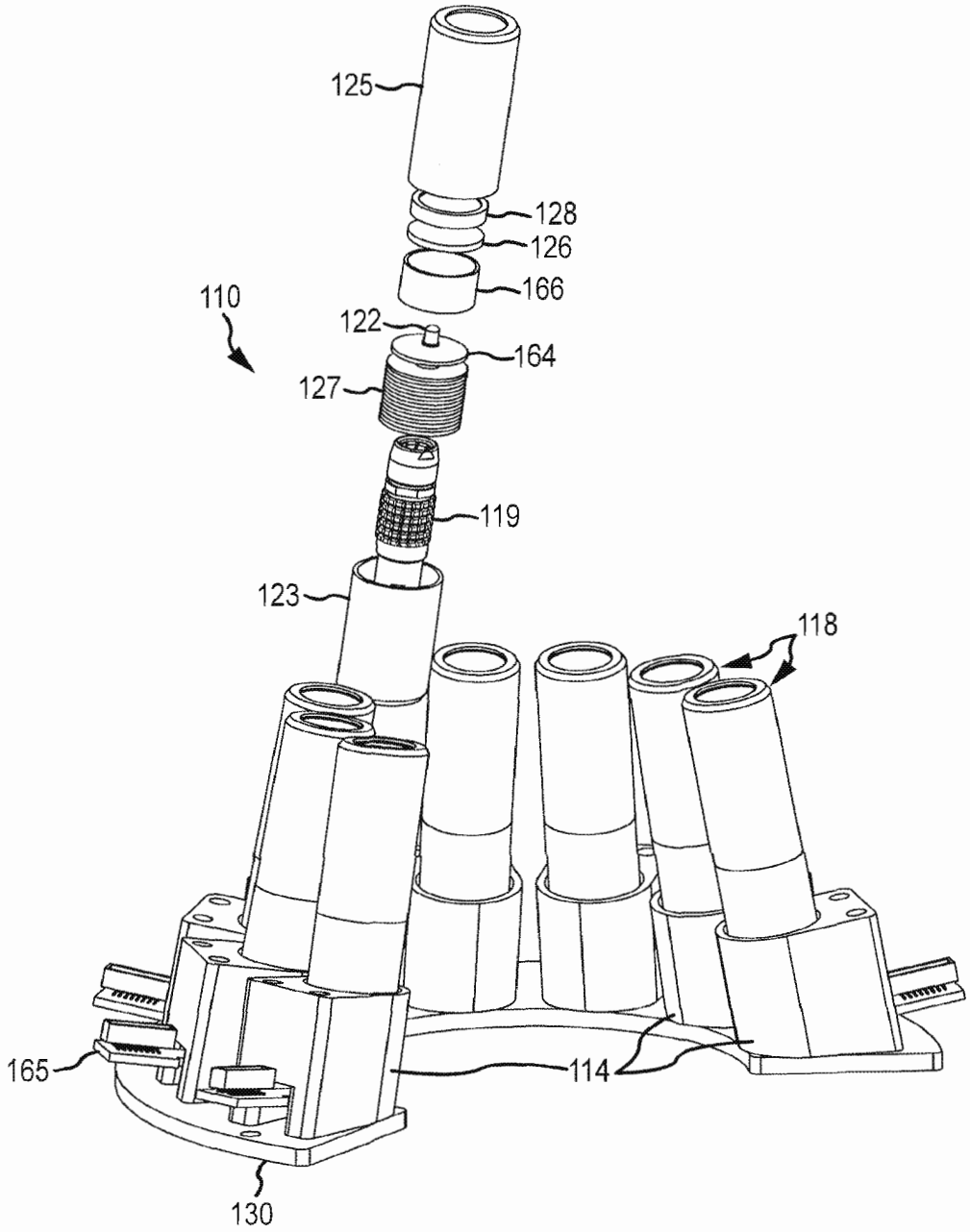


FIG.11

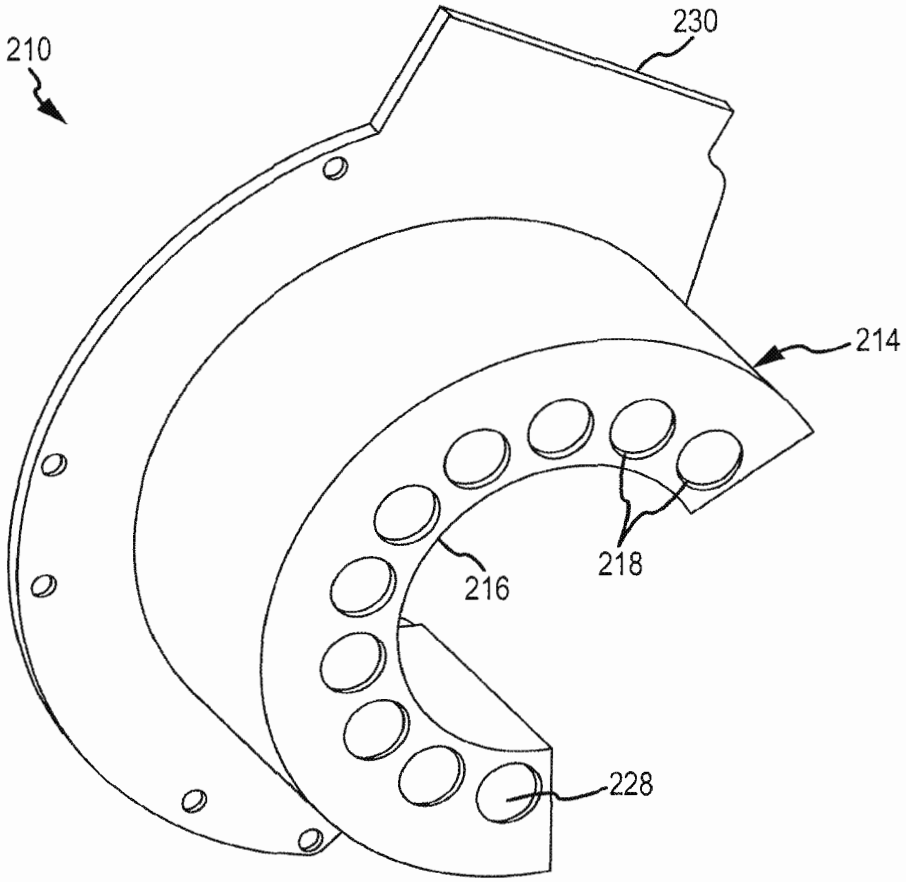


FIG.12

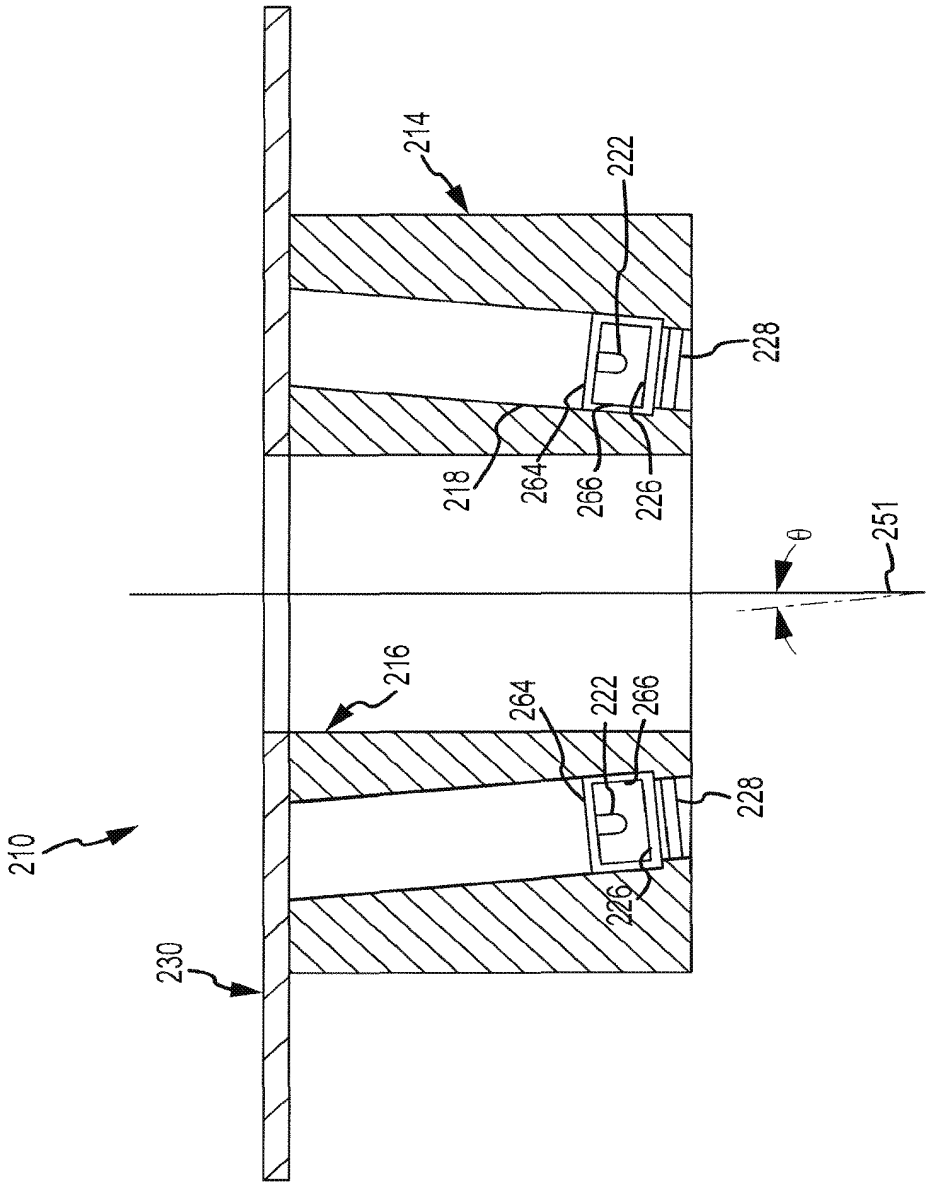


FIG.13

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	EXCITATION LIGHT SOURCE ASSEMBLY
First Named Inventor/Applicant Name:	Michael B. Nelson
Filer:	Bruce E. Dahl/Cynthia Sullivan
Attorney Docket Number:	SPEC US-4 (027560-0014)

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility filing Fee (Electronic filing)	4011	1	82	82
Utility Search Fee	2111	1	270	270
Utility Examination Fee	2311	1	110	110
Pages:				
Claims:				
Claims in excess of 20	2202	9	26	234
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	696

SPECTRAL_0000069

Electronic Acknowledgement Receipt	
EFS ID:	10855419
Application Number:	13222688
International Application Number:	
Confirmation Number:	5910
Title of Invention:	EXCITATION LIGHT SOURCE ASSEMBLY
First Named Inventor/Applicant Name:	Michael B. Nelson
Customer Number:	27104
Filer:	Bruce E. Dahl/Cynthia Sullivan
Filer Authorized By:	Bruce E. Dahl
Attorney Docket Number:	SPEC US-4 (027560-0014)
Receipt Date:	31-AUG-2011
Filing Date:	
Time Stamp:	16:36:10
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$696
RAM confirmation Number	14665
Deposit Account	060590
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees) Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees) Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		SPEC-US-4.pdf	462560 1214de5248adf3d33732fa14eb524c262d0f2847	yes	45

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Application Data Sheet	1	3
	Specification	4	26
	Claims	27	32
	Abstract	33	33
	Drawings-other than black and white line drawings	34	45

Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	36866 9994f7c83ac74cb0bdc3353463f456e8cdc52372	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	499426	

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.

SCORE Placeholder Sheet for IFW Content

Application Number: 13222688 Document Date: 8/31/2011

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawings

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions developed by SIRA.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface using the Supplemental Content tab.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces using the Supplemental Content tab.

Form Revision Date: May 1, 2009

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/222,688

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	82		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	270		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	110		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	29 minus 20 =	* 9	x 26 =	234	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x 110 =	110			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 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).			0.00			
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	806		TOTAL	

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	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
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.

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	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/222,688	08/31/2011	2828	806	SPEC US-4 (027560-0014)	29	4

CONFIRMATION NO. 5910

27104
FENNEMORE CRAIG, P.C.
1700 Lincoln Street
SUITE 2900
DENVER, CO 80203

FILING RECEIPT

OC000000049828647

Date Mailed: 09/15/2011

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**

Applicant(s)

Michael B. Nelson, Tucson, AZ;
Michael D. Cable, Tucson, AZ;

Assignment For Published Patent Application

SPECTRAL INSTRUMENTS IMAGING, LLC, Tucson, AZ

Power of Attorney: None

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/379,282 09/01/2010

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)

If Required, Foreign Filing License Granted: 09/14/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/222,688**

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No
** **SMALL ENTITY** **

Title

EXCITATION LIGHT SOURCE ASSEMBLY

Preliminary Class

372

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 (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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
13/222,688	08/31/2011	Michael B. Nelson	SPEC US-4 (027560-0014)

CONFIRMATION NO. 5910

27104
FENNEMORE CRAIG, P.C.
1700 Lincoln Street
SUITE 2900
DENVER, CO 80203

FORMALITIES LETTER

OC000000049828648

Date Mailed: 09/15/2011

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The oath or declaration is missing.
  A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
  Note: If a petition under 37 CFR 1.47 is being filed, an oath or declaration in compliance with 37 CFR 1.63 signed by all available joint inventors, or if no inventor is available by a party with sufficient proprietary interest, is required.

The applicant needs to satisfy supplemental fees problems indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- A surcharge (for late submission of filing fee, search fee, examination fee or oath or declaration) as set forth in 37 CFR 1.16(f) of $**65** for a small entity in compliance with 37 CFR 1.27, must be submitted.

SUMMARY OF FEES DUE:

Total fee(s) required within **TWO MONTHS** from the date of this Notice is $**65** for a small entity
- $**65** Surcharge.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/tle/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Electronic Acknowledgement Receipt

EFS ID:	10855419
Application Number:	13222688
International Application Number:	
Confirmation Number:	5910
Title of Invention:	EXCITATION LIGHT SOURCE ASSEMBLY
First Named Inventor/Applicant Name:	Michael B. Nelson
Customer Number:	27104
Filer:	Bruce E. Dahl/Cynthia Sullivan
Filer Authorized By:	Bruce E. Dahl
Attorney Docket Number:	SPEC US-4 (027560-0014)
Receipt Date:	31-AUG-2011
Filing Date:	
Time Stamp:	16:36:10
Application Type:	Utility under 35 USC 111(a)

09/13/2011 VVAN11 00000003 060590 13222688
01 FC:2201 110.00 DA

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$696
RAM confirmation Number	14665
Deposit Account	060590
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: Michael B. Nelson et al.	)	Confirmation No.: 5910
Application No.: 13/222,688	)	Examiner: Not yet assigned
Filing Date: August 31, 2011	)	Group Art Unit: 2828
For: EXCITATION LIGHT SOURCE ASSEMBLY	)	Atty. Docket No.: SPEC US-4 (027560-0014)

RESPONSE TO NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION FILED UNDER 37 CFR 1.53(b)

Dear Sir:

In response to the Notice to File Missing Parts of Nonprovisional Application under 37 CFR 1.53(b) mailed on September 15, 2011, for the above-identified patent application, Applicant hereby submits the executed Declaration in compliance with 37 CFR 1.63.

The $65.00 surcharge fee is also being submitted with this filing. The Commissioner is hereby authorized to credit or debit Deposit Account 06-0590 if there is an overpayment or shortage.

Respectfully submitted,

FENNEMORE CRAIG, P.C.

Bruce E. Dahl
Registration No. 33,670
Telephone: (303) 291-3200
Customer No. 27104

Date: November 2, 2011

DEN/25513

PTO/SB/01A (01-09)
Approved for use through 09/30/2010. 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.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	EXCITATION LIGHT SOURCE ASSEMBLY

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☐ The attached application, or

☑ Application No. 13/222,688 filed on August 31, 2011

☐ As amended on _____ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

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.

All statements made herein of my/our own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: Michael B. Nelson Date: 10/5/11

Signature: _____ Citizen of: USA

Inventor two: Michael D. Cable Date: 10/20/11

Signature: _____ Citizen of: USA

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

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.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Patent Application Fee Transmittal

Application Number:	13222688
Filing Date:	31-Aug-2011
Title of Invention:	EXCITATION LIGHT SOURCE ASSEMBLY
First Named Inventor/Applicant Name:	Michael B. Nelson
Filer:	Bruce E. Dahl/Cynthia Sullivan
Attorney Docket Number:	SPEC US-4 (027560-0014)

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Late filing fee for oath or declaration	2051	1	65	65
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**65**

SPECTRAL_0000084

Electronic Acknowledgement Receipt

EFS ID:	11323917
Application Number:	13222688
International Application Number:	
Confirmation Number:	5910
Title of Invention:	EXCITATION LIGHT SOURCE ASSEMBLY
First Named Inventor/Applicant Name:	Michael B. Nelson
Customer Number:	27104
Filer:	Bruce E. Dahl/Cynthia Sullivan
Filer Authorized By:	Bruce E. Dahl
Attorney Docket Number:	SPEC US-4 (027560-0014)
Receipt Date:	02-NOV-2011
Filing Date:	31-AUG-2011
Time Stamp:	18:48:04
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$65
RAM confirmation Number	4980
Deposit Account	060590
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees) Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees) Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		SPEC-4-POA-3-73b.pdf	194934 cc15a89881be2f671985b2267e8a6a1216362bbe	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Power of Attorney	1	1
	Assignee showing of ownership per 37 CFR 3.73(b).	2	8

Warnings:
Information:

2		SPEC-4-Declaration.pdf	57781 e0358a927b2c9ef9a35d54d997dabcd25d95866c	yes	2

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Applicant Response to Pre-Exam Formalities Notice	1	1
	Oath or Declaration filed	2	2

Warnings:
Information:

3	Fee Worksheet (SB06)	fee-info.pdf	30344 4ef627cef5c78732b15997c8bd25e04bc5f56a95	no	2

Warnings:
Information:

Total Files Size (in bytes):	283059

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.

PTO/SB/81 (01-09)
Approved for use through 11/30/2011. OMB 0651-0035
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.

POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Application Number	13/222,688
Filing Date	August 31, 2011
First Named Inventor	Michael B. Nelson
Title	Excitation Light Source Assembly
Art Unit	Not yet known
Examiner Name	Not yet known
Attorney Docket Number	SPEC US-4 (027560.0014)

I hereby revoke all previous powers of attorney given in the above-identified application.

☐ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

27104

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified application to:

☒ The address associated with the above-mentioned Customer Number.

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☐ Applicant/Inventor.

OR

☒ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____.

SIGNATURE of Applicant or Assignee of Record

Signature		Date	10/24/11
Name	Keith G. Copeland	Telephone	(820) 884-8821
Title and Company	CEO		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.31, 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.

PTO/SB/96 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
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.

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Spectral Instruments Imaging, LLC

Application No./Patent No.: 13/222,688 Filed/Issue Date: August 31, 2011

Titled: EXCITATION LIGHT SOURCE ASSEMBLY

Spectral Instruments Imaging, LLC , a corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.

states that it is:

1. ☒ the assignee of the entire right, title, and interest in;

2. ☐ an assignee of less than the entire right, title, and interest in (The extent (by percentage) of its ownership interest is _____ %); or

3. ☐ the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made) the patent application/patent identified above, by virtue of either:

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 027156, Frame 0495, or for which a copy therefore is attached.

OR

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☐ As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Signature — Date: 10/24/11

Keith G. Copeland — Printed or Typed Name

CEO — Title

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. 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.

UNITED STATES PATENT AND TRADEMARK OFFICE
UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

NOVEMBER 1, 2011

PTAS

FENNEMORE CRAIG, P.C.
1700 LINCOLN STREET
SUITE 2900
DENVER, CO 80203

501710008

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT RECORDATION BRANCH OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE ASSIGNMENT RECORDATION BRANCH AT 571-272-3350. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, MAIL STOP: ASSIGNMENT RECORDATION BRANCH, P.O. BOX 1450, ALEXANDRIA, VA 22313.

RECORDATION DATE: 11/01/2011 REEL/FRAME: 027156/0495
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

DOCKET NUMBER: SPEC US-4 (027560.0014)

ASSIGNOR:
NELSON, MICHAEL B. DOC DATE: 10/05/2011

ASSIGNOR:
CABLE, MICHAEL D. DOC DATE: 10/20/2011

ASSIGNEE:
SPECTRAL INSTRUMENTS IMAGING, LLC
420 BONITA AVENUE
TUCSON, ARIZONA 85745

APPLICATION NUMBER: 13222688 FILING DATE: 08/31/2011
PATENT NUMBER: ISSUE DATE:
TITLE: EXCITATION LIGHT SOURCE ASSEMBLY

ASSIGNMENT RECORDATION BRANCH
PUBLIC RECORDS DIVISION

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
Michael B. Nelson	10/05/2011
Michael D. Cable	10/20/2011

RECEIVING PARTY DATA

Name:	Spectral Instruments Imaging, LLC
Street Address:	420 Bonita Avenue
City:	Tucson
State/Country:	ARIZONA
Postal Code:	85745

PROPERTY NUMBERS Total: 1

Property Type	Number
Application Number:	13222688

CORRESPONDENCE DATA

Fax Number: (303)291-3201
Phone: 303-291-3200
Email: ip@fclaw.com
Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent via US Mail.
Correspondent Name: Fennemore Craig, P.C.
Address Line 1: 1700 Lincoln Street
Address Line 2: Suite 2900
Address Line 4: Denver, COLORADO 80203

ATTORNEY DOCKET NUMBER:	SPEC US-4 (027560.0014)

NAME OF SUBMITTER:	Bruce E. Dahl
Signature:	/Bruce E. Dahl/
Date:	11/01/2011

Total Attachments: 3
source=Assignment-SPECUS-4#page1.tif
source=Assignment-SPECUS-4#page2.tif
source=Assignment-SPECUS-4#page3.tif

RECEIPT INFORMATION

EPAS ID:	PAT1741354
Receipt Date:	11/01/2011
Fee Amount:	$40

ASSIGNMENT OF PATENT APPLICATION

WHEREAS, Michael B. Nelson and Michael D. Cable; hereinafter referred to as "Assignors," are the inventors of the invention described and set forth in the below-identified application for United States Letters Patent:

Title of Invention: EXCITATION LIGHT SOURCE ASSEMBLY

Filing Date: August 31, 2011

Application Serial No.: 13/222,688

WHEREAS, Spectral Instruments Imaging, LLC, United States of America, hereinafter referred to as "Assignee," is desirous of acquiring an interest in the invention and application and in any U.S. Letters Patent and Registrations which may be granted on the same;

For good and valuable consideration, receipt of which is hereby acknowledged by Assignors, Assignors have assigned, and by these presents does assign to Assignee all right, title, and interest in and to the invention and application and to all foreign counterparts (including patent, utility model, and industrial designs), and in and to any Letters Patent and Registrations which may hereafter be granted on the same in the United States and all countries throughout the world, and to claim the priority from the application as provided by the Paris Convention. Assignors also acknowledge that Assignors' assignment to Assignee may be transferred to any successors or assignees of Assignee, without Assignors' further consent. Assignors further acknowledge that Assignors' assignment including the entire right, title, and interest in Assignors' Letters Patent, including the right of priority in, to, and under Assignors' application for Letters Patent, the invention(s) and improvement(s) set forth therein, and any and all continuations, continuations-in-part (CIPs), divisionals, renewals, substitutions, conversions, reissues, prolongations, or extensions thereof.

Assignors additionally authorize Assignee to file applications in Assignors' names for Letters Patent in any country, to be held and enjoyed by Assignee, its successors, assigns, nominees or legal representatives, to the full end of the term or terms for which said Letters Patent respectively may be granted, reissued or extended, as fully and entirely as the same would have been held and enjoyed by Assignors had this sale, assignment, and transfer not been made.

Assignors also covenant that Assignors have full right to convey the entire interest herein assigned, and that Assignors have not executed and will not execute any agreement in conflict herewith. Assignors further covenant and agree that Assignors will, each time a request is made, and without undue delay, execute and deliver all such papers as may be necessary or desirable to perfect the title to said invention(s), improvement(s), application(s) and Letters Patent, to Assignee, its successors, assigns, nominees, or legal representatives. Assignors further agree to communicate to Assignee, or to its nominee,

all known facts respecting said invention(s), improvement(s), application(s) and said Letters Patent, to testify in any legal proceedings, to sign all lawful papers, to execute all disclaimers and divisionals, continuations, CIPs, reissues, and foreign applications, to make all rightful oaths and declarations, and generally to do everything possible to aid Assignee, its successors, assigns, nominees and legal representatives to obtain and enforce, for its or their own benefit, proper patent protection for said invention(s) or improvement(s) in any and all countries, provided the expenses which may be incurred by Assignors in lending such cooperation and assistance are paid by Assignee.

Assignors further authorize and direct the attorneys of Fennemore Craig, P.C. to insert the serial number and filing date of said application identified as set forth above as soon as the same shall have been made known to them by the United States Patent and Trademark Office.

Assignors further authorize and request the Commissioner of Patents and Trademarks of the United States, and any international or foreign official whose duty is to issue patents on applications as aforesaid, to issue to Assignee, as assignee of the entire right, title, and interest, any and all Letters Patent for said invention(s) or improvement(s), including any and all Letters Patent of the United States which may be issued and granted on or as a result of my application for Letters Patent, in accordance with the terms of this assignment.

IN WITNESS of the above representations, Assignors execute this agreement below.

_____ Date: 10/5/11
Michael B. Nelson

STATE OF Arizona)
)ss
COUNTY OF Pima)

Before me this 5th day of October, 2011, personally appeared before me Michael B. Nelson, known to me to be the individual who executed the foregoing instrument, and who acknowledged to me that he executed the same of his own free will and for the purposes set forth herein.

My commission expires: July 5, 2013

[SEAL] OFFICIAL SEAL
HANS J. MEYER
Notary Public - State of Arizona
PIMA COUNTY
My Comm. Expires July 5, 2013

_____
Notary Public

Michael D. Cable

Date: 10/20/11

STATE OF California)
)ss
COUNTY OF Alameda)

Before me this 20th day of October, 2011, personally appeared before me Michael D. Cable, known to me to be the individual who executed the foregoing instrument, and who acknowledged to me that he executed the same of his own free will and for the purposes set forth herein.

My commission expires: 08/16/2015

[SEAL]

Notary Public

SHILPA DHIR
COMM. # 1948055
NOTARY PUBLIC • CALIFORNIA
ALAMEDA COUNTY
My Comm Expires AUG 16, 2015

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	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/222,688	08/31/2011	2828	871	SPEC US-4 (027560-0014)	29	4

CONFIRMATION NO. 5910

UPDATED FILING RECEIPT

27104
FENNEMORE CRAIG, P.C.
1700 Lincoln Street
SUITE 2900
DENVER, CO 80203

OC000000050859456

Date Mailed: 11/10/2011

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**

Applicant(s)

Michael B. Nelson, Tucson, AZ;
Michael D. Cable, Tucson, AZ;

Assignment For Published Patent Application

SPECTRAL INSTRUMENTS IMAGING, LLC, Tucson, AZ

Power of Attorney: The patent practitioners associated with Customer Number 27104

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/379,282 09/01/2010

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)

If Required, Foreign Filing License Granted: 09/14/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/222,688**

Projected Publication Date: 03/01/2012

Non-Publication Request: No

Early Publication Request: No
** **SMALL ENTITY** **

Title

EXCITATION LIGHT SOURCE ASSEMBLY

Preliminary Class

372

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 (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage, facilitate, and accelerate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/222,688

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	95		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	310		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	125		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	29 minus 20 =	* 9	x 30 =	270	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x 125 =	125			
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).			0.00			
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	925		TOTAL	

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	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
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.

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
13/222,688	08/31/2011	Michael B. Nelson	SPEC US-4 (027560-0014)

CONFIRMATION NO. 5910

27104
FENNEMORE CRAIG, P.C.
1700 Lincoln Street
SUITE 2900
DENVER, CO 80203

POA ACCEPTANCE LETTER

OC000000050859428

Date Mailed: 11/10/2011

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 11/02/2011.

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.

/mbayou/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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 contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	13222688
Filing Date	2011-08-31
First Named Inventor	Michael B. Nelson
Art Unit	2828
Examiner Name	Not yet known
Attorney Docket Number	SPEC US-4 (027560.0014)

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	3800079		1974-03-26	McNeil et al.	
	2	3871767		1975-03-18	Holm-Hansen et al.	
	3	3949162		1976-04-06	Malueg	
	4	4196994		1980-04-08	de Jesus et al.	
	5	4298887		1981-11-03	Rode	
	6	4343021		1982-08-03	Frame	
	7	4585934		1986-04-29	French et al.	
	8	4593728		1986-06-10	Whitehead et al.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

	9	4687325		1987-08-18	Corby, Jr.	
	10	4863690		1989-09-05	Berthold et al.	
	11	4885544		1989-12-05	Tago	
	12	4948975		1990-08-14	Erwin et al.	
	13	5008548		1991-04-16	Gat	
	14	5039868		1991-08-13	Kobayashi et al.	
	15	5202091		1993-04-13	Lisenbee	
	16	5401465		1995-03-28	Smethers et al.	
	17	5414258		1995-05-09	Liang	
	18	5515161		1996-05-07	Blumenfeld	
	19	5542012		1996-07-30	Fernandes et al.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

	20	5587583		1996-12-24	Chin et al.	
	21	5672881		1997-09-30	Striepeke et al.	
	22	5680492		1997-10-21	Hopler et al.	
	23	5689110		1997-11-18	Dietz et al.	
	24	5818977		1998-10-06	Tansley	
	25	5840572		1998-11-24	Copeland et al.	
	26	5865754		1999-02-02	Sevick-Muraca et al.	
	27	5898802		1999-04-27	Chen et al.	
	28	5964220		1999-10-12	Boussignac et al.	
	29	5986271		1999-11-16	Lazarev et al.	
	30	6004767		1999-12-21	Crouch et al.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

	31	6038038		2000-03-14	Selby et al.	
	32	6097025		2000-08-01	Modlin et al.	
	33	6154277		2000-11-28	Snelling et al.	
	34	6205244	B1	2001-03-20	Bawolek et al.	
	35	6217847	B1	2001-04-17	Contag et al.	
	36	6377353	B1	2002-04-23	Ellis	
	37	6614452	B1	2003-09-02	Cable	
	38	6642499	B1	2003-11-04	Boni et al.	
	39	6754008	B1	2004-06-22	Wallerstein et al.	
	40	6775567	B2	2004-08-10	Cable et al.	
	41	6894289	B2	2005-05-17	Nilson et al.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

	42	6901279	B2	2005-05-31	Cable et al.	
	43	6919919	B2	2005-07-19	Nelson et al.	
	44	6922246	B2	2005-07-26	Nilson et al.	
	45	7113217	B2	2006-09-26	Nilson et al.	
	46	7116354	B2	2006-10-03	Rice et al.	
	47	7177024	B2	2007-02-13	Nilson et al.	
	48	7190991	B2	2007-03-13	Cable et al.	
	49	7196190	B2	2007-03-27	Ning et al.	
	50	7255851	B2	2007-08-14	Contag et al.	
	51	7298415	B2	2007-11-20	Nilson et al.	
	52	7299420	B2	2007-11-20	Cable	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

	53	7331341	B2	2008-02-19	Nelson	
	54	7383078	B2	2008-06-03	Cable et al.	
	55	7403812	B2	2008-07-22	Rice et al.	
	56	7449567	B2	2008-11-11	Zhang et al.	
	57	7449615	B2	2008-11-11	Contag et al.	
	58	7461652	B2	2008-12-09	Dalgetty et al.	
	59	7464707	B2	2008-12-16	Dalgetty et al.	
	60	7466418	B2	2008-12-16	Nilson et al.	
	61	7474398	B2	2009-01-06	Nilson et al.	
	62	7474399	B2	2009-01-06	Nilson et al.	
	63	7503323	B2	2009-03-17	Dalgetty et al.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

	64	7555332	B2	2009-06-30	Rice et al.	
	65	7555334	B2	2009-06-30	Coquoz et al.	
	66	7581191	B2	2009-08-25	Rice et al.	
	67	7589786	B2	2009-09-15	Nilson et al.	
	68	7595838	B2	2009-09-29	Nilson et al.	
	69	7599731	B2	2009-10-06	Rice et al.	
	70	7603167	B2	2009-10-13	Stearns et al.	
	71	7616985	B2	2009-11-10	Stearns et al.	
	72	7663664	B2	2010-02-16	Rice et al.	

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

	1					

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] ¡	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	0097060	EP	A2	1983-12-28	Electronic Pneumatic Apparatus & Controls Ltd.		☐
	2	0228877	EP	A2	1987-07-15	Artel, Inc.		☐
	3	0491131	EP	A1	1992-06-24	Hewlett-Packard Company		☐
	4	0493707	EP	A2	1992-07-08	DiPietro, Matthew, c/o Eastman Kodak Company		☐
	5	0718622	EP	A2	1996-06-26	Biosensor Laboratories Co., Ltd.		☐
	6	1478423	EP	B1	2006-07-26	Dalgetty et al.		☐
	7	1478916	EP	B1	2008-07-09	Nilson et al.		☐
	8	0049938	WO	A1	2000-08-31	Micro Medical Devices, Inc.		☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

	9	0161324	WO	A1	2001-08-23	The University of Chicago		☐
	10	0163247	WO	A2	2001-08-30	Xenogen Corporation		☐
	11	9908233	WO	A1	1999-02-18	Imaging Research Inc.		☐
	12	9400742	WO	A1	1994-01-06	Holmes et al.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	O'KANE et al., "Absolute Calibration of Luminometers with Low-Level Light Standards," Methods in Enzymology, Vol. 305, Copyright 2000 by Academic Press, pages 87-96.	☐
	2	BROWN et al., "Absolute Radiometric Calibration of Digital Imaging Systems," IS&T/SPIE Electronic Imaging, San Jose, California, January 2001, 9 pages.	☐
	3	CHEN et al., "Auaomated Calibration of a Zoom Lens CCD Image System for Videogrammetry," International Archives of Photogrammetry and Remote Sensing, Vol. XXXIII, Part B4, Amsterdam, The Netherlands, 2000, pages 180-185.	☐
	4	CAMPBELL et al., "Bioluminescence and Chemiluminescence," Proceedings of the 8th International Symposium of Bioluminescence and Chemiluminescence, Cambridge, September 1994, John Wiley & Sons, 5 pages.	☐
	5	CONTAG et al., "Bioluminescent indicators in living mammals," Nature Medicine, Vol. 4, No. 2, February 1998, http://www.nature.com/naturemedicine, pages 245-247.	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

	6	OCHS et al., "Camera Types for Low Level Light Imaging," October 1995, pages 1-6.	☐
	7	GATAN, INC., "CCD Image Acquisition Tutorial," Gatan, Inc., Pleasanton, California, May 2001, cover page and pages 1-7.	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13222688
	Filing Date	2011-08-31
	First Named Inventor	Michael B. Nelson
	Art Unit	2828
	Examiner Name	Not yet known
	Attorney Docket Number	SPEC US-4 (027560.0014)

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Bruce E. Dahl/	Date (YYYY-MM-DD)	2011-11-11
Name/Print	Bruce E. Dahl	Registration Number	33670

This collection of information is required by 37 CFR 1.97 and 1.98. 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 1 hour 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.**

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 record s.

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.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 097 060**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: **83303471.3**

(22) Date of filing: **15.06.83**

(51) Int. Cl.³: **A 61 M 16/00**

(30) Priority: **15.06.82 GB 8217354**

(43) Date of publication of application: **28.12.83 Bulletin 83/52**

(84) Designated Contracting States: **DE FR GB IT NL SE**

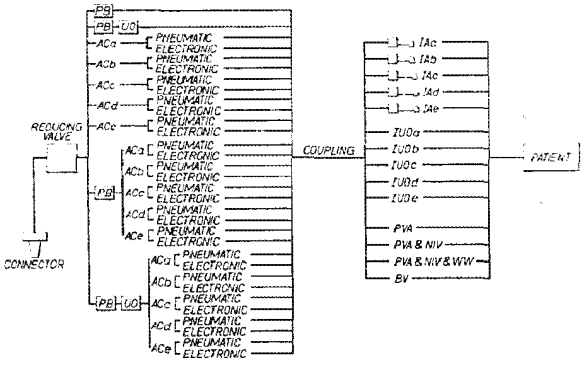
(71) Applicant: **ELECTRONIC PNEUMATIC APPARATUS & CONTROLS LIMITED, The Lanterns 24 Worrin Road, Shenfield Brentwood Essex (GB)**

(72) Inventor: **Burchell, Geoffrey Barnett, Dr., The Lanterns 24 Worrin Road, Shenfield Brentwood Essex (GB)**

(74) Representative: **Burrows, Anthony Gregory et al, Haseltine Lake & Co. Hazlitt House 28 Southampton Buildings Chancery Lane, London, WC2 1AT (GB)**

(54) **Improvements in or relating to respiratory apparatus.**

(57) A resuscitation apparatus comprises a respirable gas flow valve (REDUCING VALVE), one or more modular control devices (PB, UO, AC) connected to the valve (REDUCING VALVE) for operating the valve, and a modular administering device (IA, IUO, PVA, BV) connected to downstream of the valve (REDUCING VALVE) for administering the gas to a patient's respiratory system. The control device(s) can be selected from two manual control devices (PB, UO), a series of pneumatic automatic control devices (AC-PNEUMATIC), and a series of electronic automatic control devices (AC-ELECTRONIC); whilst the administering device can be selected from a series of injection airways (IA), a series of oxygen injection units (IUO), and a series of patient valves (PVA, BV).



EP 0 097 060 A2

SPECTRAL_0000113

IMPROVEMENTS IN OR RELATING TO RESPIRATORY APPARATUS

This invention relates to respiratory apparatus, in particular resuscitation apparatus.

Resuscitation apparatus is used to resuscitate a person whose respiration has become insufficient to maintain life for any substantial period of time. The resuscitation apparatus used depends on the particular patient (in particular his or her size) and the circumstances in which the patient is found. A large number of different types of resuscitation apparatus have thus been developed to meet the various different situations that arise in practice. However, known resuscitation apparatus tend to be of limited utility.

It is an aim of the present invention to provide versatile resuscitation apparatus.

According to a first aspect of the present invention, there is provided resuscitation apparatus comprising an inlet connectible to a source of respirable gas, an outlet connectible to a patient's respiratory system, duct means interconnecting said inlet and said outlet, a valve interposed in said duct means and operable selectively manually and automatically, manually operable means connected to said valve and operable manually to operate said valve, and automatically operable means connected to said valve and operable automatically to operate said valve.

According to a second aspect of the present invention there is provided resuscitation apparatus comprising a housing; an inlet port in a wall of the housing connectible to a source of respirable gas; an outlet port in a wall of the housing connectible to means for administering respirable gas to a patient; a flow pasasge extending within the housing from the inlet port to the outlet port; a valve in said passage, the valve being capable of being operated manually or automatically; means operable manually from outside of

the housing for opening and closing the valve; and a connector in the wall of the housing associated with the valve and suitable for connecting the valve to external means for operating the valve automatically.

The resuscitation apparatus is thus capable of being operated manually or automatically to supply pulses of respirable gas to a patient.

The connector may be electrical and the valve have a solenoid actuator so that electronic control means may be employed to operate the valve. There is provided an electronic control device for operating the valve automatically, this device having an electrical connector engageable with the electrical connector of the apparatus.

The control device may have its own power supply or may be connectible to a mains electricity supply. The control device preferably has a housing containing suitable electronic control circuits and a power supply if it is not to be operated from a mains electricity supply. The control circuits are adapted to generate electrical signals which effect opening and closing of the valve, preferably at a variable frequency. Each period for which the valve is open corresponds to the inspiratory phase of a patient's breathing, and each period for which the valve is closed corresponds to the expiratory phase of a patient's breathing. The control device is preferably adapted to give a constant inspiratory phase/expiratory phase ratio, i.e. the ratio of the duration of each pulse of gas to the interval between consecutive pulses is a constant. Preferably, the ratio is 1:2.

It is also preferred that the duration of each pulse of gas can be varied by manual setting of a suitable control. This makes it possible for the resuscitation apparatus to supply pulses of respirable gas that can be adjusted to suit the size (or lung capacity) of the patient be he or she a newly born baby

SPECTRAL_0000115

or a fully grown adult with a relatively large lung capacity. Typically, the range of settings of the manual control may vary the frequency of the gas pulses from 10 or 15 to 40 per minute.

It is to be appreciated from the aforegoing description that the resuscitation apparatus may be set to operate automatically to produce gas pulses at a chosen frequency by using the control device. It is, however, a feature of the resuscitation apparatus that it can be operated entirely manually and without connecting a control device thereto. This can be done by operating the said manually operable means for opening and closing the valve (which means is conveniently a push-button). Manual operation of the valve offers flexibility in that it is possible to produce any desired pattern of gas pulses. The resuscitation apparatus is thus usable even if there is no available power supply or automatic means for operating the valve or if the condition of the patient is so serious that there is no time to connect the resuscitation apparatus to any available means for operating the valve automatically. Moreover, the valve is preferably of a kind such that when operating automatically, the control signals it recieves are able to be manually over-ridden.

It is another feature of the resuscitation apparatus that its outlet port may be connected to any suitable means for delivering respirable gas to a patient. Such means may comprise an artificial airway. A preferred artificial airway is as claimed and described in UK Patent Specification No. 1 558 171. Such an artificial airway is anatomically-shaped and has a flange at one end to limit its insertion into a patient's airway and a fixed injector at the flanged end for injecting respirable gas at relatively high flow rates into the airway to entrain air from the atmosphere. The length of the airway is related to the

size of the patient so that the inner end of the airway, when the airway is in position, lies in the oral pharynx. Such an artificial airway obviates the need for a face mask and thus overcomes difficulty in keeping an air-tight fit between the face mask and the patient's face. The size of the artificial airway selected will depend on the size of the patient. It is alternatively possible to use more conventional means (including face masks) to deliver the respirable gas to the patient. Thus, the outlet port may be connected to a conventional non-return valve which is fitted with a suitable taper (or face mask). The non-return valve may be suitable for a child and fitted with a British Standard paediatric taper or maybe suitable for an adult and fitted with the British Standard adult taper. In both instances, there may be a flow control valve in the conduit connecting the outlet port to the non-return valve. Other alternative means for delivering respirable gas to the patient that are connectible to the outlet port include endotracheal and trachaeostomy tubes.

The resuscitation apparatus preferably has in a wall of the housing a second outlet port communicating with the flow passage in the housing upstream of the valve. The second outlet port may be used to supply respirable gas continuously to a second patient while resuscitating a first. If desired, the second outlet port may be connected to a manually operable valve, downstream thereof, to produce a second pulsed supply of respirable gas. By connecting the outlet of such manually operable valve to a means for delivering respirable gas to a patient such as has been described above it is possible to use the resuscitation apparatus to resuscitate two patients (e.g. mother and child) simultaneously, generally with the valve within the housing being operated automatically. It is alternatively possible to attach the outlet of the

SPECTRAL_0000117

manually-operable valve connected to the second outlet port to an inflatable pillow, which may then be inflated to a desired degree and used to prop the head of a patient so as to facilitate delivery of the respirable gas.

According to a third aspect of the present invention, there is provided a method of assembling a resuscitation apparatus, comprising selecting a control device from a range of modular control devices, connecting said control device to a respirable gas flow control valve to be operated by said control device, selecting a respirable gas administering device from a range of modular respirable gas administering devices, and connecting said administering device to downstream of said valve for administering said gas to a patient's respiration system.

According to a fourth aspect of the present invention, there is provided a collection of modular devices from a selection from which a resuscitation apparatus can be assembled, comprising a respirable gas flow valve, a range of modular control devices each connectible to said valve for opening the same, and a range of modular respirable gas administering devices connectible to downstream of said valve for administering said gas to a patient's respiratory system.

According to a fifth aspect of the present invention, there is provided a resuscitation apparatus, comprising a respirable gas flow valve, a modular control device connected to said valve for operating the same, and a modular respirable gas administering device connected to downstream of said valve for administering said gas to a patient's respiratory system.

According to a sixth aspect of the present invention, there is provided resuscitation apparatus comprising an inlet connectible to a source of respirable gas, an outlet connectible to a patient's

SPECTRAL_0000118

respiratory system, duct means interconnecting said inlet and said outlet, a valve interposed in said duct means and operable automatically, and electrical means connected to said valve and arranged to operate said valve automatically, said electrical means including electrical switching means arranged to detect the presence or absence of said gas under pressure upstream of said valve and accordingly to energize or de-energize said electrical means.

According to a seventh aspect of the present invention, there is provided a respiratory apparatus including first and second variable flow control valves which serve to control respective flows of gaseous fluid therethrough, and a non-pneumatic adjusting linkage interconnecting the valves so that adjustment of the first valve is accompanied by adjustment of the second valve.

According to an eighth aspect of the present invention, there is provided a valve device, comprising an inlet for fluid, an outlet for said fluid, valve closure means arranged to control flow of said fluid from said inlet to said outlet, and a pilot pressure chamber bounded by said valve closure means, the effective area of said valve closure means exposed to the pressure of said fluid between said inlet and said outlet being less than the effective area of said valve closure means exposed to the pressure in said chamber.

According to a ninth aspect of the present invention, there is provided a valve device comprising a valve housing, a valve closure member movable in said housing from an open position to a closed position, an inlet to said housing for fluid, and an outlet from said housing for fluid, the arrangement being such that, as said member moves from said open position to said closed position, initially fluid can flow via a first path from said inlet to a limited degree and via a second path from said inlet to no more than a limited extent, but

SPECTRAL_0000119

subsequently said member enables flow of said fluid via said second path from said inlet to greater than said limited extent, whereby said fluid can flow from said inlet to an increased extent.

According to a tenth aspect of the present invention, there is provided a valve device comprising a valve housing, a valve closure member movable in said housing from an open position to a closed position, a tubular inlet to said housing for fluid, and an outlet from said housing for fluid, said member being in the form of a bobbin closely encircling said tubular inlet and including first and second flanges of substantially equal external dimension transverse to the bobbin axis.

According to an eleventh aspect of the present invention, there is provided a valve device comprising a first valve including a first valve closure member arranged to open automatically upon occurrence of a predetermined pressure differential thereacross, a second valve which includes a selectively closable second valve closure member and through which the first valve exhausts when the second valve closure member is open, and a third valve which includes a third valve closure member arranged to open automatically upon occurrence of a predetermined pressure differential thereacross and through which the first valve exhausts when the second valve closure member is closed, the arrangement being such that pressure upstream of said first valve at which said third valve opens is higher than that pressure upstream of said first valve at which said first valve opens.

Resuscitation apparatus according to the present invention will now be described by way of example with reference to the accompanying drawings, in which:-

Figure 1 is a schematic perspective view of one embodiment of resuscitation apparatus according to the invention;

Figure 2 is a schematic perspective view of one

SPECTRAL_0000120

embodiment of a control device adapted to be connected to the resuscitation apparatus shown in Figure 1;

Figure 3 is a schematic side elevation of the apparatus shown in Figure 1 illustrating the inlet port;

Figure 4 is a schematic view from below of the apparatus shown in Figure 1 illustrating the electrical connector;

Figure 5 is a schematic circuit diagram of the apparatus shown in Figure 1;

Figure 6 is a graph showing the gas supply that the apparatus shown in Figure 1 is capable of supplying;

Figure 7 is a side view of an anatomically-shaped artificial airway suitable for use with the resuscitation apparatus shown in Figure 1;

Figure 8 is an end view of the airway shown in Figure 7;

Figures 9 to 13 all illustrate devices connectible to one or other of the outlet ports of the apparatus shown in Figure 1;

Figure 14 is a diagrammatic representation of a manner in which the resuscitation apparatus can be assembled by a method involving selection from ranges of modular devices;

Figure 15 shows a diagram of an electronic automatic control device for giving variable respiration frequency and a fixed inhalation/exhalation time ratio;

Figure 16 is a diagram of part of a modified version of that device;

Figure 17 is a diagram of part of another modified version of that device;

Figure 18 is a diagram of part of a further modified version of that device;

Figure 19 is a diagram of a pneumatic part of a further embodiment of the apparatus;

Figure 20 is a diagram of a mechanical part of that further embodiment;

Figure 21 shows an axial section through a

patient valve of that further embodiment;

Figure 22 shows an axial section through part of a modified version of that patient valve;

Figure 23 shows an axial section through a normally-open, pilot-controlled valve seen in Figure 19, and

Figure 24 shows an axial section through a normally-closed, pilot-controlled valve seen in figure 19.

The drawings are not to scale.

Referring to Figure 1, a resuscitation apparatus 2 includes a box-shaped housing 4. The longer opposed side walls of the box-shaped housing 4 are indicated by the reference numerals 6 and 8, and the shorter side walls by the reference numerals 10 and 12. (The side 10 is also shown in Figure 3). The top and bottom walls of the box or housing 4 are indicated by the reference numerals 14 and 16 respectively. (The bottom 16 is also shown in Figure 4). The housing 4 is conveniently formed of any suitable durable plastics material.

There is an inlet port 18 in the side wall 10 and first and second outlet ports 20 and 22 in the opposite side wall 12. The inlet port 18 is adapted to be connected to a source of respirable gas at high pressure (e.g. an oxygen cylinder fitted with a regulator). By the term 'high pressure' is meant a pressure higher than normal respirable pressures. The outlet ports 20 and 22 may comprise self-capping connectors of the Schraeder type or any other simple connecting device.

The housing 4 contains a solenoid (on-off) valve (not shown in Figure 1) which is alternatively operable by a push-button 24 projecting through the top wall 14. The valve is shown schematically in Figure 5 and is indicated by the reference 26. It is located in a main flow passage 28 connecting the inlet port 18 to the first outlet port 20. A tributary 30 from the main flow passage 28 extends from upstream of the valve 26 to the

SPECTRAL_0000122

second outlet port 22.

The valve 26 is capable of being operated manually by the push-button 24 or automatically by the solenoid 32. If the valve is operating automatically the push-button 24 may be operated to override the solenoid 32. In order to keep down the total size of the resuscitation apparatus 2, it is desirable that the valve 26 be relatively small. The valve may, for example, be a Martonair (trade mark) valve having two valve positions and three ports (with one of the outlet ports blocked).

The solenoid 32 of the valve 26 is electrically connected to an electrical socket 34 in the bottom wall 16 of the housing 4. This socket is adapted to be mated with the complementary plug 38 of an electronic valve control box or device 36 (see Figure 2). The control box 36 typically has a switch 40 for switching on its own internal power supply (not shown) which may typically comprise rechargeable or disposable batteries. The control box 36 also has a control knob 42 for adjusting the frequency with which the valve 26 may be opened and closed. The control knob 42 is associated with electronic circuits capable of generating signals effective to open and close the valve 26 so as to supply to the outlet port 20 pulses of gas as shown in Figure 6.

As can be seen from Figure 6 the interval between the generation of each pulse and the next is twice as long as the duration of each pulse. Each pulse corresponds to the inspiratory phase of a patient's breathing and the interval between one pulse and the next to the expiratory phase of the patient's breathing. Accordingly, a constant inspiratory phase/expiratory phase ratio of 1:2 is given by operating the valve automatically using the control box. Moreover, by adjusting the control knob 42 the duration of each pulse may be adjusted to suit the particular patient without

SPECTRAL_0000123

altering the inspiratory phase/expiratory phase ratio. Electronic control circuits capable of generating the necessary signals for such operation of the valve 26 are well known and will not be described in detail in this specification. Suffice it to state that typically, two integrated circuits are required, one for generating rapid electrical pulses, the other containing a gate means for selecting pulses. Typically, the control device may produce from 15 to 40 gas pulses per minute.

It is to be appreciated that instead of using the control box 36 the valve 26 may be operated manually by means of the push-button 28 to produce a pulsed gas supply such as that shown in Figure 6.

The first outlet port 20 may be connected to any suitable means for delivering the pulsed high pressure gas supply to the patient. The preferred means is an anatomically-shaped artificial airway as claimed and described in UK Patent Specification 1 558 171. Such an airway is shown in Figure 7 and 8. Connecting the airway shown in Figures 7 and 8 to the outlet port 20 of the apparatus shown in Figures 1 and 3 to 5 forms a complete manually-operable resuscitator. If the control box shown in Figure 2 is connected to the apparatus shown in Figures 1 and 3 to 5, the resuscitator may operate automatically.

The airway shown in Figures 7 and 8 consists basically of an open-ended tube 52 having at one end 60 an outwardly-directed flange 54. The tube 52 is intended to be fitted into a patient's mouth so that the tube overlies the patient's tongue and extends into the oro-pharynx. The flange 54 is intended to come into contact with either the patient's teeth or lips so as to prevent the patient from swallowing the airway and to limit the extent to which the airway projects into the patient's oro-pharynx.

Extending to the side of the flange 54 is a length of tubing 56 which is able to be connected to the

SPECTRAL_0000124

outlet port 20 of the resuscitation apparatus 2. The end of the tubing which is remote from the outlet port 20 terminates in a nozzle (or primary injector) 58 which is shaped so as to inject a jet of gas at high-pressure into the interior of the tube 54. The nozzle 58 may be of one piece with the tubing 56. The tubing 56 and nozzle 58 may be of one piece with the flange 54 and therefore with the tube 52. Alternatively, the tubing 56 may be bonded or otherwise secured in a non-detachable manner to the flange 54.

When the patient is being resuscitated, the interior of the tube 52 is open to the atmosphere through the central opening 60 in the flange 54. The pulses of respirable gas issue as jets from the nozzle (or primary injector) 58 and entrain air from the atmosphere. Each jet of gas and the entrained air are typically mixed completely by the time the mixture reaches the distal end of the airway in the patient's oro-pharynx. As the flow of gas leaves the distal end of the injector it will impinge on the soft palate tending to drive it back on to the posterior pharyngeal wall narrowing the aperture between the oro-pharynx and naso-pharynx. With the soft palate in this position the oro-pharynx flares out like the throat of a venturi. Thus, the end of the airway in the throat constituted a secondary injector entraining additional air through the nose and around the airway in the mouth. There is only a relatively weak suction but the important result is that it is not necessary to pinch the nose or cover the mouth to ensure inflation. The existence of a low suction pressure in these zones can be demonstrated by the movement of a wisp of cotton wool.

Typically, at the distal end of the airway, with no back pressure, flows of 100 ml to 1000 ml per second can be obtained with different sized airways and nozzles 58. As the back pressure rises the flow rate decreases and flow will cease at a pressure of 30 to 40 cm H_2O

according to the dimensions of the airway and nozzle 58. Typically, with pulse of oxygen at 60 psi the oxygen concentration delivered to the patient may be between 40% and 60% by volume.

Although the combination of a resuscitation apparatus such as that illustrated in Figures 1 and 3 to 5 with the artificial airway shown in Figures 7 and 8 is a preferred arrangement according to the invention, the outlet port 20 of the resuscitation apparatus illustrated in Figures 1 and 3 to 5 may be connected to alternative devices for delivering pulses of respirable gas to the patient.

Examples of suitable alternative equipment are shown in Figures 9 and 10. The equipment shown in Figure 9 comprises a flow control valve 70, a non-return valve 72 suitable for an infant or child, and a face mask 74 suitable for an infant or child. In addition, there are suitable lengths of flexible connecting tubing 76. The equipment shown in Figure 10 is analogous to that shown in Figure 9 but is adapted for resuscitation of an adult. The equipment comprises a flow control valve 80, a non-return valve 82 suitable for an adult, and a face mask 84 suitable for an adult. In addition, there are suitable lengths of flexible connecting tubing 86.

Accordingly, by keeping with the resuscitation apparatus shown in Figures 1 and 3 to 5, a range of different sized equipment of the kind shown in Figures 7 and 8 and/or 9 and 10, all sizes of patient from neolates to large adults can be resuscitated.

The continuous supply of respirable gas to the outlet port 22 of the resuscitation apparatus shown in Figure 1 may be emloyed in one of three alternative ways. First, a continuous flow of respirable gas may be supplied to assist the breathing of a patient who does not require resuscitation. Second, a pulsed flow of respirable gas may be created from the continuous flow

and supplied to resuscitate a second patient. Third, a flow of gas may be supplied to an inflatable pillow so as to inflate it to a desired degree. The pillow may then be used to prop the head of a patient. Suitable equipment for these purposes is illustrated schematically in Figures 11, 12 and 13.

The equipment shown in Figure 11 comprises a flow control valve 90, an oxygen mask 92 and suitable lengths of flexible connecting tubing 96.

The equipment shown in Figure 12 comprises a manually (e.g. push-button) operable on-off valve 102 whose inlet is connected to a suitable length of flexible tubing 104. The outlet of the valve is connectible with the artificial airway shown in Figures 7 and 8 or with the equipment shown in Figure 9 or 10. The valve 102 may thus be operated manually to resuscitate one patient while another patient is resuscitated by means of pulses of respirable gas leaving the port 20 of the apparatus shown in Figure 1. Such pulses can be supplied automatically by means of the control box shown in Figure 2. Thus one suitably qualified person can use the apparatus shown in Figure 1 to resuscitate two patients simultaneously.

The apparatus shown in Figure 13 comprises a manually operable (e.g. push-button) valve 110, an inflatable pillow 112 of suitable material and suitable lengths of flexible connecting tubing 114. The valve 110 may be kept open until the pillow 112 has been inflated to a suitable degree and then closed.

The tubing employed in the airway shown in Figures 7 and 8 and the tubing employed in the equipment shown in Figures 9 to 13 may be provided at the end to be connected to the port 20 or 22 of the apparatus shown in Figure 1 with suitable connectors (not shown). Preferably, the ports 20 and 22 have connectors of different sizes from one another so that equipment intended to be connected to the port 20 cannot be

connected to the port 22, and vice versa.

The equipment shown in Figures 11 to 13 may be kept or stored with the resuscitation apparatus shown in Figures 1 and 3 to 5, the control box shown in Figure 2 and suitable airways of the kind shown in Figures 7 and 8 (or equipment of the kind shown in Figures 9 and 10) to provide a resuscitation kit which may be used to resuscitate patients ranging from neonates to fully grown adults in a number of different situations. Moreover, hospitals, rescue services and ambulance services may select only those parts of the whole range of apparatus and equipment illustrated in the drawings so as to meet their own particular requirements.

Referring to Figure 14, the manufacturer of the resuscitation apparatus wouldhave in stock and purchasable individually the following modules:-

A standard connector to a gas supply and called "connector" in Figure 14.

A pressure reducing valve called "reducing valve"

A push-button manual control device for the reducing valve and reference "PB" and operable to give manually controlled pulses of respirable gas.

A manual control device referenced "UO" and operable to caused the reducing valve to give a continous, i.e. unpulsed, flow of respirable gas, usually oxygen.

A series of pneumatic automatic control devices for the reducing valve and referenced "ACa pneumatic" to "ACe pneumatic". ACa refers to a device giving a constant volume flow rate at a fixed respiration frequency and with a fixed inhalation/exhalation time ratio. ACb represents a device giving a constant flow rate with a variable frequency and a fixed inhalation/exhalation time ratio. ACc refers to a device for giving a variable flow with a variable frequency and a fixed inhalation/exhalation time ratio. ACd refers to a device giving a constant flow with a variable frequency

SPECTRAL_0000128

and a variable inhalation/exhalation time ratio. ACe represents a device for giving a variable flow with a variable frequency and a variable inhalation/exhalation time ratio.

A series of electronic automatic control devices referenced "ACa electronic" to "ACe electronic". The references ACa to ACe have the same meaning as in the case of the pneumatic devices.

A quick-connect and quick-release coupling referenced "coupling".

A series of injection airways referenced IAa to IAe and of various sizes.

A series of injection units designed for oxygen and referenced IUOa to IUOe and which can comprise face masks or endotracheal/tracheostomy tubes.

An adult patient valve referenced "PVA".

An adult patient valve combined with a non-inhalation valve and referenced "PVA/NIV".

An adult patient valve with a non-inhalation valve and a warning whistle and referenced "PVA/NIV/WW".

A baby patient valve referenced "BV".

The patient is also indicated in Figure 14.

A purchaser would buy the connector; the reducing valve; at least one of the valve control devices "PB", "UO" and "AC"; the coupling; and at least one of the respirable gas administering devices "IA", "IUO", "PVA", "PVA/NIV", "PVA/NIV/WW", and "BV". In practice, he would purchase a considerable part of the ranges available. At the place where the resuscitation apparatus is to be used, such as at a hospital, the connector, the reducing valve, a valve control device, the coupling, and an administering device would be assembled together, the valve control device and the administering device being selected according to the circumstances and the type of patient.

This method of assembling of the apparatus enables one apparatus with additional interchangeable

modules to cope with a wide variety of patients in a wide variety of circumstances.

Particularly in the case of resuscitation apparatus applicable to new-born babies, it is especially advantageous for the apparatus to be so designed that the first few breaths can be at a high flow rate, in order to expand the lungs properly, but the following breaths at a low flow rate more corresponding to normal breathing. For this purpose, the manual control device PB is advantageously designed to open the reducing valve sufficiently to give a high rate of flow, whilst the automatic valve control devices AC are advantageously designed to give a low rate of flow from the valve.

Figures 15 to 17 show possible designs of the device ACb electronic or ACe electronic.

Referring to Figure 15, the device naturally requires a power source 100. This power source can be any supply of electrical energy, for example dry cells, rechargeable batteries or the mains. The power source 100 is connected to an oscillator 101, a ratio divider 102 and a solenoid-operated valve 103. The oscillator is a variable frequency oscillator and can be any circuit capable of producing a digital train of pulses, which may be varied in frequency by the setting of a control 104. The ratio divider 102 can be any circuit which may be programmed to operate the solenoid-operated valve 103 for 1 out of n of the incoming pulses from the oscillator 101. The process is cyclic and repeats every n pulses. The valve 103 comprises an elctro-mechanical actuator assembly which when energised allows a change in the state of flow of the gas to be controlled.

Referring to Figure 16, the oscillator, now referenced 105, is a fixed frequency oscillator with a fixed control 106. The output from the oscillator 105 is fed to a frequency divider 107 also connected to the power source 100. A selector 108 is used to select a

SPECTRAL_0000130

desired frequency signal from the divider 107 and feed it to the ratio divider 102. The frequency divider 107 is a circuit which can take the incoming pulse train from the oscillator 105 and produce a series of frequency divided outputs.

The circuit may be extended to allow for modification of the output wave form of the ratio divider 102 in response to a stimulus from an outside source. Such an arrangement is shown in Figure 17, wherein a control signal from the ratio divider 102 is fed to a trigger device 109. On receipt of an external stimulus, the trigger device 109 emits an electrical response signal to modify the output from the ratio divider 102.

In some circumstances, it is particularly advantageous if the power supply to the electronic control device is not energised until the gas supply is switched on and is switched off immediately the gas supply is switched off. An arrangement for achieving this is illustrated in Figure 18. A gas bottle 110 is shown connected via a bottle valve 111 to a cycling unit 112. Also arranged downstream of the valve 111 is a slide valve 113 and, downstream thereof, a piston-in-cylinder device 114 arranged to control a pair of electrical contacts 115 arranged in the power supply circuit of the electronic control device. In the condition shown, the piston 116 of the device 114 has been pressed by a return spring 117 into a position corresponding to an open position of the contacts 115, the gas in the cylinder having been expelled via an exhaust port 118 of the valve 113. On switching on of the bottle valve 111, the downstream gas pressure of the valve 111 increases and is applied to the left-hand end of the slide valve 113 to press it against the action of a return spring 119 into a position in which a through passage 120 of the valve 113 communicates the gas pressure downstream of the valve 111 to the device 114,

so forcing the piston 116 against the action of the spring 117 to close the contacts 115. On closing of the bottle valve 111, the pressure downstream of the valve 111 falls and the spring 119 returns the valve 113 into its exhaust condition shown.

Referring to Figure 19, the pneumatic circuit part shown therein includes three conduits 202, 203 and 204 which are supplied from the nutrient gas supply 201. The conduit 202 divides into two conduits 205 and 206, of which the conduit 205 leads to the inlet of a normally-open, pilot-controlled valve 207, and the conduit 206 to a normally-closed, pilot-controlled valve 208. On commencement of flow from the supply 201, the nutrient gas passes straight through the valve 207 and through a non-return valve 209 to a pilot pressure chamber of the valve 208, so enabling nutrient gas to flow through the valve 208 from the conduit 206 to a conduit 210 which leads via an adjustable flow restrictor 211 to a patient valve, which is shown in Figure 21. Nutrient gas in the conduit 210 can flow via a conduit 212 containing a non-return valve 213 to a pilot pressure chamber of the valve 207. Nutrient gas is trapped in the pilot conduits by the non-return valves 209 and 213 and fills reservoirs 214 and 215 connected upstream of the non-return valves 209 and 213, respectively. The reservoirs 214 and 215 can empty only through respective adjustable bleeds 216 and 217. The adjustable bleeds 216 and 217 and the adjustable flow restrictor 211 are linked by some non-pneumatic means such that their throughflow cross-sections remain in a fixed ratio relative to one another. An example of such non-pneumatic means is shown diagrammatically in Figure 20, in which inter-meshing gear wheels 218, 219 and 220 are connected to adjusting shafts of the restrictor 211 and the bleeds 216 and 217, respectively. Inserted in the conduit 203 is a manually adjustable flow restrictor 221. The conduits 203 and 210 downstream of the

restrictors 221 and 211, respectively, may remain independent of each other, or may be joined together to give higher flow rates. The conduit 204, which contains a manually adjustable flow restrictor 222, can give a continuous flow of nutrient gas to the patient. The outlets from the flow restrictors 211, 221 and 222 are preferably in the form of quick-release couplings to allow easy change from one mode of treatment to another. These three outlets may all be used simultaneously on three respective patients, or two of these outlets may be used on two respective patients, or one outlet on a single patient.

In a case where the conduits 203 and 210 are joined together downstream of the restrictors 221 and 211, respectively, the restrictor 221 may be arranged to be non-pneumatically linked for adjustment with the restrictor 211, such linkage being instead of or as a selectable alternative to manual adjustment of the restrictor 221. As shown in Figure 20, such linkage may be achieved by means of connecting to the gear wheel 211 by way of an intermediate gear wheel 223, a gear wheel 224 connected to an adjusting shaft of the restrictor 221.

The apparatus described with reference to Figures 19 and 20 has an advantage of being a single apparatus usable for oxygen therapy (or therapy utilizing another gaseous substance), manual intermittent positive pressure ventilation, or automatic intermittent positive pressure ventilation, and enabling up to three patients to be treated at the same time. For example, in midwifery, the baby may need manual or automatic intermittent positive pressure ventilation, while the mother has oxygen therapy.

Although the pneumatic circuit shown in Figure 19 comprises two variable bleeds 216 and 217 and two fixed-volume reservoirs 214 and 215, it could instead comprise two fixed-flow-rate bleeds and two

variable-volume reservoirs. Alternatively, it could have two variable bleeds giving coarse control of the flow rates and two variable reservoirs giving fine control of the flow rates.

It will be appreciated that the reservoir 214 and the bleed 216 determine the inhalation time period, whilst the reservoir 215 and the bleed 217 determine the exhalation time period.

In most normal circumstances, the ratio of the inhalation time period to the exhalation time period is advantageously about 1:2. Owing to the non-pneumatic linkage between the restrictor 211 and the bleeds 216 and 217, not only is it possible to ensure that this ratio remains constant, but also the respiration frequency varies automatically with adjustment of the flow rate to the patient, so that the apparatus is usable with simple adjustment for either a large adult or a small child. In practice, with a decrease in the flow rate to the patient, there is an increase in the ventilation frequency, and vice-versa.

The patient valve shown in Figure 21 comprises a bobbin 230 which slides with clearance in a shell 231 which has an inlet 232 for nutrient gas and an outlet 233 for exhaled gas. The shell 231 also comprises an opening 234 leading to the patient and an opening 235 receiving a screw-in positive pressure relief valve 236. The nutrient gas entering the inlet 232 flows along an inlet tube 237 encircled by the bobbin 230. The pressure of the gas urges the bobbin 230 against the action of a return spring 238 into a position in which it seals the outlet 233. The pressure of the nutrient gas in the bobbin 230 is the inlet pressure of that gas, until a hole 239 through the tube 237 is exposed by the movement of the bobbin 230 towards its position sealing the outlet 233, whereupon the pressure in the bobbin 230 drops, but at the same time the pressure against the inside face of the closure plate 240 of the bobbin 230

rises, and this constitutes a substantial part of the sealing force, especially at low flow rates. Exposure of the hole 239 allows high flow rates of nutrient gas to be transmitted to the patient. The two flanges of the bobbin are of equal external diameter to each other. The valve 236 is a screw-in unit which can be exchanged for another simple valve or a compound valve device such as that illustrated in Figure 22, which is for neonates.

Referring to Figure 22, the valve device includes a threaded inlet stem 250 which is screwed into the opening 235. The device also includes a primary valve 251 which opens at comparatively low pressure in the stem 250, for example 30 cm. H_2O. The gas from the valve 251 exhausts through a port 252 of another valve 253 and thence through openings 254 to atmosphere. The valve 253 is normally kept open by its spring 255. However, when a knob 256 of the valve 253 is depressed, the port 252 is sealed and the gas is then voided through a port 257 of a valve 258 and thence through openings 259 to atmosphere. The closure member of the valve 258 is urged to close the port 257 by its spring 260, which is set to give an opening pressure for the valve 258 corresponding to a comparatively high pressure in the stem 250, for example 55 cm. H_2O. An advantage of this valve device is that it is possible to obtain positive pressure relief at two differing positive pressures without disturbing the setting of the valve closure springs.

The normally-open, pilot-controlled valve 207 is seen in more detail in Figure 23. It comprises a valve housing 270 with a nutrient gas inlet 271 and a nutrient gas outlet 272 connected in the conduit 205. The housing 270 has a hollow interior 273 to and from which lead the inlet 271 and the outlet 272, the interior 273 containing co-axially a valve closure member 274 in the shape of a pin with a cylindrical head and a conical tip. A seal 275 around the head of the member 274 and

an annular seal 276 encircling the shank of the member 274 divide the interior 273 into a pilot pressure chamber 277, an atmospheric pressure chamber 278, and a nutrient gas line pressure chamber 279. The chamber 277 communicates via a connection 280 with the conduit 212, the chamber 278 with atmosphere through an aperture 281, and the chamber 279 with the inlet 271 and the outlet 272. Near the end of the chamber 279 further from the chamber 277 the housing 270 carries co-axially an annular seal 282 which is of considerably smaller diameter than is the seal 276. An annular compression spring 283 encircling the shank of the member 274 and acting between the housing 270 and the head of the member 274 urges the member 274 into a fully open position. In the open condition of the valve 207, the line pressure in the chamber 279 is applied over the relatively small effective area of the member 274 at its tip, whilst the pilot pressure in the chamber 277 is applied over the relatively large effective surface area of the member 274 at its head. Thus the pilot pressure may be well below the line pressure. The valve member 274 is kept open by the spring 283 which is relatively weak. As the member 274 nears the seating seal 282, the conical shape of its tip gives a decreasing surface area exposed to the full line pressure, so giving a rapid change of state from the open condition to the closed condition. Moreover, in the closed condition, the line pressure is applied over only the relatively very small effective area defined by the seal 282. As the member 274 begins to open, the shape of the tip gives an increasing area exposed to the full line pressure, so giving a rapid change of stage from the closed condition to the open condition.

The pilot-controlled normally-closed vlave 208 shown in Figure 24 is identical to the valve 207, except that the aperture 280' leads to atmosphere, so that the chamber 277' is the atmospheric chamber; the aperture

281' is connected to the conduit 212, so that the chamber 273' becomes the pilot pressure chamber; and the spring 283' is situated in the chamber 277' and so urges the member 274 into its fully closed condition. In the closed condition of the valve 208, the seal 282 defines the relatively very small surface area over which the line pressure acts on the member 274. As the member 274 begins to open, the relatively large area now exposed to the line pressure and defined by the seal 276 compensates for any drop in the line pressure. The pilot pressure in the chamber 273' still acts over a yet larger area, so that the valve responds to a relatively low pilot pressure.

If it is desired to permit electrical operation of the valve 207 or 208, an inductive coil 284 can encircle the housing 270, which would be made of non-ferrous material, whilst the member 274 would be made of ferrous material. Such valve device may be used with either pneumatic or electrical pulsing, whereby it is possible to have an electrical override of the pneumatic circuit, or pneumatic override of the electrical circuit.

By providing a longer shank of the member 274, the member can be used to open and close further control inlets and outlets, thereby providing a three-, four- or more- port valve according to need, but keeping the principle of an inlet pilot.

The bobbin 230 has its two flanges of virtually equal external diameter, so that the ratio of the exposed areas of the flanges is not a significant factor in the motion of the bobbin.

CLAIMS

1. Resuscitation apparatus comprising an inlet (18, 203) connectible to a source (201) of respirable gas, an outlet (20) connectible to a patient's respiratory system, duct means (28) interconnecting said inlet (18, 203) and said outlet (20), and a valve (26, 221) interposed in said duct means (28) and operable manually by manually operable means (24) connected to said valve (26, 221) characterized in that said valve (26, 221) is also operable selectively automatically by automatically operable means (32, 223, 224) connected to said valve (26, 221).

2. Apparatus according to claim 1, wherein said automatically operable means (32,223, 224) comprises an electronic control device (36) and said valve (32, 223, 224) is operable automatically electrically.

3. Apparatus according to claim 1 or 2, wherein said automatically operable means (32, 223, 224) operates said valve (26, 221) to give a constant inspiratory phase/expiratory phase ratio.

4. Apparatus according to any preceding claim, and further comprising adjusting means (42, 104, 107, 108) arranged to adjust the duration of the inspiratory phase produced by said automatically operable means (32, 223, 224)..

5. Apparatus according to any preceding claim, wherein said manually operable means (24), on operation thereof, overrides said automatically operable means (32, 223, 224)..

6. Apparatus according to any preceding claim, and further comprising a second outlet (22, 204) communicating with said duct means (28) at a location upstream of said valve (26, 221).

7. Apparatus according to claim 6, and further comprising a manually operable valve (222) connected downstream of said second outlet (22, 204).

8. Apparatus according to any preceding claim, and further comprising a housing (4) containing said valve (26, 221) and said duct means (28), said automatically operable means (32, 223, 224) including a connector (34) in a wall (16) of said housing (4) for connecting the valve (26, 221) to an external automatic control device (36).

9. Apparatus according to any preceding claim, and further comprising a modular gas administering device (52, 74) connected downstream of said outlet (20) for administering said gas to said system, said manually operable means (24) comprising a modular manual control device, and said automatically operable means (32, 223, 224) comprising a modular automatic control device (any one of Figures 15 to 19).

10. A method of assembling a resuscitation apparatus, characterized in that it comprises selecting a control device from a range of modular control devices (PB, 36, Figures 15 to 19), connecting said control device to a respirable gas flow control valve (26, 221) to be operated by said control device, selecting a respirable gas administering device from a range of modular respirable gas administering devices (52, 74 etc.), and connecting said administering device to downstream of said valve (26, 221) for administering said gas to a patient's respiration system.

11. A method according to claim 10, and further comprising selecting an automatic control device from said range of modular control devices (PB, 36, Figures 15 to 19) and connecting said automatic control device to said valve (26, 221), the first-mentioned control device being a manual control device (PB).

12. A collection of parts from which a resuscitation apparatus can be assembled, characterized in that it comprises a collection of modular devices from a selection from which said resuscitation apparatus can be assembled, comprising a respirable gas flow valve (26,

221), a range of modular control devices (PB, 36, Figures 15 to 19) each connectible to said valve (26, 221) for operating the same, and a range of modular respirable gas administering devices (52, 74 etc.) connectible to downstream of said valve (26, 221) for administering said gas to a patient's respiratory system.

13. A collection according to claim 12, wherein said range of modular control devices (PB, 36, Figures 15 to 19) comprises a manual control device (PB), a pneumatic automatic control device (Figure 19) and an electrical automatic control device (Figure 15).

14. A collection according to claim 12 or 13, wherein said range of modular administering devices (52, 74 etc.) comprises an injection airway (IA), an injection unit (IUO) designed for oxygen, an adult patient valve (PVA), and a baby patient valve (BV).

15. A resuscitation apparatus, comprising a respirable gas flow valve (26, 221), a control device (PB, 36, Figures 15 to 19) connected to said valve (26, 221) for operating the same, and a respirable gas administering device (52, 74 etc.) connected to downstream of said valve (26, 221) for administering said gas to a patient's respiratory system, characterized in that said control device (PB, 36, Figures 15 to 19) and said administering device (52, 74, etc.) are modular.

16. An apparatus according to claim 15, and further comprising a modular automatic control device (36, Figures 15 to 19) connected to said valve (26, 221) for operating the same, the first-mentioned control device being a modular manual control device (PB).

17. Resuscitation apparatus comprising an inlet (18, 203) connectible to a source of respirable gas, an outlet (20) connectible to a patient's respiratory system, duct means (28) interconnecting said inlet (18, 203) and said outlet (20), a valve (26, 221) interposed

in said duct means (28) and operable automatically, and electrical means (36) connected to said valve (26,221) and arranged to operate said valve (26,221) automatically, characterized in that said electrical means (36) includes electrical switching means (114, 115) arranged to detect the presence or absence of said gas under pressure upstream of said valve (26, 221) and accordingly to energize or de-energize said electrical means (36).

18. A respiratory apparatus including first and second variable flow control valves (211, 221) which serve to control respective flows of gaseous fluid therethrough, characterized in that a non-pneumatic adjusting linkage (218, 223, 224) interconnects the valves (211, 221) so that adjustment of the first valve (211) is accompanied by adjustment of the second valve (221).

19. A valve device, comprising an inlet (271) for fluid, an outlet (272) for said fluid, valve closure means (274) arranged to control flow of said fluid from said inlet (271) to said outlet (272), and a pilot pressure chamber (277, 273) bounded by said valve closure means (274), characterized in that the effective area of said valve closure means (274) exposed to the pressure of said fluid between said inlet (271) and said outlet (272) is less than the effective area of said valve closure means (274) exposed to the pressure in said chamber (277, 273').

20. A valve device comprising a valve housing (231), a valve closure member (230) movable in said housing (231) from an open position to a closed position, an inlet (232) to said housing (231) for fluid, and an outlet (234) from said housing (231) for fluid, characterized in that, as said member (230) moves from said open position to said closed position, initially fluid can flow <u>via</u> a first path from said inlet (232) to a limited degree and <u>via</u> a second path (239) from said

inlet to no more than a limited extent, but subsequently said member (230) enables flow of said fluid via said second path (239) from said inlet 232) to greater than said limited extent, whereby said fluid can flow from said inlet (232) to an increased extent.

21. A valve device comprising a valve housing (231), a valve closure member (230) movable in said housing (231) from an open position to a closed position, a tubular inlet (237) to said housing (231) for fluid, and an outlet (234) from said housing (231) for fluid, said member (230) being in the form of a bobbin (230) closely encircling said tubular inlet (237), characterized in that said bobbin (230) includes first and second flanges of substantially equal external dimension transverse to the bobbin axis.

22. A valve device comprising a first valve including a first valve closure member (251) arranged to open automatically upon occurrence of a predetermined pressure differential thereacross, and a second valve which includes a second valve closure member (253) and through which the first valve exhausts when the second valve closure member (253) is open, characterized in that the second valve closure member is selectively closable and in that there is a third valve which includes a third valve closure member (258) arranged to open automatically upon occurrence of a predetermined pressure differential thereacross and through which the first valve exhausts when the second valve closure member (253) is closed, the arrangement being such that that pressure upstream of said first valve at which said third valve opens is higher than that pressure upstream of said first valve at which said first valve opens.

1/9

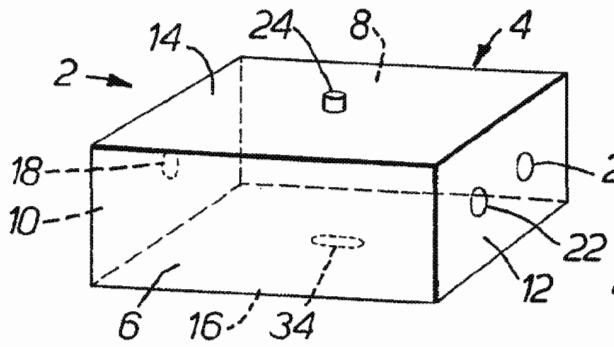


FIG. 1.

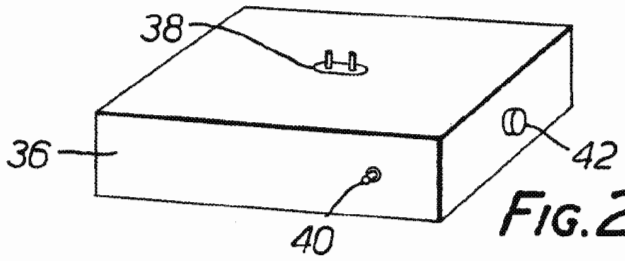


FIG. 2.

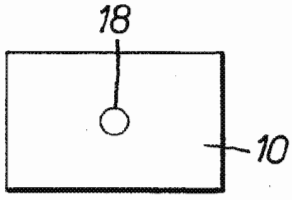


FIG. 3.

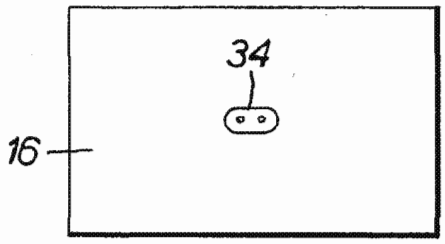


FIG. 4.

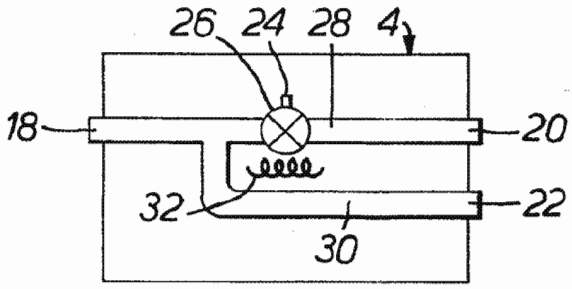


FIG. 5.

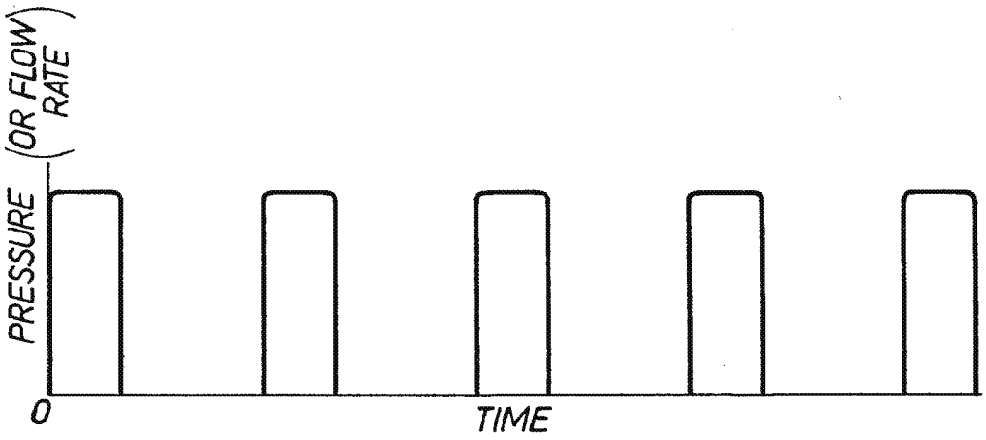


FIG. 6.

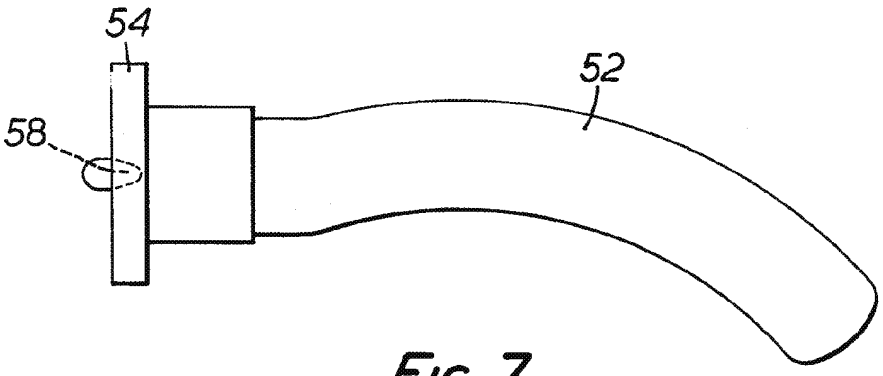


FIG. 7.

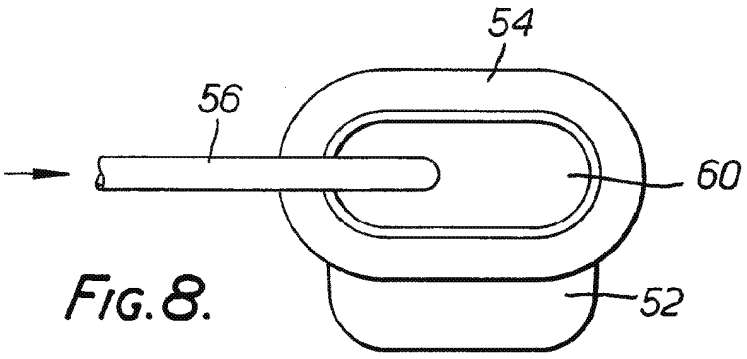


FIG. 8.

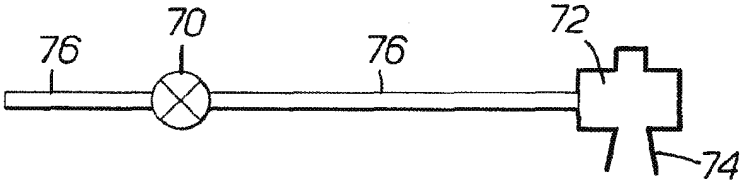


FIG. 9.

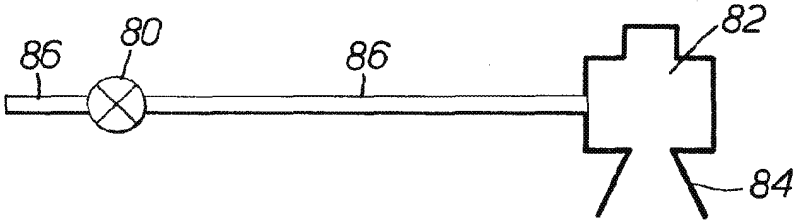


FIG. 10.

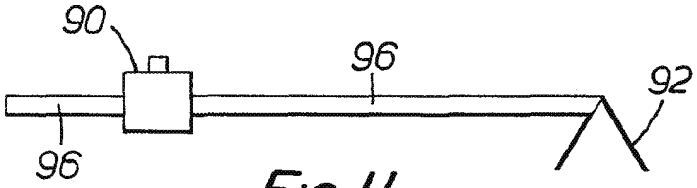


FIG. 11.

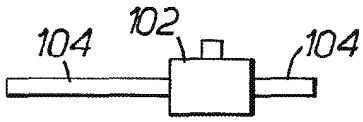


FIG. 12.

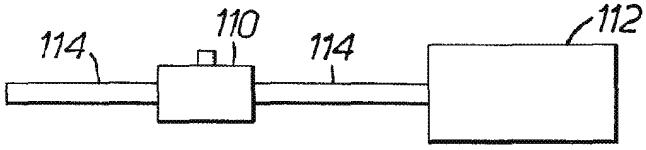


FIG. 13.

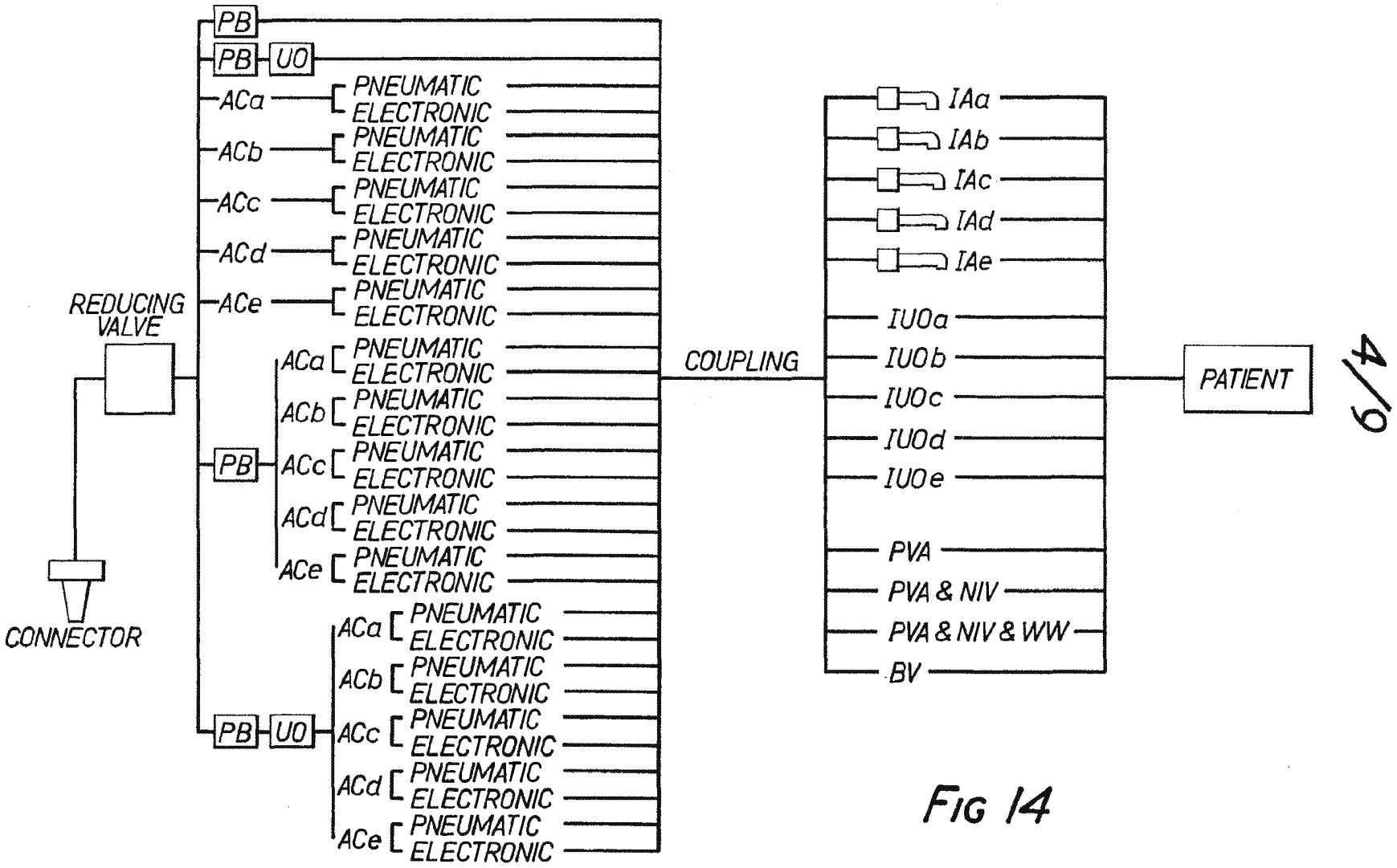


FIG 14

4/9

009706O

SPECTRAL_0000146

5/9

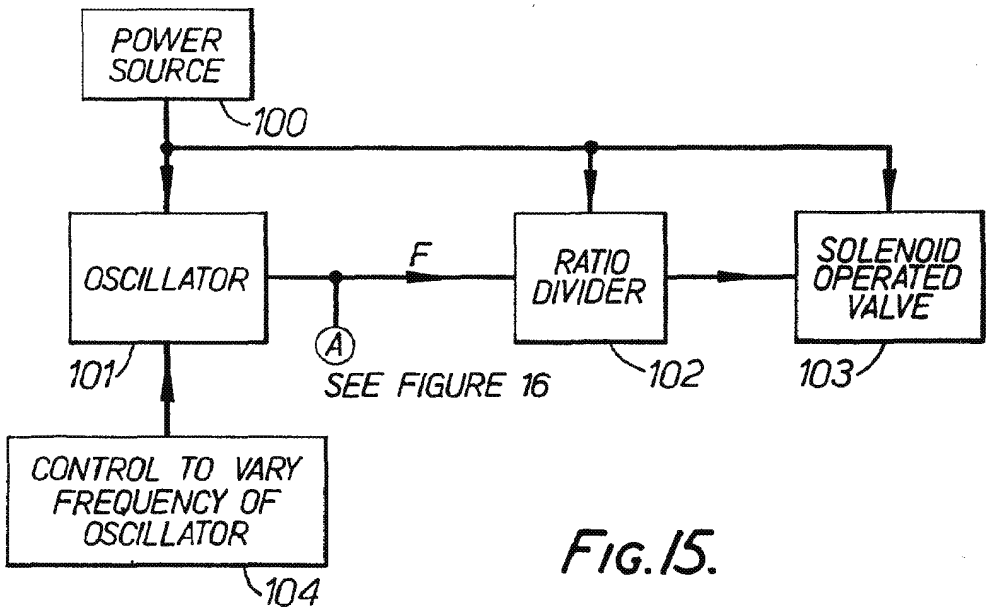


FIG. 15.

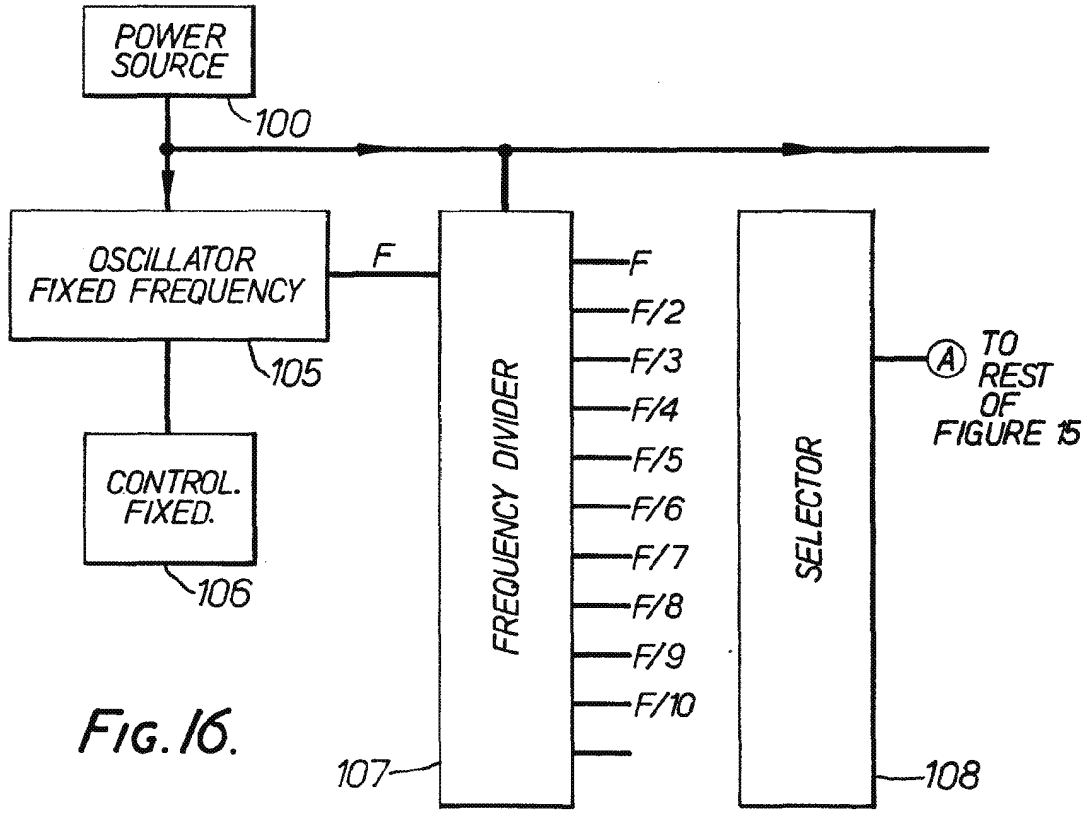


FIG. 16.

SPECTRAL_0000147

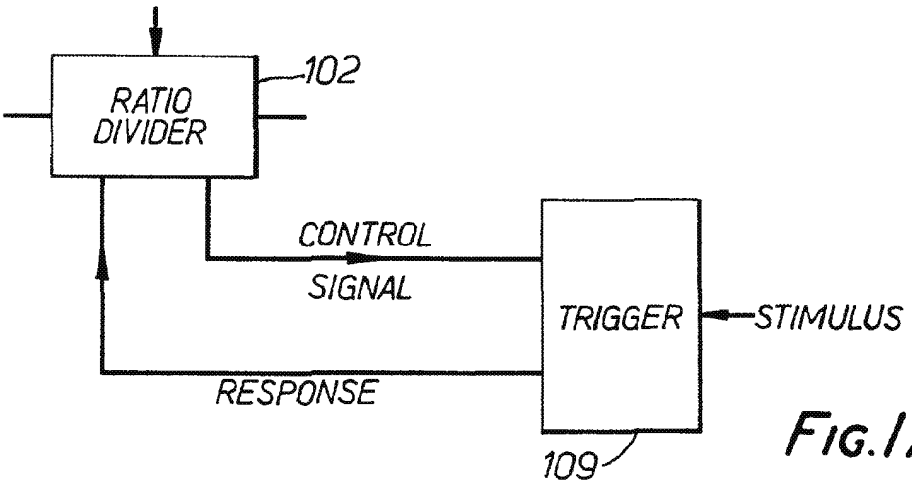

RATIO DIVIDER
102
CONTROL SIGNAL
TRIGGER
STIMULUS
RESPONSE
109

FIG. 17.

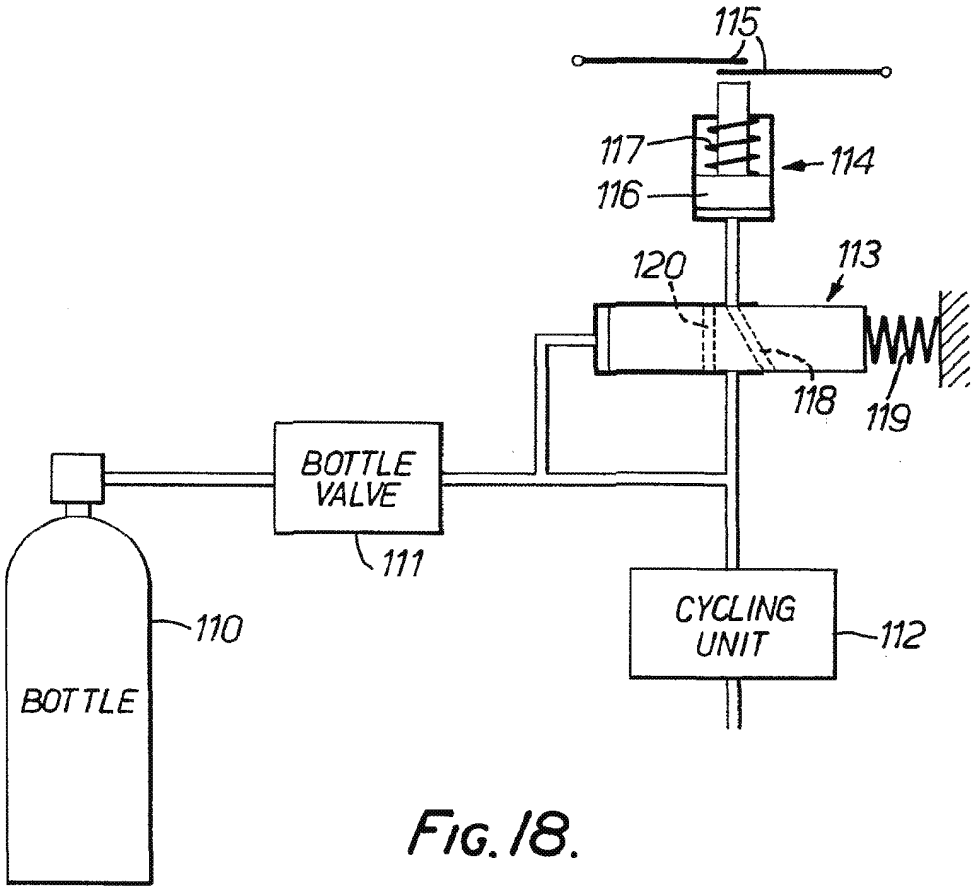

115
117
114
116
120
113
118
119
BOTTLE VALVE
111
110
BOTTLE
CYCLING UNIT
112

FIG. 18.

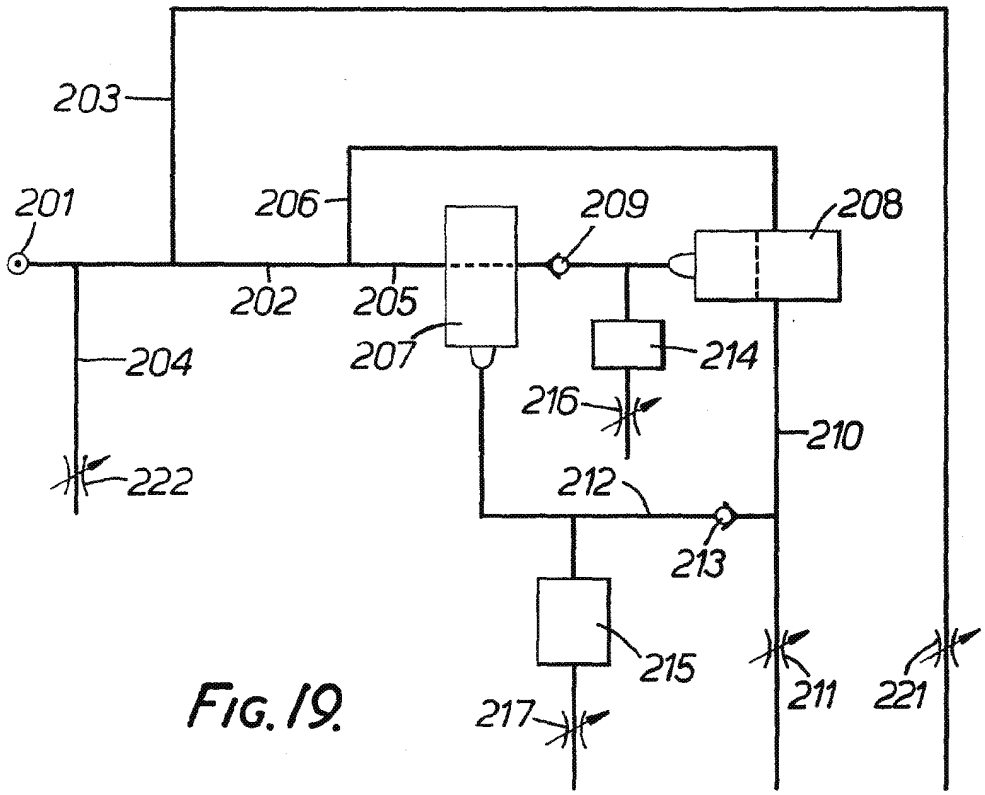

203
201
206
209
208
202
205
207
214
204
216
210
222
212
213
215
211
221
217

FIG. 19.

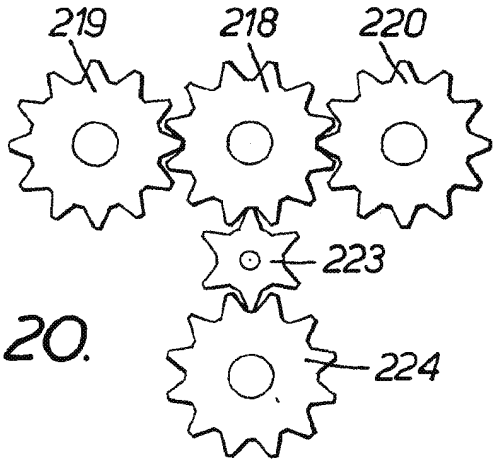

219
218
220
223
224

FIG. 20.

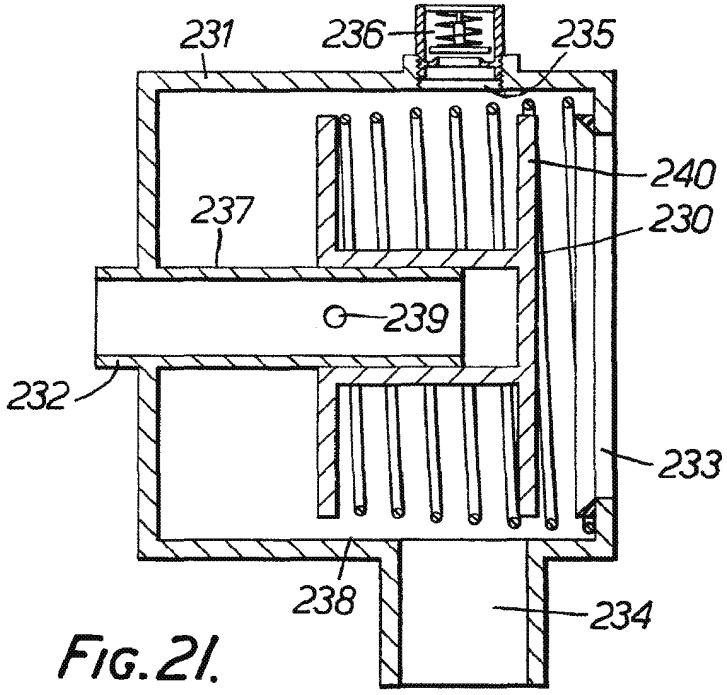

231
236
235
240
237
230
239
232
233
238
234

FIG. 21.

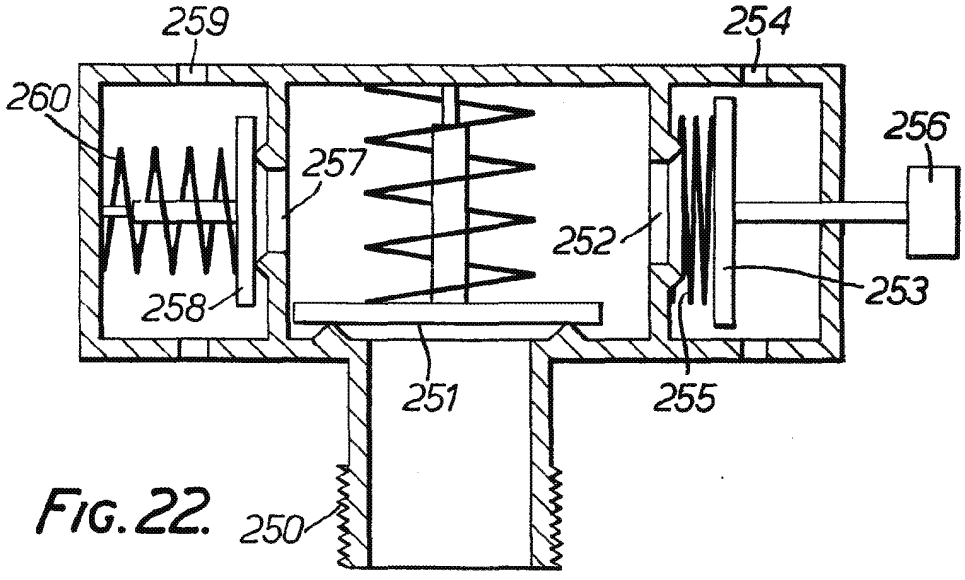

259
254
260
256
257
252
253
258
251
255
250

FIG. 22.

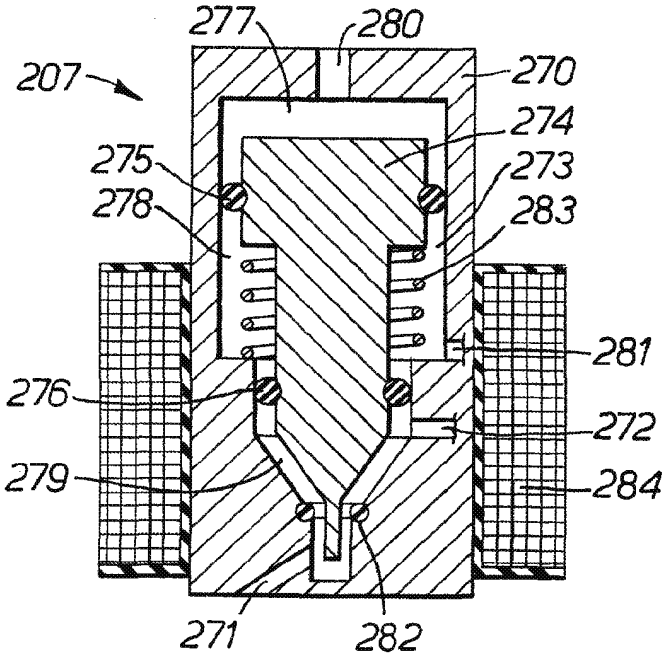

277
280
207
270
274
275
273
278
283
281
276
272
279
284
271
282

FIG. 23.

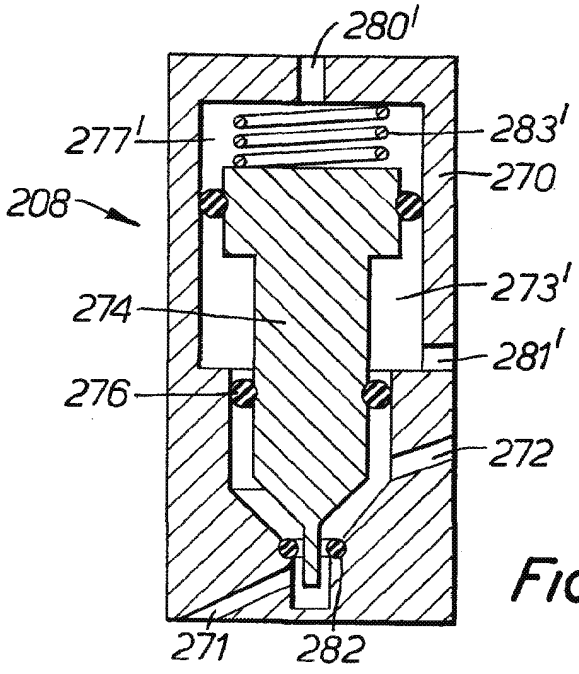

280'
277'
283'
270
208
273'
274
281'
276
272
271
282

FIG. 24.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 228 877 A2**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: **86309923.0**

(22) Date of filing: **18.12.86**

(51) Int. Cl.⁴: **G01N 21/25** , G01N 21/27 , G01J 1/16 , G01R 19/165

(30) Priority: **02.01.86 US 815522**

(43) Date of publication of application: **15.07.87 Bulletin 87/29**

(84) Designated Contracting States: **BE CH DE FR GB IT LI NL SE**

(71) Applicant: **ARTEL, INC.**
28 Depot Street
S. Windham Maine 04082(US)

(72) Inventor: **Curtis, Richard H.**
Wards Hill Road RR2 Box 606
Gorham Maine 04033(US)

(74) Representative: **Read, Matthew Charles et al**
Venner Shipley & Co. 368 City Road
London EC1V 2QA(GB)

(54) **Comparative colorimeter.**

(57) A comparative colorimeter for use in the field which simultaneously compares the color densities of two liquid samples and designates the degree of difference. The apparatus includes two major subsystems, optical and electronic. The optical subsystem is designed to provide identical light beams through both the sample and standard solutions and to minimize the effect which imperfect sample tubes have on the output. The electronic subsystem includes a log conversion and differential amplifier circuit for generating a difference signal representative of the difference between the optical densities of the sample and standard solutions. The difference signal is evaluated by a comparative circuit consisting of a resistive chain having a plurality of node voltages and hex inverters connected to the node voltages. The hex inverters drive a series of LED indicator lights to designate the relative degree of difference between the optical densities of the sample and standard. A correction circuit is provided for referencing the difference signal to the threshold voltage of the hex inverters and for compensating for initial differences in the optical paths of the sample and standard solutions.

EP 0 228 877 A2

COMPARATIVE COLORIMETER

This invention relates to a comparative colorimeter, and more specifically to an instrument which simultaneously compares the color (i.e., optical) densities of two liquid samples and designates the degree of difference.

A colorimeter is useful for conducting:

(a) medical diagnostic tests based on enzyme immuno assays (EIA), e.g., the test for Leutenizing Hormone as an indication of ovulation in humans;

(b) medical diagnostic tests based on Enzyme Multiplied Immuno assay Technique (EMIT), e.g., tests for drugs of abuse such as barbituates;

(c) veterinary diagnostic tests based on either (a) or (b), e.g., progesterone level in dairy cows;

(d) environmental tests based on either (a) or (b), e.g., residual Chlorodane levels after extermination around residences; and

(e) determination of the concentration of certain chemicals based on absorption of a given wavelength of light, e.g., concentration of the herbicide Dynaseb™.

In the above-identified tests, it is generally not the absolute value of optical density of a solution which is of interest, but rather a comparison of a sample solution with a standard solution. Further, while all of these tests can be satisfactorily performed in the laboratory using a standard off-the-shelf spectrophotometric instrument, such instruments are delicate and expensive and are not adapted for use in the field. For example, a colorimeter is useful in the field to determine whether the residual level of a pesticide applied to a crop has declined sufficiently to allow safe entry of personnel into the field for harvesting operations. A known colorimeter used in the field performs separate quantitative determinations of the optical densities of a sample solution and a standard solution. The human operator then computes the difference between the quantitative determinations. This device has several disadvantages. First, since the operator is generally interested in only the relative difference between the sample and standard, it is a waste of effort to make a quantitative determination for each of the sample and standard. Further, since the quantitative determinations of the sample and standard are made at different times, there exists a large potential for error if either of the solutions changes in optical density with respect to time or temperature.

It is an object of the present invention to provide a relatively low-cost comparative colorimeter for use in the field for comparing the optical densities of two solutions and indicating the relative degree of difference.

It is another object of this invention to provide a comparative colorimeter for simultaneously evaluating the optical densities of sample and standard solutions prepared at the same time in order to eliminate any error caused by changes in the optical densities of the sample and standard with respect to time.

It is a further object of this invention to provide an optical subsystem for a colorimeter which reduces the error caused by defects in the sample tube such as scratches, irregularities or striations in the tube wall, and lack of concentricity.

It is still another object of this invention to provide an electronic subsystem, including low-cost digital integrated circuits, for quantifying the difference between the optical densities of the sample and standard solutions.

It is a still further object of this invention to provide a correction circuit to compensate for variations in the threshold voltage of the digital IC components with respect to batch, time, temperature, and supply voltage.

It is still another object of this invention to provide a correction circuit to compensate for differences in the optical paths of the sample and standard solutions.

The apparatus of this invention provides a means for simultaneously comparing the optical densities of two solutions and generating an output indicative of the degree of difference. The apparatus includes two major subsystems, optical and electronic.

The optical subsystem is designed to provide identical light paths through two liquid samples disposed in optically transparent sample tubes and to minimize the effect which imperfect sample tubes have on the measurement. The optical subsystem includes a light source means for transmitting a pair of substantially equal light beams, one to each of a pair of photodetectors. Each of the two liquid samples are simultaneously and identically positioned between the light source means and an associated photodetector. The photodetectors generate a pair of optical density output signals representative of the amounts of light passing through the samples and reaching the photodetectors. The optical system is designed to spread the light over a substantial portion of each sample tube to minimize the effect of defects in the tubes.

In one embodiment of the optical system of this invention, a collimating lens is positioned between the sample tube and light source and spaced slightly closer to the light source than the focal point of the lens so that the lens transmits a slightly divergent beam of light to the tube. The tube is spaced with respect to the lens so that the outer diameter of the divergent beam at the center plane of the tube is substantially equal to the inner diameter of the tube. The sample tube acts as a cylinder lens to focus the beam at a vertical focal line, and the photodetector is spaced slightly ahead of the focal line so that the beam strikes a substantial portion of the photodetector. The photodetector output is substantially independent of defects in the sample tube.

Preferably, the optical system further includes a combination filter/diffuser positioned between the sample tube and photodetector. The filter/diffuser includes a filter for selecting a predetermined wavelength of light and a light diffusing layer for averaging the effect of individual defects in the sample tube so that the photodetector output is less affected by such defects. Preferably, the light diffusing layer is disposed over the surface of the filter adjacent the photodetector. In a further preferred embodiment, a plurality of such combination filter/diffusers are connected in serial arrangement and the photodetector is positioned adjoining a light diffusing layer at one end of the series.

In a second embodiment of the optical system of this invention, a first lens set is positioned between the sample tube and light source. The first lens set includes a first collimating lens disposed adjacent the light source and having a focal point at the light source, and a first converging lens disposed adjacent the sample tube and having a focal point at the center of the sample tube. A second lens set is positioned between the sample tube and photodetector. The second lens set includes a second collimating lens disposed adjacent the sample tube and having a focal point at the center of the tube, and a second converging lens disposed adjacent the photodetector and having a focal point at the photodetector. Because in this second embodiment the light rays pass perpendicularly through the tube and the tube has no focusing effect, refraction is reduced and irregularities in the tube do not affect the focus point. Thus, the accuracy of this second embodiment is less dependent on the spacing between the lenses and tube than that of the first embodiment. A filter may be positioned between the two lenses of the second lens set, and because the light passes through the filter in parallel rays, there is minimal reflection at the filter surface and thus minimal light loss.

The electronic subsystem of this invention provides a high level of accuracy and stability despite the stringent cost limitations imposed by the intended application. In particular, the circuit utilizes low-cost digital components to achieve a quantified output indicative of the degree of difference in optical densities of the two samples. A correction circuit is provided to compensate for variations in the threshold voltage of the digital IC components and to compensate for differences in the optical paths of the two samples.

The electronic subsystem includes a differential circuit means for generating a difference signal representative of the difference between the optical density output signals of the photodetectors. The system further includes a comparative circuit including means for comparing the difference signal to a plurality of predetermined references and a means for indicating the relative value of said difference signal with respect to said predetermined references.

In a preferred embodiment, the current outputs of a pair of photodiodes are logarithmically converted to voltage signals and the resultant voltage signals are differentially amplified to produce a difference signal. The difference signal is sent to a resistive chain including a plurality of nodes, each node having a different predetermined reference voltage. A plurality of digital gate means each having the same threshold voltage are each connected to one of said nodes. The outputs of the gate means activate a plurality of indicator means to indicate the relative value of the difference signal with respect to the predetermined reference voltages.

In addition, a correction circuit is provided for referencing the difference signal to the threshold voltage of the gate means. The correction circuit includes an additional gate means from the same batch used in the comparative circuit. The output of the correction circuit, which is referenced to the threshold voltage of the gate means, is applied to the comparative circuit so that the difference signal is referenced to the threshold voltage. Further, the correction circuit includes a zeroing mechanism to compensate for differences in the light paths of the two sample solutions.

Fig. 1 is a perspective view of the comparative colorimeter of this invention.

Fig. 2 is an illustrative top view of a first embodiment of the optical subsystem of this invention.

Fig. 2A is an illustrative top view of the filter/diffuser and photodetector combination of this invention.

Fig. 3 is an illustrative top view of a second embodiment of the optical subsystem of this invention.

Fig. 4 is an illustrative top view of a prior art optical system.

Fig. 5 is a schematic diagram showing the circuit means for logarithmically converting the current outputs of the photodiodes to voltage signals and means for differentially amplifying the resultant voltage signals to produce a difference signal.

Fig. 6A is a schematic diagram of three major portions of the electronic subsystem of this invention, including the log conversion and differential amplifier circuit, the correction circuit, and the comparative circuit.

Fig. 6B is a schematic diagram of the supply regulator circuit and an incandescent light source.

Fig. 6C is a schematic diagram of an alternative light source comprising two LED's.

Fig. 7 is an illustration of the transfer characteristic for an IC digital hex inverter.

Fig. 1 is a perspective view of the comparative colorimeter of the present invention. A housing 10 contains the optical and electronic subsystems of this invention. A pair of sample tube holders 11, 12 consist of tubular bores disposed vertically in the housing and having closed lower ends and open upper ends in the top wall 13 of the housing. The holders snugly engage two cylindrical sample tubes 5, 6 which contain the sample and standard solutions respectively. A row of five indicator lights 15, 16, 17, 18, 19 is provided on the front wall 14 of the housing. An on-off button 20 is provided on top wall 13 of the housing. The housing is made of a nonbreakable plastic such as acrylonitrile-butadiene-styrene polymer. The device shown in Fig. 1 is compact and portable, being 5" (127 mm) wide, 2.5" (64 mm) high, 3" (76 mm) deep, and weighing about 200 grams. Typically, sample tubes 5, 6 are made of polystyrene and have outer dimensions of 12 mm in diameter and 75 mm in length, and an inner diameter of 9 mm.

The comparative colorimeter of this invention consists of two major subsystems, optical and electronic. The important features of the optical subsystem include:

(a) it must provide equal light paths through both of the sample tubes; to the extent that the light paths are not identical, then the ratio of light going through them must be constant despite: the buildup of dirt and contamination; thermal effects; warping or flexing of the printed circuit board or other structural members; mechanical shock and abuse; and aging of various components, e.g., light bulb and filters;

(b) it must provide adequate light intensity at the photodiodes under all operating conditions;

(c) it must minimize the effects of stray or ambient light; and

(d) it must minimize the effect of imperfect sample tubes.

In a laboratory spectrophotometric instrument, the samples are held in square cuvettes with ground and polished plane sides so that the sample container contributes little or no uncertainty to the measurement. In the field it is desirable to use inexpensive and disposable cylindrical plastic test tubes. However, these test tubes may contribute significantly to the uncertainty of the measurement. A typical plastic tube suffers three principal defects:

(a) it may be scratched to a greater or lesser degree, depending on the care with which it is handled; these scratches tend to scatter and diffuse light, making the light path indeterminate;

(b) it may contain striations or irregularities in the wall of the tube, which will refract light in an indeterminate way; and

(c) the tube's exterior and interior may not be concentric, so that the actual path length and direction of a light ray passing through the sample depends on the orientation of the tube.

The degree to which these imperfections contribute uncertainty is highly dependent on the details of the optical design.

In the prior art design shown in Fig. 4, a narrowly collimated or focused beam of light from a lamp 200 passes through a sample tube 201 and then through a lens 202 which focuses the beam on a photodetector 203 which is sensitive to a predetermined wavelength. A screen 205 having an aperture 206 is disposed between the lamp and tube to prevent stray light from reaching the photodetector. A filter 204 is disposed between the lens and photodetector for selecting light of a predetermined wavelength. In this prior art design, it is desirable to pass a very narrow beam of light through the center of the tube only so that the light beam passes through a sample area having a roughly uniform thickness. This is required where a quantitative determination of absorbance is being made and it is necessary to know the dimensions of the sample through which the beam passes. However, individual defects in the area of the sample tube through which the beam passes have a significant effect on the photodetector output.

All of the above-mentioned defects in the plastic sample tubes affect the measurement results of this prior art design very severely. Typically, a measurement in absorbance may contain an error of .05 to .1 as a result of tube defects alone. The amount of error is highly dependent on the individual tube under test and its orientation.

Two designs have been found to be highly effective at minimizing the errors caused by sample tube defects. These designs are shown in Figs. 2 and 3.

A first embodiment of the optical subsystem is shown in Fig. 2. A pair of photodetectors 30, 31 are positioned at opposite ends of an axial line. A light source means 32, such an incandescent lamp, is centered at the midpoint of the axial line. A first sample tube 5 and a second sample tube 6 are identically positioned on the axial line on opposite sides of the lamp, between the lamp and an associated photodetector. A collimating lens 33A(33B) and a screen 34A(34B) having an aperture 35A(35B) are identically positioned on either side of the lamp between the associated sample tube 5(6) and lamp 32. A filter/diffuser 36A(36B) is positioned between the associated sample tube 5(6) and associated photodetector 30(31).

The object of this first design is to spread the illumination over substantially the entire width of the sample tube so that the net light transmitted to the photodetector is averaged over many scratches and defects and is minimally dependent on the lack of concentricity of the inner diameter and outer diameter of the tube. While the operation of the optical system with respect to sample tube 5 is described herein, the operation with respect to sample tube 6 is identical.

The collimating lens 33A is positioned between the sample tube 5 and light source 32 and spaced slightly closer to the light source than the focal point of the lens so that the lens transmits a slightly divergent beam to the sample tube. The screen 34A prevents stray light beams from reaching the photodetector 30. Further, the height of the aperture 35A is restricted to prevent light from hitting the meniscus of the liquid sample in the tube or the bottom of the tube and reflecting into the photodetector. The sample tube 5 is spaced with respect to the lens 33A such that the outer diameter of the divergent beam at the center plane of the tube is equal to or slightly less than the inner diameter d of the tube. The sample tube acts as a cylinder lens to focus the beam at a vertical focal line and the photodetector is spaced slightly ahead of the focal line so that the beam strikes a large area of the photodetector. This insures a photodetector signal of large amplitude and averaged value.

The combination filter/diffuser 36A disposed between the sample tube 5 and photodetector 30 consists of a filter glass 39 for selecting a predetermined wavelength of light and a diffusive sheet or film 40 covering the surface of the filter glass adjacent the photodetector. For example, film 40 may be Mylar™ film frosted on both sides and about .006" (.15 mm) thick. Because scratches and defects in the sample tube cause light to bounce in a random manner, the diffusive sheet 40 is provided to produce an averaging effect so that the photodetector output is less dependent on defects in the sample tube. Preferably, the photodetector 30 is sensitive to the same predetermined wavelength as the filter glass 39.

A preferred embodiment of the filter/diffuser is shown in Fig. 2A. Two pieces of filter glass 41, 43 and two sheets of diffusive film 42, 44 are sandwiched together and disposed adjoining a photodetector 45. Diffusive sheet 42 is sandwiched between filter glasses 41 and 43, and diffusive sheet 44 is sandwiched between filter glass 43 and photodetector 45. This filter/diffuser is preferred because the addition of a second diffusive film separated from the first aids in a more uniform diffusion of the light. The use of a second filter glass is necessary in most cases in order to limit the bandwidth of the light transmitted. Further, adjoining the photodetector 45 to diffusive layer 44 eliminates light loss and increases the amplitude of the photodetector output.

A second embodiment of the optical subsystem of this invention is shown in Fig. 3. Again, the design spreads the light beam over a large area of possible defects in the tube. A first lens set 50A includes a pair of diametrically opposed lenses 51A, 52A positioned on an axial line between the sample tube 5 and light source 32. Collimating lens 51A is disposed adjacent the light source and has a focal point at the light source. Converging lens 52A is disposed adjacent the sample tube and has a focal point at the center of the sample tube. A second lens set 54A includes a second pair of diametrically opposed lenses 55A, 56A positioned on the axial line between the sample tube 5 and photodetector 30. Collimating lens 55A is disposed adjacent the sample tube and has a focal point at the center of the sample tube and converging lens 56A is disposed adjacent the photodetector and has a focal point at the photodetector.

In the second embodiment, because the light rays pass perpendicularly through the sample tube and the tube has no focusing effect, irregularities of the tube do not affect the focal point. Thus, the space between the lenses and sample tube is less critical in this design. Furthermore, in the second embodiment a filter 57A can be placed between the two lenses 55A, 56A of the second lens so that the light rays pass through the filter as parallel rays. This minimizes refractions at the surface of the filter and thus reduces light loss.

In Fig. 3, the first and second lens sets 50B and 54B between the lamp 32, sample tube 6 and photodetector 31, are identical to those of 50A and 54A previously described.

To evaluate the effectiveness of the designs shown in Figs. 2 and 3, in comparison with the prior art design of Fig. 4, a series of measurements was taken with one particular sample tube filled with a sample solution. Eight different orientations of the tube were tried for each design to find the range of readings resulting from the one sample and tube. The resulting inaccuracy can be expressed as an error in absorbance as follows:

$\Delta A = \log[(I_{max} - I_{min})/2I_{av}]$ where I_{max} = maximum light intensity resulting from the series of readings for a given sample, tube and optical design;

I_{min} = minimum light intensity resulting from the series of readings for a given sample, tube and optical design; and

I_{av} = average light intensity resulting from the series of readings for a given sample, tube and optical design.

Design 1 was that shown in Fig. 2 with lens 33A having a diameter of 5/16" and a focal length of 3/8". Lens 33A was spaced .27" from lamp 32, screen 34A, having an aperture .125" x .275", was spaced .40" from lens 33A, tube 5 was spaced .35" from screen 34A, and photodiode 30 was spaced .65" from tube 5 - (all distances are from center point of element). The filter/diffuser used was that shown in Fig. 2A wherein 41 was a 2 mm thick filter glass, model GG-435 by Schott, imported by ESCO Products, Inc. of Oak Ridge, New Jersey, and 43 was a 4.0 mm thick filter glass, model 5-58 by Corning Glass Works of Corning, New York. Each filter glass had a diameter of 5/16". The diffuser sheets 42 and 44 were Mylar™ film, each .006" thick and frosted on both sides. The photodiode was adjoining sheet 44.

Design 2 was that shown in Fig. 3 with lenses 51A, 52A, 55A and 56A each having a diameter of 3/8" and a focal length of 1/2". The first lens set was spaced .5" from lamp 32, tube 5 was spaced .5" from the first lens set, lens 55A was spaced .5" from tube 5, lens 56A was spaced .25" from lens 55A, and photodiode 30 was spaced .5" from lens 56A (all distances are from center point of element). Two filter glasses, the same as 41 and 43 described in design 1 (but without diffusive sheets 42 and 44) were used. In design 2, the distance between the lenses and sample tube could be changed up to 1/16" (1.6 mm) without changing the photodetector reading significantly. In comparison, in design 1, the sample tube had to be spaced within 5/1000" (.13 mm) with respect to the lens and with respect to the axial line extending between the lamp and photodetector to insure a consistent photodetector reading.

The prior art design was that shown in Fig. 4 with lens 202 having a diameter of 5/16" and a focal length of 3/8". Screen 205 had an aperture 206 of 1/10" in height. The same two filter glasses described for design 2 were used.

In all designs an incandescent lamp was used, model 158X sold by Gilway of Woburn, Massachusetts. The sample tube was made of polystrene having a length of 12 mm, an outer diameter of 75 mm, and an inner diameter of 9 mm. The photodiode was model S-1133 sold by Hamamatsu of San Jose, California.

The following values of ΔA were obtained for the prior art design and designs 1 and 2 of the present invention:

PRIOR ART DESIGN ΔA = .05

DESIGN 1 ΔA = .01

DESIGN 2 ΔA = .004

Thus, it has been shown that both design 1 and design 2 of this invention give substantially better results than the prior art design. While it would appear that design 2 is preferable over design 1, other factors such as cost, size, complexity and the required accuracy of construction mitigate in favor of design 1.

The electronic subsystem of the present invention, as shown in Fig. 6A, performs the following functions:

(a) the current outputs I_{D1} and I_{D2} of the two photodiodes PD1 and PD2 are logarithmically converted to voltage signals;

(b) the two voltage signals are differentially amplified at A3 to produce a difference signal E_o;

(c) the difference signal E_o is compared to a plurality of predetermined reference voltages with a series of voltage comparators, consisting of digital gate means G_1-G_5 and resistive chain R12-R17;

(d) the digital gate means drive a plurality of indicator LEDs 1-5 (corresponding to indicator lights 15-19 in Fig. 1) to indicate the relative value of the difference signal with respect to the reference voltages; and

(e) a correction circuit is provided so that the difference signal E_o is referenced to the threshold voltage E_T of the digital gate means.

The indicator lights or LEDs 15-19 of Fig. 1, which correspond to LED's 1-5 of Fig. 6A respectively, indicate the degree of difference in optical density between the samples in the tubes. If the sample and standard solutions have equal optical densities, then a middle green LED 17 turns on to indicate the same. If the solution of tube 5 has a lesser optical density than the solution of tube 6, when the difference exceeds

a first predetermined level the comparator will trigger a right center red LED 18 and simultaneously extinguish the green LED, and if the difference exceeds a larger sound predetermined level, then a right red LED 19 will light. At no time is more than one indicator LED on. If the solution of tube 5 has a greater optical density than the solution of tube 6, then the left center red LED 16 and left red LED 15 will light in the same manner as described for those to the right of center. A typical configuration is for the right center LED 18 (and left center LED 16) to come on at ΔA = .03 O.D. (difference in absorbance between the tubes is .03), and the right LED 19 (and left LED 15) to comes on at ΔA = 0.1 0.D.

The operating voltage V_{REG} for the lamp and the electronic subsystem is stabilized at 2.000 volts by the supply regulator circuit shown in Fig. 6B. The circuit includes amplifier A4, capacitors C1, C2, C7, C8, resistors R1, R2, R3 (variable), R4, R7, R22, R23, transistors Q1, Q2, battery V+, and internal regulator V_{REF}. A range of battery voltages V+ from 3.0 to 2.1 volts can be accommodated without degrading performance. Lamp life is extended substantially by operating the lamp 32 at a constant 2.0 volts.

The regulator circuit is built around one section of a amplifier A4 specifically intended for voltage regulation. A4 in this preferred embodiment is amplifier U4 LM10CLN sold by National Semiconductor of Santa Clara, California. The LM10CLN drives transistors Q1 and Q2 as required to regulate V_{REG} to exactly 2.000 volts. An internal regulator provides a constant V_{REF} of .2 volts. V_{REG} is divided down to .2 volts and then compared to this reference. V_{REF} is also used to determine the operating points of the operational amplifiers A1 and A2 as shown in Fig. 6A.

To compute the difference signal E_o, the two diode currents I_{D1} and I_{D2} are logarithmically converted to voltage signals and the two voltage signals are differentially amplified. The stability of the logarithmic conversion and differential amplification is such that an overall electronic error budget of .005 O.D. is not violated over the life of the instrument.

Fig. 5 shows the log conversion and differental amplifier circuit for generating the difference signal E_o. Light from light source 32 which passes through sample tube 5 and strikes photodiode PD1 generates a current I_{D1}. Likewise, light from light source 32 which passes through sample tube 6 and strikes photodiode PD2 generates a current I_{D2}. The photodiode currents can be expressed as:

$I_{D1} = k_1 g_1 f_1 I$ (1)

$I_{D2} = k_2 g_2 f_2 I$ (2)

where I is the total intensity of light source 32, f_1 and f_2 are the fractions of light absorbed by the samples in tubes 5 and 6 respectively, g_1 and g_2 are the fractions of light passing through tubes 5 and 6 which actually reach the photodiodes PD1 and PD2 respectively, and k_1 and k_2 are the efficiencies with which the photodiodes PD1 and PD2 convert the light reaching their surfaces respectively.

The diode currents I_{D1} and I_{D2} constitute optical density signals representative of the optical densities of the samples in tubes 5 and 6 respectively. The diode currents I_{D1} and $I_{D2}$are logarithmically converted to voltage signals E_1 and E_2 by a pair of identical log conversion circuits shown in Figs. 5 and 6A. Thus, amplifier A1, capacitor C11, and transistor Q4, arranged in parallel, convert I_{D1} to E_1, and similarly, amplifier A2, capacitor C12, and transistor Q5, arranged in parallel, convert I_{D2} to E_2. A1 and A2 are from the same package and are identically powered by connection to the regulated supply voltage V_{REG} and decoupling capacitor C3 (as shown for A2) and grounded (as shown for A1). Because the log conversion circuits are identical, the votages E_1 and E_2 may be expressed as:

$E_1 = K\log I_{D1}$ (3)

$E_2 = K\log I_{D2}$ (4)

where K is a constant.

The voltage signals E_1 and E_2 are differentially amplified at A3 to produce a difference signal E_o as set forth below:

$E_o = G(E_2 - E_1) + E_c$ (5)

where G is the amplifier gain and E_C a correction voltage. The correction voltage E_c is added to the positive input of A3 for reasons discussed hereinafter. The differential amplifier circuit includes differential amplifier A3, capacitor C4, and resistors R8, R9, R10, and R11, where R8 = R10 = R_A and R9 = R11 = R_B. The amplifier gain $G = R_B/R_A$ is maintained constant through careful selection of the gain resistors. Selecting the value of the gain is also described hereinafter.

By substituting equations 1-4 into equation 5, the following equation for E_o is obtained:

$E_o = K\log[(k_2 g_2)/(k_1 g_1)] + K\log(f_2/F_1) + E_c$ (6)

The ratio of photodiode efficiencies k_2/k_1 is constant over time given equal wavelength of the incident light, equal temperature and equal reverse bias. Dark current will drift somewhat but is not a limitation in this application.

The ratio g_2/g_1 of optic path efficiencies depends on the details of the design and its implementation. If we assume perfect sample tubes and an unchanging rigid geometry, then the ratio g_2/g_1 is fixed.

Since the amplifier gain G is maintained constant through careful selection of gain resistors, and K is a constant, the entire first term in (6) is a constant C. Thus, the difference signal E_o can be represented as follows:

$E_o = C + KGlog(f_2/f_1) + E_c$ (7)

C can be compensated for by a simple additive offset applied at the input of the differential amplifier A3. This is accomplished by the correction circuit discussed hereinafter. The connection circuit further references the difference signal E_o to the threshold voltage E_T of the hex inverters used in the comparative circuit so that $E_o = E_T$ when the optical densities of the two samples are equal.

The difference signal E_o generated by A_3 is sent to the comparative circuit shown in Fig. 6A, wherein E_o is compared to a series of predetermined reference voltages to determine to the relative value of the difference signal with respect to the reference voltages. The act of comparison is performed by a plurality of low cost digital integrated circuits. In the preferred embodiment, hex inverters such as the 74HC04 sold by Motorola of Austin, Texas are used as the digital IC gates means. The hex inverters are used in the configuration shown in Fig. 6A in order to economize on parts count and cost.

The output from the differential amplifier E_o is divided by a resistive chain so that the voltage at each node of the chain is directly related to E_o. The resistive chain consists of resistors R12, R13, R14, R15, R16 and R17 arranged in series between V_{REG} and ground. Connected to each node is an input of one of hex inverter gates G1, G2, G3, and G4. Gates G1-G4 are all in the same package and each is connected to the regulated supply voltage V_{REG} and to decoupling capacitor C5 (as shown for G3) and to ground (as shown for G4). If the node happens to be above the switching threshold of the gate, which for the 74HC04 is about 1/2 of the regulated supply voltage V_{REG}, then the gate output is low. Conversely, if the node is below the threshold, then the gate output is high. All of the gates are selected from a single package so they have very nearly the same threshold for a given temperature and supply voltage.

The outputs of gates G1-G4 are connected to drive indicator LEDs 1-5. Thus, in addition to the resistive chain and hex inverter gages G1-G4, the comparative circuit further includes indicator LEDs 1-5, resistors R19, R20 and R21, diode D3, transistor Q3, and hex inverter gate G5 as shown in Fig. 6A. The indicator LEDs are arranged such that only one LED is on at a time. The operation of the comparative circuit is thus understood by determining its operation under three circumstances, when the optical densities of the two samples are equal, when the optical density of the first sample is greater than the second, and when the optical density of the second sample is greater than the first.

Thus, when $I_{D1} = I_{D2}$, i.e., the optical densities of the two samples are equal, then the voltage at TP4 - (E_o) will be E_T. The gate outputs of G2 and G1 will be low so that the right center red LED 2 and right red LED 1 will be off. The gate outputs of G3 and G4 will be high so that the left center red LED 4 and left red LED 5 will also be off. However, because the gate output of G5 and G3 will both be high, transistor Q3 will conduct and the green middle LED 3 will be on indicating that the two solutions have equal optical densities.

By way of further example, if I_{D1} is greater than I_{D2} by up to a first predetermined value, e.g., .03 O.D., then the outputs of G2, G3 and G4 will be high and the output of G1 will be low. All LED's will be off except for the right center red LED 2 which will be on to indicate that the absorbence of the sample in tube 6 is greater than the absorbence of the sample in tube 5, but by no more than .03 O.D. If the absorbence of the sample in tube 6 is greater than that of the sample in tube 5 by at least a second predetermined value, e.g., 0.1 O.D., then the left red LED 1 will be on, and all other LED's off.

Similarly, if I_{D1} is less than I_{D2} by up to .03 O.D., then the outputs of G1, G2 and G3 will be low and G4 will be high. Thus the left center red LED 4 will be on to indicate that the absorbence of the sample in tube 5 is greater than the absorbence of the sample in tube 6 by no more than .03 O.D. Further, if the absorbence of the sample in tube 5 is greater than that of the sample in tube 6 by at least 0.1 O.D., then the left red LED 5 will be on and all other LED's off.

The correction circuit provides both a zeroing mechanism to account for the fact that the two light paths and photodetectors are not truly identical and further provides a compensation factor to account for variations in the threshold voltage E_T of the hex inverters used in the comparative circuit. The output of the correction circuit shown in Fig. 6A is a correction voltage E_c which is applied to the positive input of A3.

The correction circuit includes a hex inverter G6 from the same package as the hex inverters G1-G5 used in the comparative circuit. A trim potentiometer R18, having an output voltage E_p, is provided along with resistors R25 and R26 and capacitors C9 and C10.

The switching threshold of the hex inverter G6 is about one-half of the regulated supply voltage V_{REG}. This logic gate is quite abrupt in its action and all of the gates in the package have very nearly the same threshold voltage E_T for a given temperature and supply voltage. The transfer characteristic of the hex inverter is shown in Fig. 7. The slope of the transfer characteristic in the region of E_T is the gain of the hex

inverter if it is considered as an amplifier. With the gate output tied to the input, the input and output will stabilize at a value which is called the threshold or switching point E_T. The absolute value of E_T varies from one batch of devices to another, and varies according to time, temperature, and supply voltage. All of the inverters in a package have the same threshold within a few milivolts. By utilizing G6 in the correction circuit as an amplifier as shown in Fig. 6A, the difference signal E_o is referenced to the threshold voltage of G6.

Another function of the correction circuit is to compensate for differences between the optical paths during the initial set-up procedure. For this purpose, the trim-potentiometer R18 is introduced to the circuit. The circuit will stabilize with the input voltage at E_T if the trim-pot is adjusted to a value Ep, where

$$(E_p - E_c)R_d = (E_p - E_T)(R_c + R_d) \quad (8)$$

where $R26 = R_c$ and $R25 = R_d$.

During initial calibration of the device, f_2 is made equal to f_1 (equal samples) so that $KGlog(F_2/f_1) = 0$. The trim-pot is adjusted until the voltage $E_o = E_T$. Now, if E_T changes following calibration, E_o will follow the change in E_T as set forth below:

$$E_o = E_T + KGlog(f_2/f_1) \quad (9)$$

Thus, if the value of E_T drifts upward, the output E_o will also. At the same time, the voltage inputs for each of the comparator circuits will be going up by the same amount. The output of the differential amplifier is thus fully and exactly compensated for drift in E_T. At the same time the initial differences in the optical paths are corrected with one trim-pot adjustment.

While the incandescent lamp 32 shown in Fig. 6B is the preferred light source, alternatively, a pair of diametrically opposed LED's can be used as light sources, one to illuminate each of sample tubes 5 and 6. Fig. 6C shows a circuit for driving red LED 6 and red LED 7.

While the invention is not so limited, the following table of values for the circuit elements shown in Figs. 5, 6A, 6B and 6C are an example of an operative circuit according to this invention.

TABLE 1

A1	U1 25L2 CP
A2	U1 25L2 CP
A3	U4 LM10C LN
A4	U4 LM10C LN
C1	.01uF
C2	10uF
C3	.01uF
C4	.22uF
C5	1uF
C6	.01uF
C7	.1uF
C8	1uF
C9	.01uF
C10	.01uF
C11	100pF
C12	100pF

* ultra-stable 1% resistors, matched to .1%, 25 PPM
** 1% metal film resistors

TAPLE 1 (cont'd)

D3	CR1 1N6263
G1	U3 74HC04
G2	U3 74HC04
G3	U3 74HC04
G4	U3 74HC04
G5	U3 74HC04
G6	U3 74HC04
Q1	PN2222
Q2	TIP32C
Q3	PN2222
Q4	U2 LM394N
Q5	U2 LM394N
R1	470 Ω
R2	10K Ω
R3	2K Ω, 20 turn
R4	9.1K Ω**
R5	100 Ω
R6	100 Ω
R7	1K Ω **
R8	7.68K Ω*
R9	499K Ω*
R10	7.68K Ω*
R11	499K Ω*
R12	9.1K Ω**
R13	1.0K Ω**
R14	1.0K Ω**
R15	1.0K Ω**

TABLE 1 (cont'd)

R16	1.0KΩ**
R17	9.1KΩ**
R18	10KΩ, 20 turn
R19	100Ω
R20	10KΩ
R21	100Ω
R22	3.9KΩ
R23	10KΩ
R24	--
R25	100KΩ**
R26	56KΩ**

---

* ultra-stable 1% resistors, matched to .1%, 25 PPM

** 1% metal film resistors

The operational amplifier U1 25L2 CP is sold by Texas Instruments of Dallas, Texas. The differential amplifier U4 LM10CLN is sold by National Semiconductor of Santa Clara, California. The diode CR1 1N6263 is sold by Hewlett-Packard of Palo Alto, California. The hex inverter U3 74HC04 is sold by Motorola of Austin, Texas. The transistors PN2222, TIP32C, and U2 LM394N, are all sold by National Semiconductor of Santa Clara, California.

In designing the electronic subsystem of this invention, it is necessary to set the gain of differential amplifier A3 by selecting appropriate values for the gain resistors R8, R9, R10, and R11. The selection is made so that the indicator LEDs 1-5 come on for predetermined differences in absorbance which are of importance for the test at hand. One method for setting the gain of A3 is to use known sample solutions which have been accurately measured in a laboratory spectrophotometric instrument. Alternatively, the required gain can be calculated. To do so, one must understand the difference between the concepts of absorbance and absorptivity. An example of the calculations required to set the gain of A3 is described hereinafter.

The definition of absorbance is:

$A = \log I/I_o$

where I_o is the intensity of light transmitted when the sample tube is filled with completely clear fluid and I is the light transmitted when the actual sample is in place. See Skoog and West, "Principles of Instrumental Analysis," Saunders College, Philadelphia, Pa., 1980.

If one compares two samples which transmit amounts of light I_1 and I_2, then the differential absorbance ΔA is of interest where:

$\Delta A = \log I_2/I_o - \log I_1/I_o = \log I_2/I_1$ (10)

Beer's law relates light absorbance to the properties of the sample under test:

$A = abc$

where a = absorptivity of the solution, b = path length, and c = concentration of the solution.

Specific absorptivity is defined as:

$\alpha = ac$ (11)

This is the measure of absorbance per unit path length, and is dependent on the sample's nature and concentration. Thus:

$A = \alpha b$

If one compares two different solutions in identical sample containers, then the differential absorbance is:

$\Delta A = A_2 - A_1 = b(\alpha_2 - \alpha_1) = b\,\Delta\alpha$ (12)

When a sensitivity of .03 units of absorbance is desired, what is really required is a specific differential absorptivity $\Delta\alpha$ = .03 since this is the quantity which is independent of the means of measurement. Combining equations (10) and (12) produces:

$\Delta\alpha = (1/b)\Delta A = (1/b)\log(I_2/I_1)$

It is convenient to use natural logarithms (1n), so that

$\Delta\alpha = (1/2.3026b)1n(I_2/I_1)$ (13)

Further one must consider the path length b for a particular instrument. Thus, for the optical configuration of Fig. 2, one may consider as an approximation that the light rays pass directly through the sample tube without refraction. Then, it can be shown geometrically that the average path length through the solution is

$b_{av} = (\pi/4)d$

where d is the inner diameter of the sample tube. This is the value of path length which must be used in a calculation for specific differential absorptivity:

$\Delta\alpha = [4/(2.3026\pi d]1n(I_2/I_1)$ (14)

Equation (14) relates specific differential absorptivity to light intensities. In addition one needs to determine the relationship between light intensities and E_o, the output of the differential amplifier.

The chracteristic of the LM394 log conversion circuit is that of an ideal silicon diode, for which

$V_f = (T/11,600)1n(I_f/I_s)$

where V_f = forward voltage, T = absolute temperature °K, I_f = forward current and I_s = saturation current for that diode.

The two LM394s which are used in the two log amplifiers are housed in the same package and come very well matched from the factory, so that I_s is the same for both. The forward current I_f is just equal to the photodiode current caused by the incident light:

$I_{f1} = I_{D1}$

$I_{f2} = I_{D2}$

The differential amplifier extracts the difference in voltage between the two log conversion circuits and amplifies it by a factor G (gain):

$E_o = G(V_{f2} - V_{f1})$

which reduces to:

$E_o = (GT/11,600)1n(I_{D2}/I_{D1})$

Diode current I_D is proportional to light intensity I at the diode:

$I_{D2} \alpha I_2$

$I_{D1} \alpha I_1$

$E_o = (GT/11,600)1n(I_2/I_1)$

Combining this result with equation (5):

$\Delta\alpha = [4/(2.3026\pi d)][(11,600\ E_o)/GT]$

or

$E_o = (2.3026\pi dGT\Delta\alpha)/[(4)(11,600)]$

For this example, we want to have $\Delta\alpha$ = .03 when the first red light (i.e., LED2 or LED4) comes on. The first red light comes on when E_o = .100 volts, as is determined by the resistor chain to which the hex inverter inputs are attached.

Given

$\Delta\alpha$ = .03

T = 298°K (room temperature)

d = 9 mm = 0.9 cm

E_o = .100 volts

Then equation (15) can be solved to give G = 79.7 as the required gain. Thus we need to choose

R11/R10 = R9/R8 = 79.7

This calculation provides results as close as those which have been determined by experimental means.

Although a preferred embodiment of the invention has hereinbefore been described, it will be appreciated that variations of this invention will be perceived by those skilled in the art, which variations are nevertheless within the scope of this invention as defined by the claims appended hereto.

Claims

1. Apparatus for comparing the optical densities of two liquid samples and indicating the degree of difference, said apparatus comprising:
a pair of photodetectors;
a light source means for transmitting a pair of substantially equal light beams, one to each of said photodetectors, so that each of two liquid samples can be simultaneously and identically positioned between an associated photodetector and said light source means;
said photodetectors generating a pair of optical density output signals representative of the amounts of light passing through said samples and reaching said photodetectors;
a differential circuit means receiving said optical density signals and generating a difference signal representative of the difference between said optical density signals;
a comparative circuit means receiving said difference signal including comparator means for comparing said difference signal to a plurality of predetermined references and an indicator means driven by said comparator means for indicating the relative value of said difference signal with respect to said predetermined references.

2. A method for simultaneously comparing the optical densities of two liquid samples and indicating the degree of difference, said method comprising the steps of:
preparing two liquid samples at the same time;
identically positioning each of said samples between a light source means and an associated photodetector so that the photodetectors simultaneously generate a pair of optical density signals representative of the amounts of light passing through the samples and striking the photodetectors;
generating a difference signal representative of the difference between said optical density signals;
comparing said difference signal with a plurality of predetermined references; and
indicating the relative value of said difference signal with respect to said predetermined references.

3. Apparatus for comparing the optical densities of two liquid samples and indicating the degree of difference, said apparatus comprising:
a pair of photodetectors;
a light source means for transmitting a pair of substantially equal light beams, one to each of said photodetectors, so that each of two liquid samples can be simultaneously and identically positioned between an associated photodetector and said light source means;
said photodetectors generating a pair of optical density current signals representative of the amount of light passing through said samples and reaching said photodetectors;
conversion circuit means receiving said optical density current signals for converting said optical density current signals to a pair of optical density voltage signals;
differential circuit means receiving said optical density voltage signals for generating a difference signal representative of the difference between said optical density voltage signals;
comparative circuit means receiving said difference signal for comparing said difference signal to a plurality of predetermined reference voltages and indicating the relative value of said difference signal with respect to said predetermined reference voltages, said comparative circuit means comprising:
a resistive chain including a plurality of nodes each having a different predetermined reference voltage; and
a plurality of digital gate means each of which has the same threshold voltage and is connected to one of said voltage nodes; and
a plurality of indicator means activated by the outputs of said gate means to designate the relative value of said difference signal with respect to said predetermined reference voltages.

4. The apparatus of claim 3, wherein each of said plurality of digital gate means is an inverter and each of said plurality of indicator means is a light-emitting diode.

5. The apparatus of claim 4, wherein said plurality of inverters are arranged so that only one light-emitting diode is activated at a time.

6. The apparatus of claim 5, wherein:
there are five light-emitting diodes;
a first of said five diodes being activated when said optical density voltage signals are equal;
a second of said five diodes being activated when a first of said optical density voltage signals is greater than a second of said optical density voltage signals by no more than a first predetermined value;
a third of said five diodes being activated when said first optical density voltage signal is greater than said second optical density voltage signal by at least a second predetermined value;
a fourth of said five diodes being activated when said first optical density voltage signal is less than said

second optical density voltage signal by no more than said first predetermined value; and
a fifth of said five diodes being activated when said first optical density voltage signal is less than said second optical density voltage signal by at least said second predetermined value.

7. The apparatus of claim 6, wherein said five light-emitting diodes are serially arranged with the first diode in the middle, the second and fourth diodes adjacent opposite sides of the first diode, the third diode adjacent the second diode at one end of the series and the fifth diode adjacent the fourth diode at an opposing end of the series.

8. The apparatus of claim 7, wherein said first diode is green and said second, third, fourth and fifth diodes are red.

9. The apparatus of claim 3, wherein:
said conversion circuit means logarithmically converts said optical density current signals to said optical density voltage signals; and
said differential circuit means includes a differential amplifier for generating said difference signal.

10. Apparatus for comparing the optical densities of two liquid samples and indicating the degree of difference, said apparatus comprising:
a pair of photodetectors;
a light source means for transmitting a pair of substantially equal light beams, one to each of said photodetectors, so that each of two liquid samples can be simultaneously and identically positioned between an associated photodetector and said light source means;
said photodetectors generating a pair of optical density current signals representative of the amount of light passing through said samples and reaching said photodetectors;
conversion circuit means receiving said optical density current signals for converting said optical density current signals to a pair of optical density voltage signals;
differential circuit means receiving said optical density voltage signals for generating a difference signal representative of the difference between said optical density voltage signals;
comparative circuit means receiving said difference signal for comparing said difference signal to a plurality of predetermined reference voltages and indicating the relative value of said difference signal with respect to said predetermined reference voltages, said comparative circuit means comprising:
a resistive chain including a plurality of nodes each having a different predetermined reference voltage; and
a plurality of digital gate means each of which has the same threshold voltage and is connected to one of said voltage nodes; and
a plurality of indicator means activated by the outputs of said gate means to designate the relative value of said difference signal with respect to said predetermined references voltages; and
a correction circuit for referencing the difference signal to the threshold voltage of said gate means, said correction circuit including an additional digital gate means from the same batch as the plurality of gate means in said comparative circuit, said additional gate means functioning as an amplifier so that the output of said correction circuit is referenced to the threshold voltage of said digital gate means, and said correction circuit output being applied to said comparative circuit so that the difference signal is referenced to said threshold voltage.

11. The apparatus of claim 10, wherein said difference circuit includes a differential amplifier having the optical density voltage signals as inputs and further having the correction circuit output added to one input so that the difference signal is referenced to the threshold voltage.

12. The apparatus of claim 11, wherein said correction circuit further includes an adjustable potentiometer for initial calibration of the apparatus to set the difference signal equal to the threshold voltage.

13. An improved optical system for a colorimeter of the type wherein the optical density of a liquid in a cylindrical optically transparent sample tube is determined by passing a beam of light from a light source through the liquid and onto a photodetector, said optical system comprising:
a collimating lens positioned between the sample tube and light source and spaced slightly closer to the light source than the focal point of the lens so that the lens transmits a slightly divergent beam to said sample tube;
said sample tube being spaced with respect to the lens so that the outer diameter of said divergent beam at the center plane of said tube is substantially equal to the inner diameter of said tube; and
said sample tube acting as a cylinder lens to focus said beam at a vertical focal line and said photodetector being spaced slightly ahead of said focal line so that the beam strikes a substantial portion of said photodetector.

14. The optical system of claim 13, further including a combination filter/diffuser positioned between the sample tube and photodetector, said combination filter/diffuser comprising a filter for selecting a predetermined wavelength of light and a light-diffusing layer for averaging the effect of individual defects in the sample tube so that the photodetector output is substantially unaffected by said defects.

15. The optical system of claim 14, wherein said light-diffusing layer is disposed over the surface of said filter adjacent the photodetector.

16. The optical system of claim 14, wherein a plurality of said combination filter/diffusers are connected in serial arrangement and said photodetector adjoins a light-diffusing layer at one end of the series.

17. The optical system of claim 14, wherein said light-diffusing layer is Mylar™ film which is frosted on both sides.

18. An improved optical system for a colorimeter of the type wherein the optical density of a liquid in an optically transparent sample tube is determined by passing a beam of light from a light source through the liquid and onto a photodetector, said optical system comprising:

a first lens set positioned between the sample tube and light source, said first lens set including a first collimating lens disposed adjacent the light source and having a focal point at the light source and a first converging lens disposed adjacent the sample tube and having a focal point at the center of the sample tube; and

a second lens set positioned between the sample tube and photodetector, said second lens set including a second collimating lens disposed adjacent the sample tube and having a focal point at the center of the sample tube and a second converging lens disposed adjacent the photodetector and having a focal point at the photodetector.

19. The optical system of claim 18, further including a filter for selecting a predetermined wavelength of light positioned between the second collimating lens and the second converging lens such that the light beam passes through said filter as parallel rays.

SPECTRAL_0000166

Fig. 1

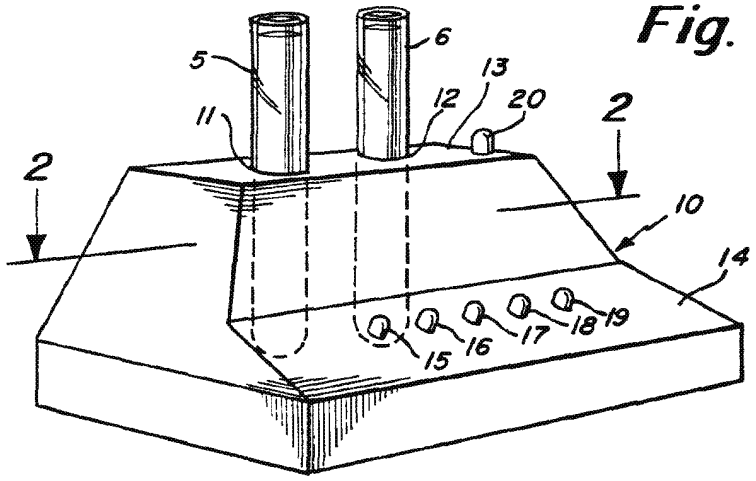
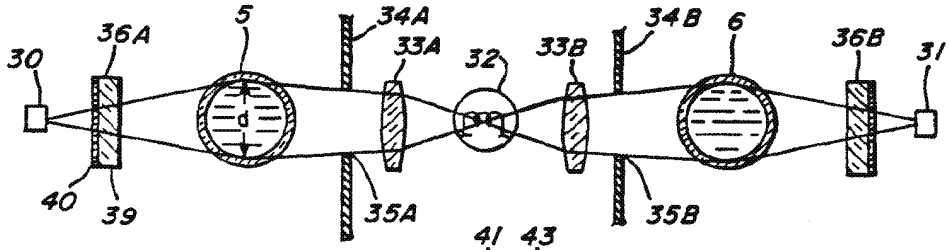


Fig. 2



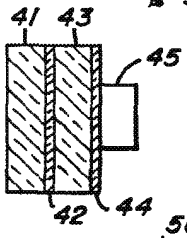


Fig. 2A

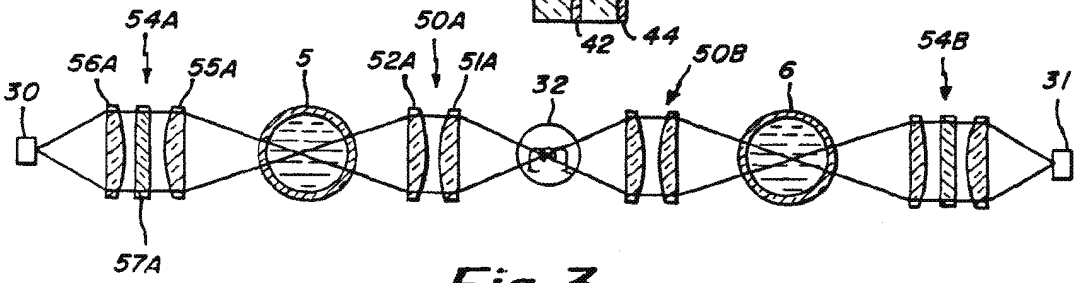


Fig. 3

SPECTRAL 0000167

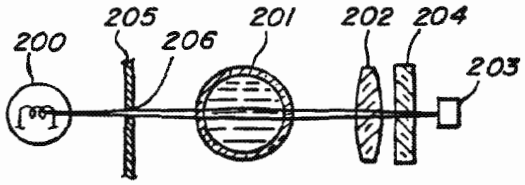
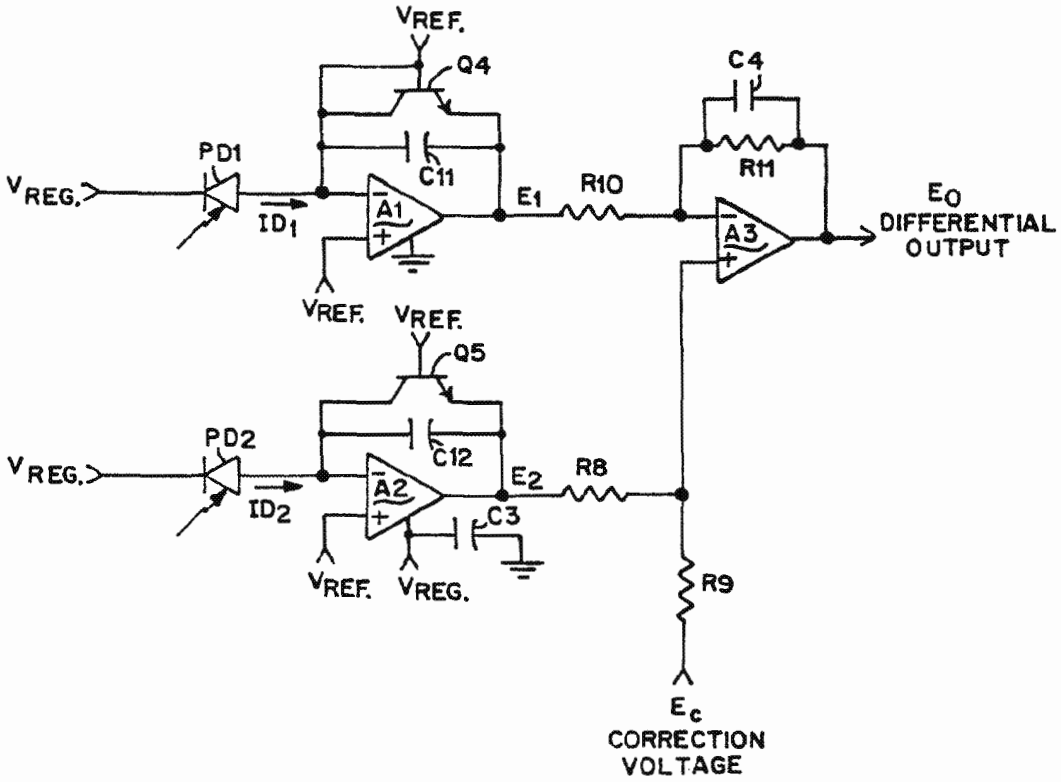


Fig.4
(PRIOR ART)

Fig. 5



SPECTRAL_0000168

Fig. 6A

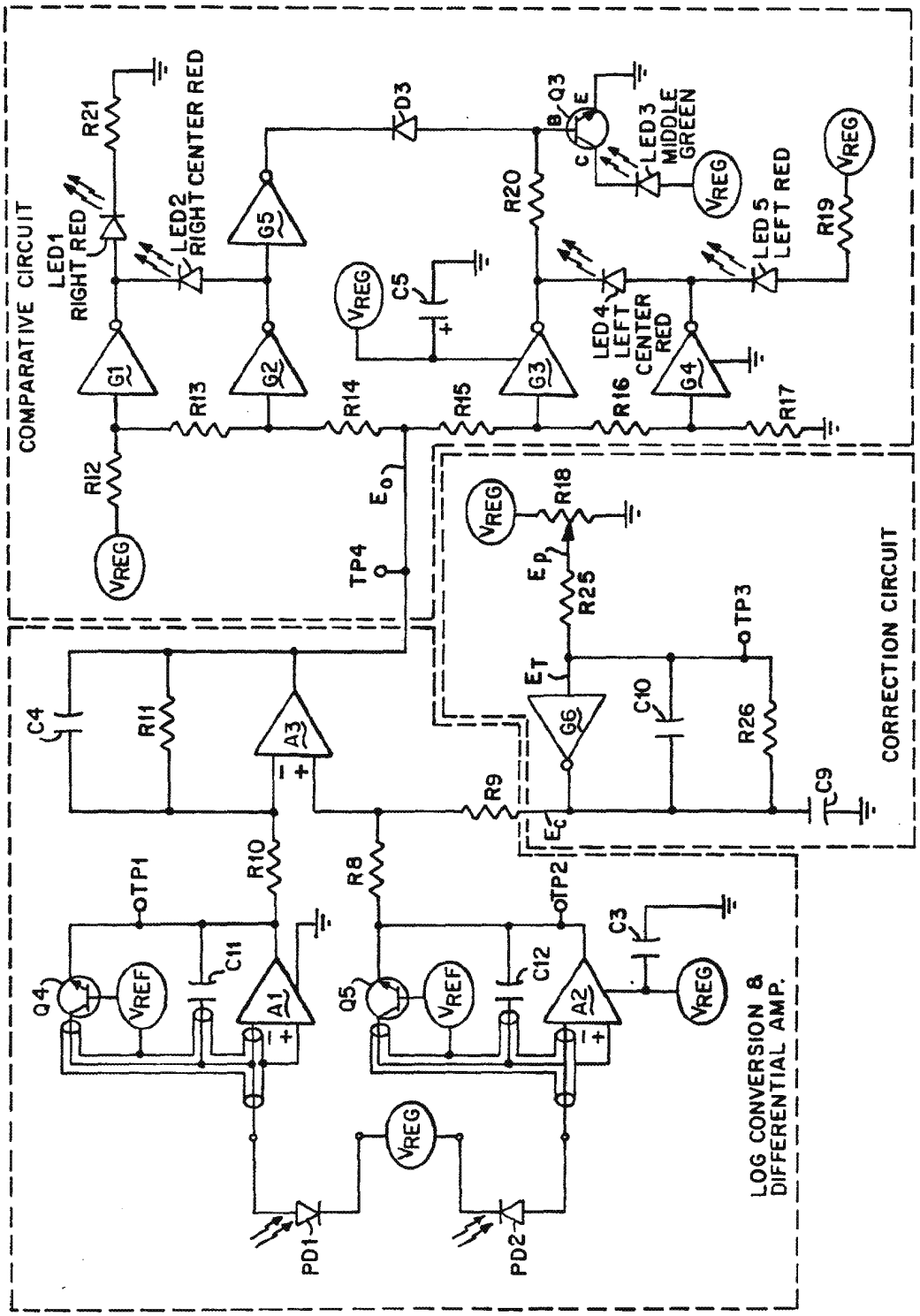

COMPARATIVE CIRCUIT
LED1 RIGHT RED
LED2 RIGHT CENTER RED
LED3 MIDDLE GREEN
LED4 LEFT CENTER RED
LED5 LEFT RED
R21
R20
R19
R13
R14
R15
R16
R17
R12
G1
G2
G3
G4
G5
D3
Q3
C5
VREG
TP4
Eo
R18
Ep
R25
ET
G6
C10
R26
C9
TP3
CORRECTION CIRCUIT
C4
R11
A3
R9
EC
TP1
TP2
R10
R8
C11
C12
C3
Q4
Q5
VREF
A1
A2
PD1
PD2
LOG CONVERSION & DIFFERENTIAL AMP.

Fig. 6C

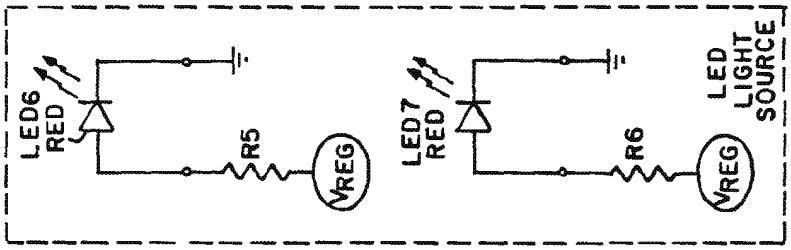

LED6
RED
R5
VREG
LED7
RED
R6
VREG
LED
LIGHT
SOURCE

Fig. 6B

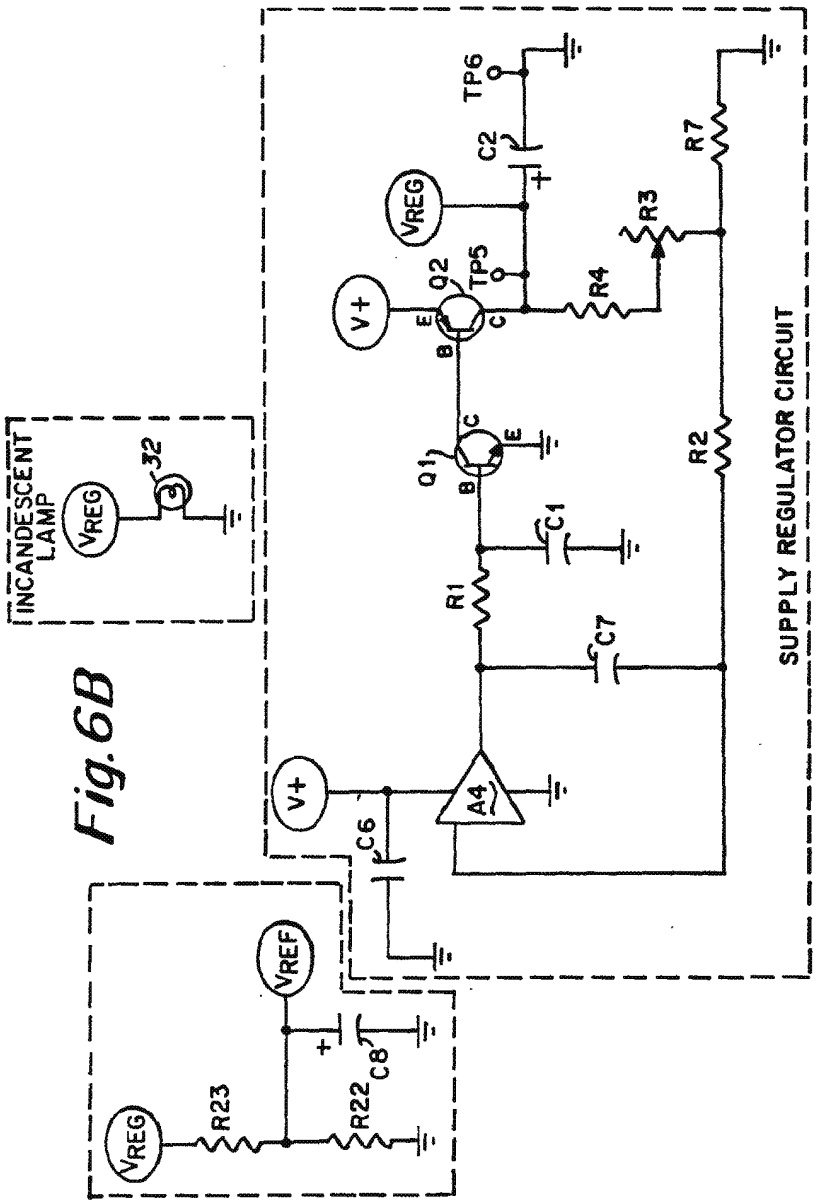
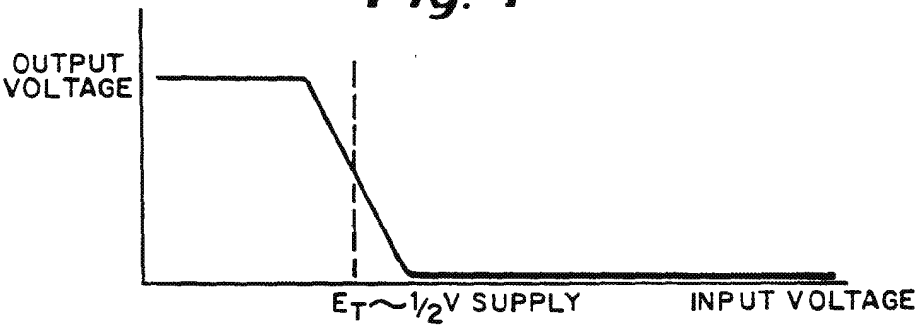

INCANDESCENT
LAMP
VREG
J2
TP6
C2
VREG
R3
R7
V+
Q2
TP5
R4
Q1
C1
R1
R2
C7
SUPPLY REGULATOR CIRCUIT
V+
A4
C6
VREF
VREG
R23
R22
C8

Fig. 7



SPECTRAL_0000171

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 491 131 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **91117745.9**

(51) Int. Cl.5: **G01J 3/51**

(22) Date of filing: **17.10.91**

(30) Priority: **17.12.90 US 629862**

(43) Date of publication of application:
24.06.92 Bulletin 92/26

(84) Designated Contracting States:
DE FR GB IT

(71) Applicant: **Hewlett-Packard Company**
3000 Hanover Street
Palo Alto, California 94304(US)

(72) Inventor: **Vincent, Kent D.**
20863 Sala Street
Cupertino, CA 95014(US)

(74) Representative: **Liesegang, Roland, Dr.-Ing. et al**
FORRESTER & BOEHMERT
Franz-Joseph-Strasse 38
W-8000 München 40(DE)

(54) Colorimeter and calibration system.

(57) Apparatus for monitoring, converting and calibrating the spectra displayed by a colored object (14), using wavelength dispersion provided by a variable wavelength filter (17). The apparatus may serve as a spectrophotometer, as a colorimeter, or as a spectroradiometer or other device that monitors and calibrates a light signal by decomposition of the signal into a wavelength distribution. The apparatus may also be used as part of a feedback network to monitor and correct colors displayed by a color monitor (61), color printer (63), color scanner (65) or other similar peripheral device controlled by a computer (56) that is part of the network.

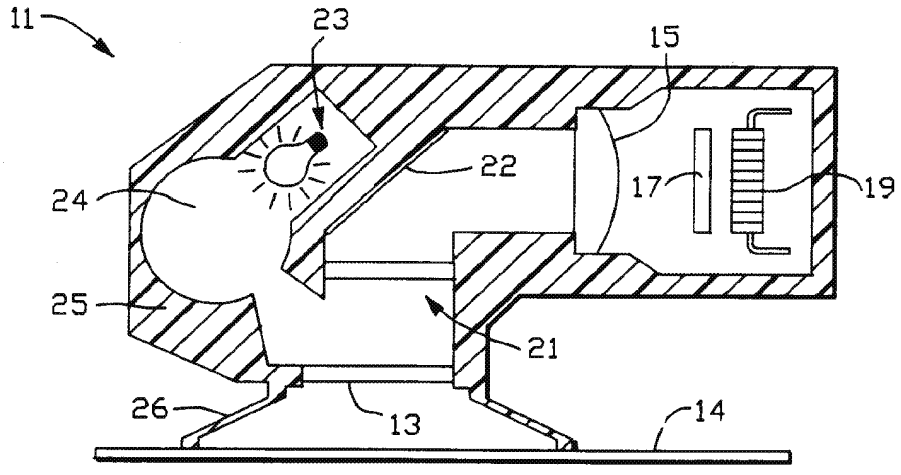


FIG.-1A

EP 0 491 131 A1

Technical Field

This invention relates to color calibration devices for self-luminous and non-self-luminous objects.

Background Art

A spectrophotometer is usually the most accurate and versatile of the devices used for measuring or monitoring color of an object based upon a dispersion of light emitted or reflected from the object and dispersed into a plurality of separate wavelengths. A colorimeter belongs to a subclass of spectrophotometers that is concerned primarily or solely with the strengths of the different wavelengths when compared with a CIE standard for color such as 1931 CIE XYZ. Standard colorimetry determines tristimulus values received by a color discrimination module with reference to certain standard values such as the widely accepted 1931 CIE XYZ standards. Colorimetry may be used to assess the colors produced on a video screen, in paints, at solid surfaces such as paper and plastics, as transmitted by or reflected from liquids, in dyed fabrics and textiles, in natural materials such as leather and wool, and as produced by computer peripheral devices such as color monitors, color printers and color scanners. Ideally, a colorimeter will perform accurate color measurements for any self-luminous body and for any light issued from a body illuminated by a separate light source.

The invention disclosed herein is applicable to spectrophotometers generally and more particularly to colorimeters. For convenient reference, the apparatus disclosed herein will be referred to as a colorimeter, without intending to limit the scope of application of the apparatus.

Hambleton, in U.S. Pat. No. 3,490,849, discloses a colorimeter in which light from a sample is diffused in a sphere and passed through a filter to a photo-multiplier tube. A plurality of filters must be inserted, one at a time, in order to adequately cover the spectral range of interest.

U.S. Pat. No. 3,525,572, issued to Hunter et al., discloses reflection of light from the surface of a fabric or similar light-reflecting object, and passage of this light to a sequence of photomultiplier tubes covered with tristimulus color filters. The tube signals are passed through certain circuits for analysis and determination of the color of the light received by the tubes. Two or more light sources may be used to separately illuminate the sample for purposes of color determination.

U.S. Pat. No. 3,880,523, issued to Thomas, discloses a multi-channel colorimeter in which a diffraction grating is used to decompose an incoming light beam into a plurality of non-overlapping spectral bands, with each such band being directed along a separate light waveguide for use in colorimetry.

Suga discloses use of three filter mirrors, each corresponding to a different color, as part of a colorimeter in U.S. Pat. No. 4,150,898. Light produced by the sample is separately reflected from each filter mirror and is received as a first light component by a phototube, which also receives a second light component directly from a light source used to illuminate the sample. The first and second light components are compared to determine the composition of light from the sample that is reflected by the particular filter mirror. Three or more filter mirrors are used, and each filter must be positioned separately to reflect light to the phototube. Simultaneous filtering at all wavelengths of light from the sample is not available.

A trichromatic colorimeter for obtaining CIE chromaticity coordinates, disclosed by Yuasa in U.S. Pat. No. 4,402,611, separates a light beam into three components and passes each beam component through a separate color filter corresponding to one of the tristimulus spectra. The three filtered light beams are then passed to separate photosensor arrays to produce electronic output signals that may be analyzed to determine the spectrum of the incoming light beam.

Kurandt, in U.S. Pat. No. 4,838,697, discloses use of two or three dichroic mirrors that receive light and filter it in different portions of the visible spectrum. The filtered light is then analyzed colorimetrically.

Steenhoek, in U.S. Pat. No. 4,917,495, discloses use of 12 photodetector elements that decompose an incoming light beam into separate spectral bands, for subsequent color analysis by computation of the tristimulus integrals for the incoming light beam.

In using a spectrophotometer, it is usually necessary to disperse the incident light beam into a plurality of different wavelengths or wavelength intervals in order to fully analyze the content of the incident light beam. Wavelength dispersion, using a plurality of beamsplitters, each functioning in a different part of the wavelength spectrum, is disclosed by Perkins in U.S. Pat. No. 4,681,445.

Crane, in U.S. Pat. No. 4,743,114, discloses the use of Fabry-Perot interferometer scanning using a nutating etalon in which the incidence angle of the light beam relative to the interferometer is caused to vary periodically in two perpendicular directions of rotation.

Use of a blazed diffraction grating or similar means of wavelength dispersion is disclosed in U.S. Pat.

No. 4,758,090, issued to Schuma, in U.S. Pat. No. 4,718,764, issued to Fink, and in U.S. Pat. No. 4,776,696, issued to Hettrick et al.

Rodine, in U.S. Pat. No. 2,960,015, discloses a method of making variable transmission light filters in a two-dimensional, radially symmetric configuration in which filter transmissivity varies with radial distance from the center of a circular pattern.

A variable color filter is disclosed by Illsley et al. in U.S Pat. No. 3,442,572, using a wavelength filter positioned on the circumference of a large circle, where the filter thickness increases linearly with increase of the azimuthal angle θ ($0 \leq \theta < 2\pi$) of the position on the circle circumference. This method relies on a fabrication method and apparatus, disclosed and claimed in two division patents, Nos. 3,530,824 and 3,617,331, but probably cannot be used to fabricate a filter whose thickness is not linearly increasing with increase in a spatial coordinate.

Fein et al. disclose an optical radiation translating device in U.S. Pat. No. 3,498,693. The Fein et al. apparatus in one embodiment (Fig. 3) uses two spaced apart planar reflectors of light that are inclined at a non-zero angle relative to one another, with a wedge-shaped dielectric material occupying the volume between the two reflectors. Light is transmitted through the wedge-shaped filter, requiring constructive interference of the light waves, only at positions along the device where the one way optical path length of the light beam through the dielectric material is an integral multiple of one half of the wavelength λ_0 of the light. The light is assumed to be monochromatic.

In U.S. Pat. No. 3,552,826, Hanes et al. disclose a variable thickness, multi-layer light reflector with a thickness h(x) that decreases exponentially with increase in a spatial coordinate x, measured in a predetermined direction in a plane of the reflector. The reflectance R of the reflector at any point x is a function of the single variable $w = \lambda/h(x)$, where λ is the wavelength of light incident on the reflector. The exponential decrease of thickness h(x) with the coordinate x is required in order to ensure that $\delta^2R/\delta\lambda\delta x = 0$ and $\delta^2R/\delta x^2 = 0$.

Bates, in U.S. Pat. No. 3,929,398, also discloses use of a wedge-shaped interference filter to produce a line of light at a particular coordinate position x that varies with the wavelength of the incident monochromatic light. The position x of the line of light is variable and is controlled by the operator's choice of wavelength of the incident light. A sequence of masks is used to selectively mask portions of the line to produce an ordered sequence of dark and light regions on the illuminated line that characterizes the light (e.g., each wavelength).

A color sensing device using a group of adjacent, non-overlapping light filters with different pass bands is disclosed by Hinoda et al. in U.S. Pat. No. 4,547,074. Each light filter consists of an interference filter with a plurality of separated wavelength pass bands plus a color filter with a sharp cutoff band that falls within one of the interference filter pass bands. The serial combination of these two filters selects a particular, fixed narrow wavelength band for transmission of light therethrough. A photodiode, positioned beneath the serially combined interference filter and color filter, receives the transmitted light and determines the relative intensities of light in each of several wavelength pass bands. Photodiode light-receiving faces may have different areas to reflect the light sensitivity of the photodiodes in different, fixed wavelength regions. A subgroup of such filters may be configured to sense the relative amount of light in each of a set of adjacent wavelength bands, to thereby produce color matching capability according to the CIE XYZ colorimetric system. The incident light is not assumed to be monochromatic, but it appears that each interference filter/color filter pair must be carefully matched to provide a fixed wavelength pass band.

Owen, in U.S. Pat. No. 4,054,389, discloses a spectrophotometer in which light is first collected by a bundle of optical fibers, each of which delivers light to a separate position on a light-receiving surface. The light-receiving surface may be a first surface of a wedge-shaped linear variable interference filter that has a second opposing surface at which an array of photosensors is positioned to receive the light passed through various sections of the interference filter. Each photosensor responds to light of a different wavelength delivered by one of the optical fibers.

U.S. Pat. No. 4,822,998, issued to Yokota et al., discloses use of an array of light sensors, each sensor being sensitive to a different wavelength range in receiving light transmitted through a light filter with a transmission wavelength band pass corresponding to the wavelength band to which the light sensor responds. In one embodiment, shown in Fig. 1 of the Yokota et al. patent, the light filter array is arranged in a double staircase configuration, with the filter thickness increasing from one plateau of constant thickness to another plateau of greater constant thickness. A first filter staircase and second filter staircase have filter thicknesses chosen to correspond to optical interference orders $m = 1$ and $m = 2$, respectively, according to well known optical relations for a Fabry-Perot etalon. By separating the visible spectrum (wavelengths λ = 0.4-0.7 μm) into two smaller wavelength ranges, the sidebands of each interference order, other than the order $m = 1$ or $m = 2$ that is desired, are caused to appear at wavelengths well removed from the visible

spectrum and can be attenuated with simple fixed band pass ultraviolet and/or infrared filters. Low order Fabry-Perot interference bands are usually not narrow enough by themselves for most spectrophotometer applications. As Fig. 7 of the Yokota patent illustrates, the full width at half maximum ("FWHM") for a low order interference band, with a central wavelength λ_c = 400 nm, is 15 nm and 9.6 nm for surface reflectivities of R = 0.23 and 0.62, respectively. The FWHM will increase with increase in wavelength. These FWHM values are much too wide for many applications of such technology in colorimeters and radiometers. Increasing the reflectivity R of the surfaces of the Fabry-Perot etalon will narrow the FWHM by a modest amount, but the FWHM is still too large for some spectrophotometer applications, and the transmissivity T = 1-R may already be so low that the signal-noise ratio for the photosensor signals becomes a concern. The wavelength skirts that extend beyond the FWHM wavelength region may also be too broad to allow sharp wavelength discrimination.

A wedge-shaped filter spectrometer is disclosed by Pellicori et al. in the patent application PCT/US88/03898, published circa 15 June 1989. The Pellicori et al. spectrometer provides a sequence of wedge-shaped order-wavelength thickness dielectric layers of alternating refractive indices high (H), low (L), high (H), low (L),... that are deposited on one another in accordance with a sequence H LL HLHLHL HH LHLHLH LL HL. The thickness of each of these layers has a constant slope as a spatial position coordinate x changes in a selected direction across the face of the filter. Pellicori et al. note that undesirable sidebands, appearing at wavelengths removed from a central wavelength λ_c, are present and suggest the use of an interference filter blocking stack, with an apparently fixed pass band, to prevent the appearance of the side bands in the transmitted light beam.

Monitor-specific calibrators are available which measure the brightness of a portion of a CRT screen under specific conditions. The sensor is usually a single silicon photodiode, and each CRT gun is turned on individually (e.g., red, then green, then blue) over a sequence of successively greater brightnesses to determine the gamma, offset and gain parameters for that color and that device. This information is fed into an associated computer to correct the CRT amplifiers in order to correct any errors present. The phosphor set used with the color monitor must be known exactly so that such a device is not universally applicable to CRTs of other manufacturers or even other products of the same manufacturer. In particular, the monitor-specific color calibrator is not useful for color printers, for general color calibration, or for use of ambient lighting rather than the specific lighting for which the color calibrator is set. Examples of such devices are light calibrators offered by Barco Industries for their color monitors, and by Radius, Inc. for the Apple Macintosh color monitors. These devices function as calibration probes that are electronically tethered to a host computer.

An example of a tristimulus colorimeter and calibration system is shown in U.S. Pat. No. 4,500,919, issued to Schreiber. This patent discloses use of a color scanner as a calibration device to produce a set of three tristimulus signals dependent on the colors sensed in the original document. The scanner measures red, green and blue components as usual and is used to calibrate the colors of print samples. The RGB signals are not colorimetrically correct, unless matched with the eye for specified lighting conditions, the scanner produces color errors. Here, a color scanner is used as a tristimulus color input device without comparison to the basic XYZ color standards. A scanner would be unable to determine the color transfer function of a color monitor, as required for a general purpose colorimeter.

Many of the devices of the prior art are large and bulky and do not make full use of or analyze all wavelengths in a continuous wavelength interval of the incident light beam. The cost of these devices is usually great, due in part to the delicate optical systems used. Further, no controllable means has been disclosed for compensating, at the same time, for the non-uniform sensitivity, as a function of wavelength, of photodetector elements or for compensating for use of a non-standard light source for illumination of an object whose color is to be measured or monitored. Finally, the devices in the prior art compensate for the presence of undesirable bands, if at all, only through use of a fixed pass band filter that is used in combination with the wavelength dispersion means.

Several other technology-based problems recur, including the problems that: (1) color errors arise because the filters cannot provide a perfect match of the XYZ tristimulus response of the human eye; (2) use of a set of fixed filters is applicable only to a fixed ambient lighting condition, with no flexibility in use of other available light sources; and (3) the XYZ tristimulus values sensed by the colorimeter must be read and manually entered into a computer for further computations.

Each peripheral device, such as a color monitor, color printer or color scanner, produces colors using intrinsically different colorants, such as phosphors used in monitors, color dyes and pigments used in printers used, and filters used in color scanners. The rendition of colors, given the same color input signals, thus varies with the device (device dependence). Some workers have attempted to electronically correct for intrinsic wavelength-dependent differences, such as lower sensitivity of photosensors at the blue end of the

visible spectrum vis-a-vis the red end of the spectrum. However, color errors still persist because the color transfer functions of these devices are not entirely stable: amplifier drift occurs in a color monitor; printer colors change with the paper or substrate upon which the printing occurs; and color scanners also have drift problems. Further, the manufacturer cannot predict the ambient lighting conditions under which the computer system will operate. The net result is that the rendered images of the peripheral device may vary with time and may be incorrect over all. Data sent from one computer to another may not provide matching colors between the two systems.

A need also exists for a means to add "real" colors to CAD rendering software. The computer industry has gone to great lengths to add realism to CAD rendering programs such as ray tracing algorithms. To date, this greater realism has been realized only through the use of synthetic color palettes with computer-generated colors. It would be more appropriate to be able to input the color values of a real object that the CAD software is rendering.

What is needed is a compact color calibration apparatus that (1) efficiently disperses a light beam into a continuous interval of wavelengths and analyzes the content of the light beam throughout this interval; (2) allows shifting of augmentation of the wavelength interval to be analyzed; (3) allows flexibility and alteration of the light sources and light beam intensity distribution received by a plurality of wavelength-sensitive photodetector elements; (4) provides sharply defined, very narrow bands of light of different wavelengths at each photodetector element with no side band problems; (5) allows construction of the apparatus on a single chip that is compact and rugged and has low cost; (6) allows use of this apparatus for spectrophotometry or colorimetry work on a variety of self-luminous or non-self-luminous objects whose color is to be measured or monitored; and (7) allows automatic entry of the tristimulus values sensed into a computer for further analysis and computation.

Summary of the Invention

These needs are met by providing a colorimeter for determining the color composition of the small predetermined area at an arbitrary position of an object, where the colorimeter includes a variable wavelength light filter having a light-receiving surface to receive a light beam that has a plurality of wavelengths in a selected wavelength band. The light filter disperses the beam into a plurality of wavelengths that increase monotonically with position x in a selected direction lying in the light-receiving surface, and the light filter either reflects the dispersed beam from, or transmits the dispersed beam through, the filter. For any position x, the light reflected from or transmitted through, as the case may be, the light filter is confined to a very narrow wavelength pass band with no side bands being transmitted at that position x. The colorimeter apparatus also includes light restriction and focusing means for receiving and admitting light from a restricted portion of the object that defines the small predetermined area and for directing this light to the light-receiving surface of the light filter. An array of photosensors is also provided, positioned to receive light reflected from or transmitted through the light filter so that each photosensor in the array receives light in a very narrow band of wavelengths having a central wavelength $\lambda_c(x)$ for light received and reflected (or transmitted) at the position x on the light filter, where $\lambda_c(x)$ increases monotonically with the value of the position coordinate x.

Where color of a non-self-luminous object is to be measured or monitored, the apparatus may optionally include a light source to illuminate different portions of the object. If this light source has a wavelength distribution that does not coincide with a reference, or ambient illumination, wavelength distribution, the photosensor array may include electronics or software that compensates for the non-standard nature of the wavelength distribution or light source.

The colorimeter apparatus may optionally be provided in a movable "mouse-like" color monitoring probe that measures or monitors the color content at an arbitrary position on the object as the apparatus is moved over the surface of this object. The mouse or probe may be tethered to the computer by a cord or wire that transports communications between the mouse or probe and the computer. Alternatively, the mouse or probe may be non-tethered and may communicate with the computer in a wireless manner, using infrared or other signals for communication of data and control information.

The colorimeter-based computer system may further include color transform electronics that alters image pixel data to compensate for various color transfer functions of appended color imaging devices, such as printers, monitors and scanners, as measured by the colorimeter of the present invention.

The invention provides apparatus to sense and store data to match paint colors, fabric colors, film colors and dye colors for foods and perfumes, as well as compensation for different lighting conditions.

Brief Description of the Drawings

Figs. 1A, 1B, 1C and 1D are cutaway sectional views of four embodiments of the colorimeter invention.

Fig. 2 is a block diagram of a system for color monitoring, conversion and correction in which the colorimeter invention may be used.

Figs. 3A and 3B illustrate illumination of a non-luminous object by an ambient source and non-ambient light source, respectively.

Fig. 4 is a block diagram illustrating provision of different color formatting for each of two peripheral devices.

Fig. 5 graphically illustrates the change in R, G and B phosphor intensities as the input voltage to the corresponding electron gun is varied.

Figs. 6 and 7 illustrate the use of a colorimeter probe, constructed according to the invention, to sense color on a color monitor (luminous source) and on a color printer printout (non-luminous source), respectively.

Fig. 8 is a side view of one embodiment (transmission mode) of the filter invention in which the distance of separation of two reflecting planes increases monotonically with a spatial coordinate x.

Fig. 9 is a plot of the spectrum of transmitted intensity versus wavelength of light for two positions, $x = x_1$ and $x = x_2$, for the embodiment shown in Fig. 8.

Figs. 10, 11 and 12 are side views of three embodiments of the invention that operate in the reflection mode.

Figs. 13A, 13B, 13C and 13D are plots illustrating light transmission through a high pass edge filter, a low pass edge filter, and combinations of these two filters, as used in the invention.

Fig. 14 is a side view of an embodiment that uses a serial combination of a high pass edge filter and a low pass edge filter.

Figs. 15 and 16 are side views of other embodiments of the invention in which one of the planar reflecting layers is replaced by a reflecting layer having a staircase configuration.

Figs. 17A and 17B are cross-sectional views of two versions of a multilayer thin film filter configuration suitable for implementing an embodiment of the invention.

Figs. 18A and 18B are plots of transmittance versus inverse wavelength for two thin film filters implemented according to the invention.

Fig. 19 is a plot of reflectance of a light beam as a function of wavelength for the embodiment shown in Fig. 17B.

Fig. 20 is a cross-sectional view of another multi-layer thin film filter constructed according to the invention.

Fig. 21 is a plot of several possible variations with distance x of the separation distance h(x) of two adjacent reflecting surfaces within the filter in a selected direction.

Figs. 22 and 23 are perspective views showing the use of one filter or two filters laid end-to-end, respectively, according to the invention, together with an array of photosensitive elements.

Fig. 24 is a plot illustrating partial overlap of selected wavelength bands versus spatial displacement of two filters constructed according to the invention in Fig. 23.

Fig. 25 is a perspective view of an embodiment that utilizes a two-dimensional array of filters and photosensors.

Figs. 26A, 26B and 26C are side views of three embodiments that allow a wavelength filter and a photosensor array to be positioned contiguous to one another, according to the invention.

Best Mode for Carrying Out the Invention

With reference to Fig. 1A, one embodiment of the color calibration device (spectrophotometer, spectroradiometer, colorimeter, etc., herein referred to simply as a "colorimeter") 11 includes a transparent window 13 that is placed adjacent to a local plane 14 of the viewed object (not explicitly shown in Fig. 1A) to receive light with wavelengths representing the object color. The window 13 may have a controllably variable aperture to limit the portion of the object from which light is admitted. This light from the object is transmitted through the window 13 through a lens or other optical focusing means 15, which focuses and directs this light to a variable wavelength filter ("VWF") 17 (transmission mode or reflection mode). A mirror or prism 22 may optionally be positioned in the beam's optical path to reflect or bend the light beam to conform to the lens focal length and packaging constraints. The light that issues from the variable wavelength filter 17 is received by a photosensor array 19, each photosensor receiving light from a single narrow band of wavelengths centered around a central frequency $\lambda_c(x)$, where x is a spatial coordinate measured in a selected direction along the light-receiving surface of the VWF 17. The photosensor array 19 may include on-board processing electronics (not explicitly shown in Fig. 1A) to determine the wavelength

distribution of light received from the object and to convolve this wavelength distribution with certain filter functions, such as the standardized 1931 CIE $\bar{x}\bar{y}\bar{z}$ color matching functions of the eye, to produce color values such as XYZ. Alternatively, the electronics for determination of the wavelength distribution and convolution integrals with an appropriate filter function, positioned elsewhere, may receive a sequence of electronic signals from the photosensor array 19 or from the colorimeter 11. Alternatively, the filter 17 may be three or more filters that individually match the wavelength passing characteristics of a color sensor for the eye.

The colorimeter 11 may optionally include a light diffuser 21, positioned between the window 13 and the lens or other focusing system 15 in Fig. 1A, or positioned between the lens 15 and the VWF 17, to diffuse the light received by the VWF from the portion of the object 14 that appears beneath the window 13 of the colorimeter 11. The window itself may be a light diffuser, as illustrated by the window/diffuser 13/21 in Fig. 1B, or may function as a wavelength trimmer by filtering or otherwise removing undesirable wavelength components from an incident light beam. Use of a light diffuser is intended to redistribute light in the incident light beam to remove any light "hot spots" and to provide a light beam of more uniform intensity incident on the VWF 17. Preferably, the diffuser is positioned at a focal plane for the lens 15. If the object is not self-luminous and requires illumination in order to adequately display its color(s), a light source 23 may be optionally provided to illuminate the portion of the object 14 that appears beneath the window 13. Light from the source 23 should be shielded so that it is not directly received by the light diffuser 21 (Fig. 1A), the lens 15, the VWF 17 or the photosensor array 19. Typically, light issuing from the source 23 will be distributed by an appropriate dispersion reflector 24, such as an integrating cylinder or sphere, to uniformly distribute the light beam intensity over the monitored area of the plane 14. These reflectors are well known in the colorimetry art. Optionally, the light source 23 may be located in a separate device into which the remainder of the probe apparatus 11 is inserted for color measurements of the object.

If the object is self-luminous, the light source 23 and dispersion reflector 24 are not used, as illustrated in the embodiment 12 in Fig. 1B. The colorimeter 11 may optionally be provided with a container or housing 25 that surrounds and contains all the components of the colorimeter 11, with the window 13 forming a portion of one wall of the housing 25. Optionally, the apparatus 11 may include a hood 26 that extends from the housing 25 to the plane 14 to shield the window 13 and all internal components from any external stray light that might otherwise enter through the window 13 and reach the filter 17 and/or photosensor array 19.

Those skilled in colorimetry will appreciate that the angle of incidence of light rays issuing from the light source 23 and passing to the object plane 14 for capture by the photosensor array 19, may vary according to specific applications. Commonly, the incidence angle may be adjusted to about 45° and the reflected cone angle may be adjusted to 2° or 10°.

The colorimeter 11 may optionally be included as part of a "mouse" 27, as illustrated in Fig. 1C, that operates similarly to the mouse introduced by Apple Computer, Hewlett Packard, Microsoft and others for use as indicator and selector in entering computer commands. The mouse 27 would include a first position sensor 29 that senses motion in a first coordinate direction(s) by sensing rotational motion of a sphere or cylinder or similar object about an axis perpendicular to this direction. A second, analogous position sensor (not explicitly shown in Fig. 1C) senses position in a second perpendicular coordinate direction. The colorimeter 11 and the position sensor(s) such as 19 are contained in a mouse housing 31, and the mouse itself receives power and generates position signals and color wavelength distribution signals through a power cord 33 that may be attached to other processing electronics that are not shown here.

A fourth embodiment 35 of the colorimeter is illustrated in Fig. 1D. Here, the colorimeter uses only ambient light admitted through a second window 37 to illuminate a part of the object 14 that lies beneath the first window 13. The lens or other focusing system 15, the VWF 17 and the photosensor array 19 are now positioned to receive light from the object sensed through the first window 13 and so that these components do not interfere with illumination of the object by ambient light admitted through the windows 37 and 13. Again, optionally, a light diffuser 21 may be provided to diffuse light received from the object.

An alternate embodiment can be used that allows shuttering of the light source (ambient or other) so that both self-luminous and non-self-luminous objects to be analyzed using the colorimeters discussed here. In the embodiment of Fig. 1D, for example, a shutter 28 (optional) can be included for this purpose. The shutter 28 would be fully opened to admit light from the light source 23 in order to illuminate a non-self-luminous object; and the shutter 28 would be closed if the object whose colors are to be analyzed is self-luminous or if illumination through the second window 37 in Fig. 1D is not desired.

If the spectrum emitted by the light source 23 does not coincide with the ambient spectrum for the intended ambient illuminating light, the processing electronics associated with the photosensor array 19 may include electronic filters or other calibration means for adjusting the wavelength distribution received from the object by the array of photosensors to compensate for use of a non-ambient light source 23. The

light spectrum received from the light source provided by the colorimeter and the light spectrum received from the ambient light source are analyzed separately for color content by a colorimeter device. These light spectral data are used to determine the appropriate compensation to be applied for the non-ambient light source. Typically, the spectrophotometer output signals will be multiplicatively factored by the ratio of ambient spectrum divided by lamp or light source spectrum for each measured wavelength interval, as shown in Fig. 3B. The desired result is a spectrum representing the visual stimulus that would enter the eye if a human being, rather than the colorimeter, viewed the colored object. The desired output from the colorimeter is a tri-stimulus set of color values such as those set forth in 1931 CIE XYZ. The subjects of colorimetry, photometry and visual matching of colors are well set forth in the text Color Science, Gunter Wyszecki and W.S. Stiles, John Wylie & Sons, 1982, chapters 3, 4 and 5, which are incorporated herein by reference. In a preferred embodiment, much of the processing of signals generated by the colorimeter will be done in a host computer that is located elsewhere and receives such signals from the colorimeter by means of a bus in a well known manner.

The word "light" here is taken to refer to any portion of the electromagnetic spectrum. The word "stratum" here refers to a material with at least one light receiving surface, which can be a bare surface or a surface with a filter layer deposited on it.

A computer system in which the colorimeter is used, is complicated by the number of color corrections and other color transformations that must be executed for measurement and compensation purposes, usually in real time. Each object whose color(s) or wavelength distribution is determined by the colorimeter (e.g. a color monitor, color printer or color scanner), uses different colorants. Color monitors use color phosphors, colored printers use colored dyes and pigments, and color scanners use different colored filters with fluorescent lamps comprising various colored phosphors, each set of colors being spectrally unique from the others. Some devices, such as color monitors and color scanners, may exhibit amplifier drift that provides constantly changing color transfer functions within the device. One task of the signal processing device is to rapidly translate color pixel data from one color representation to another in order to correct or alter color data that are currently provided for a non-perfect peripheral device. Another task is to transform color pixel data from one color space to another.

Colorimeter System

Fig. 2 illustrates, in block diagram form, color calibration and correction tasks that may be performed in use of the colorimeter such as shown in any of Figs. 1A, 1B, 1C or 1D. A colorimeter 11 receives spectral data from one or more objects and transmits this data to an analog-to-digital converter ("ADC") 41 that transforms the data to digital form. This digital form data is received from the ADC by an intensity converter 42 (optional) that converts digitized optical energy E to intensity $I = E/\tau$, (where τ is the exposure time interval for the object) and transmits the intensity signal I to a filter alignment module 43, then to a dark current determination module for determination and subtraction of dark current signal values from each photosensor signal, then to a calibration module 45 that corrects the spectrum or wavelength intensity distribution information received from the colorimeter 11 with a reference spectrum or spectra. Optionally, the information generated by the calibration module 45 may be passed through an electronic filter 46 before this information is passed to an input/output port 47 that delivers such information to an information bus 48. The optical energy signal E produced by the ADC 41 is (optionally) also received by an auto exposure control ("AEC") module 41A (optional) that determines what the exposure time τ should be. The AEC module 41A then issues a control signal that is received by a clock module 41B (optional) that controls the exposure times τ for the wavelength intervals sensed by the colorimeter 11. A bypass signal path 49 may optionally be provided to transport the signal issued by the calibration module 45 directly to the I/O port 47 for delivery of this signal to the information bus 48.

In some applications, such as computer peripheral color calibration, the colorimeter must measure spectral irradiance over a 1000-to-1 or more dynamic range. This dynamic range is best accommodated in practical digital systems through exposure control. The exposure time for a particular photosensor, corresponding to a predetermined, small wavelength interval, may be varied to decrease the error and increase the cumulative optical signal received at that photosensor so that the signal falls between two predetermined thresholds that define a region of maximum photosensor sensitivity. The optimum exposure time τ for a given photosensor, or for all photosensors, may be determined by the auto exposure control module 41A and converted to an equivalent frequency to drive a clock 41B that controls the exposure(s). Intensity I in a particular wavelength interval is obtained from cumulative optical energy E received in that interval by the relation $I = E/\tau$, as noted above.

An appropriate exposure time τ for a particular wavelength interval may be determined as follows.

Assume the cumulative optical energy for the peak wavelength (the interval receiving the maximum cumulative energy in a test time interval of length Δt) has a value E_p, and assume that the photosensors can measure cumulative optical energies E most satisfactorily in a range given by $E_{min} \leq E \leq E_{max}$. If $Ep > E_{max}$, reduce the exposure time τ to $r_1 \Delta t$ where $r_1 \leq E_{max}/Ep$. If the cumulative optical energy E_p exceeds a saturation value E_{sat} the exposure time τ might be reduced by successive factors of two until the new E_p satisfies $E_p < E_{sat}$. If $E_p < E_{min}$, increase the exposure time τ to $r_2 \Delta t$ where $r_2 \geq E_{min}/E_p$.This exposure time adjustment procedure can be applied to any wavelength interval. The accumulated energy range E for a photosensor signal may have a large dynamic range. This quantity E must be adjusted, by adjustment of exposure time τ so that the adjusted value of E falls in a range allowing optimal resolution, based on the quantization or bit resolution allowed by the analog-to-digital converter. By distributing the energy optimally over the available bit resolution range, quantization error is reduced or minimized.

The filter alignment module is used to determine which wavelength interval of the VWF 17 in Figs. 1A, 1B, 1C and 1D corresponds to each photosensor in the array 19. As an example, a Hg lamp emission line such as the λ = 546.1 nm line may be transmitted to the VWF, and it may be determined that this spectral line is received by photosensor no. 121 in a 256-photosensor array 19. The two photosensors corresponding to wavelength cutoff, for example, λ = 385 nm at the violet end of the spectrum and λ = 725 nm at the red end, may be determined. If light of wavelengths λ = 385 nm and λ = 725 nm is received predominantly at photosensor nos. 14 and 248, respectively, signal information received at photosensors no. 1-13 and 249-256 would be discarded and not processed further. The wavelength intervals between λ = 385 nm and λ = 725 nm would then be distributed among the photosensors no. 14-248, with photosensor no. 121 receiving light in a narrow wavelength interval including the wavelength λ = 546.1 nm. These wavelength intervals would be distributed linearly or non-linearly between the two cutoff wavelengths depending on whether the central wavelength $\lambda_c(x)$ increases linearly or non-linearly with increasing values of the position variable x.

The dark current subtraction module 44 includes memory to store the measured intensity response of each photoelement in photosensor array 19 in the absence of any light exposure. The dark current values are obtained by blocking light entrance to the sensor and storing the measured intensities. The measured dark current values for each photoelement are then subtracted from the respective photoelement intensities obtained from all subsequent spectral measurements. As is well known in the photosensor art, photosensors produce charge in the absence of light as well as in its presence. Dark current is primarily thermally induced, and rises rapidly with increasing temperature T. For accurate color measurements, dark current must be measured on a regular basis by the user. The necessity for frequent measurements can be reduced by monitoring the temperature of the photoelements and adjusting the dark current values with temperature, as the dark current of a given photoelement rises exponentially with temperature. The temperature may be monitored by providing photoelements along the array that are protected from light exposure, by an opaque covering. These elements would only be capable of producing dark current, and the relative changes in their response can be applied to dark current values of the exposed photoelements. Temperature measurement can also be obtained by use of thermistors and other similar devices.

Not all photosensors have a linear dark current response with exposure time. With auto exposure control, it may be possible to calibrate, read dark current, and measure color at more than one exposure time, leading to a small level of color measurement error. The characteristic non-linearity in dark current contribution $E_d(\tau,T)$ to intensity may be minimized by developing a table of compensation values $K_d(\tau,T)$ for each exposure time τ or for a number of exposure time ranges, such that the computed dark current contribution to intensity I may be correctly computed as $I_d = E_d/K_d\tau$, which does not vary much with change of exposure time τ. The compensation value K_d for each exposure range is obtained by recording the dark current intensity response (E_d/τ) of the sensor over the desired exposure range at a chosen temperature T, or by computing the ratio of spectral and dark current responses for a covered photosensor. In all subsequent measurements (including calibration), the appropriate K_d value is retrieved from memory and applied to the computation of each photoelement intensity response for a given exposure time. The compensated average light intensity then becomes $I' = (E(\tau)/\tau)-(E_d(\tau)/K_d(\tau)\tau)$. The forementioned compensation would be part of the function of the intensity converter 42 and the dark current module 44.

Calibration module 45 includes memory to store calibration factors for each photosensor and means to multiply each photosensor intensity by the respective calibration factor stored in memory. Calibration is an essential step for accuracy in spectral measurement. The intensity values obtained from the photosensor array 19 typically have errors due to photoresponse non-uniformities along the array, such as wavelength-dependent responsivity, bandpass shape and transmission variation along the length of VWF 17, and non-uniformities in the optics. These device-dependent and fixed non-uniformities can be compensated for by calibration. Through measurement, a calibration factor for each photosensor is determined that corrects the

colorimeter response to a source with a precisely known spectrum, for example a black body radiator. The factors for each photoelement are digitally stored in a calibration lookup table of calibration module 45.

The electronic filter module 46 includes a first sub-module 50 containing the ambient wavelength spectrum, wavelength interval-by-interval, that represents the preferred illumination source for non-luminous objects. The filter module 46 also includes a second sub-module 51 containing the wavelength spectrum of the light source supplied by or in association with the colorimeter. As noted above, the ambient spectrum is divided wavelength interval-by-interval by the colorimeter lamp spectrum for use in compensating for use of a non-ambient light source in illumination of a non-luminous object. The filter module 46 also includes sub-modules 52, 53 and 54 for receiving the uncompensated or compensated photosensor intensity signals and for forming the tristimulus convolution integrals X, Y and Z, respectively. Optionally, the filter module 46 may also include a sub-module 55 that determines the photosensor channel with the maximum intensity, and the maximum intensity thereof.

The electronic filter module 46 might be used, for example, to compensate for the use of a non-ambient light source in illumination of a non-luminous object or objects monitored by the colorimeter 11. The signal value received by each photosensor in the array 19 (Figs. 1A, 1B, 1C and 1D) represents the component of incident light lying in a predetermined, small wavelength range. Signal values from direct illumination of the VWF 17 by the colorimeter light source 23 are compared with one another and with relative photosensor signal values produced by direct illumination of the VWF 17 by an ambient (reference) light source 23, with these two sets of signal values being provided in a memory. The presently received photosensor signal value (assumed to be non-zero) may be multiplied by the ratio of the direct illumination, ambient source signal value divided by the direct illumination, non-ambient source value within the electronic filter module 46, to compensate for illumination of a non-luminous object by the non-ambient light source 23. These compensated signal values, rather than the presently received signal values, may be used for subsequent colorimeter computations. The compensated (or uncompensated) signal values $I(\lambda)$ may be convolved with the CIE color matching functions $\bar{x}(\lambda)$, $\bar{y}(\lambda)$ and $\bar{z}(\lambda)$ stored in memory to relate the spectrum to tristimulus convolution integral values X, Y and Z by the equations

$$X = \int I(\lambda)\,\bar{x}(\lambda)\,d\lambda, \quad (1)$$

$$Y = \int I(\lambda)\,\bar{y}(\lambda)\,d\lambda, \quad (2)$$

$$Z = \int I(\lambda)\,\bar{z}(\lambda)\,d\lambda. \quad (3)$$

Illumination/compensation of the object by the ambient and colorimeter light sources are illustrated in Figs. 3A and 3B.

Information placed on a bus 48 is received by a central processing unit ("CPU") 56, part of a computer system, for further processing if necessary. Information carried on the bus 48 is also received by a color map module 57 and by a color transform engine 58 that perform appropriate color transformations upon the information received based upon the nature of the object that produced the color data. The CPU 56 controls data distribution to and from a plurality of peripheral devices such as a monitor 61, a printer 63, a scanner 65 and any network connection 67. These three peripheral devices 61, 63 and 65 and network connection 67 have the respective input/output ports 62, 64, 66 and 68, respectively, connected to the bus 48 and thereby to the CPU 56. Color or wavelength distribution information from the monitor 61, the printer 63 and the scanner 65 is optically collected and is read by the colorimeter 11 to complete the circuit. Data sent along the network channel would be converted to a standard color data format, preferably based on a system such as the CIE Lab, YUV or LUV coordinates. Color conversion or compensation information can be processed in real time by the system shown in Fig. 2.

The CPU 56, or an alternative co-processor or state machine, computes a table of color conversion values for each peripheral device measured by the colorimeter. In some instances color conversion may be processed using standard matrices where CPU 56 would determine the conversion matrix coefficients to convert color data from one tri-stimulus representation to another. The non-linear behavior of many peripheral devices, such as printers, degrades the accuracy afforded by simple matrix conversion. In such instances, the conversion values preferably include a mapping of color data values from a given data representation to the representation used by the particular peripheral device. Normally, the mapping will also include color gamut compression or other compensation for the best mapping of colors in a data representation that cannot be exactly produced by the given peripheral device. The color conversion values loaded into the color map module 57 are changed in content for each color conversion process by the CPU 51 that controls it. Normally, color image data will be stored in a color data module 70 associated with the CPU 56 and transmitted along the network between computer systems in a standard format, preferably according to a colorimetric standard such as the CIE Lab luminance/chrominance values. These data may

be converted to a device-dependent color format, such as RGB or CYMK, adjusting the conversion map to include the device calibration. As illustrated in Fig. 4, color data stored in the color data module 70 may be converted by a first look-up table 59, calibrated in RGB color format, for display on a specific monitor 61, and the formatted color data may then be displayed on that monitor 61. The color data stored in the color data module 70 may also be converted by a second look-up table 60, calibrated in CYMK color format, for printing on a specific printer 63.

One task of the color map module 57 may be to convert a particular set of color tri-stimulus device-independent values such as CIE XYZ, Lab, YUV or LUV to a set of color tri-stimulus device-dependent values such as RGB (red/green/blue) or CYM (cyan/yellow/magenta), or conversely, to evaluate video or other color presentations. A color map used to evaluate an RGB color monitor will differ from a color map used to convert or monitor colored objects produced by a CYM color printer 63. The color map can also be used to convert uncorrected image color format, such as RGB or CYM, to a device-dependent, corrected image color format, for display of the image on a particular monitor, printer or scanner. The CPU 56 will compute the color map requirements based on accepted conversion algorithms, will determine color map contents from experimental data held in memory, or will compute or interpolate alterations to this experimental data based upon calibration information received from the colorimeter 11.

The contents of each color map produced by the color map module 57 may represent a small fraction of the number of possible colors represented in the image data. Intermediate color values not specifically contained in a given color map may be interpolated by using the color transform engine 58 and a linear or non-linear three-dimensional interpolation scheme.

Each peripheral device is calibrated by comparing the ideal or reference response of the device to a given stimulus with the actual response of the peripheral device. The monitor 61 and the printer 63, for example, receive commands for outputs from the CPU 56 under control of software. The response of the displayed colors on the monitor 61 and printed colors on the printer 63 are accurately measured by the colorimeter 11 and are fed back as XYZ tri-stimulus values to the CPU 56. Any color errors produced by the peripheral device being monitored are used to generate or modify the contents of the color map module 57 so that, when subsequent output commands for color are given by the CPU 56, the output of this peripheral device will be corrected and made as near to the ideal or reference values as possible. The network shown in Fig. 2 thus represents a feedback loop in which the spectral or wavelength distributions associated with a given peripheral device are first monitored and then corrected.

Calibration of a scanner device 65 is accomplished by scanning and colorimetrically measuring a color test chart, feeding the colorimeter output values to the CPU 56, comparing the values sensed by the colorimeter 11 with the values from the scanner, and determining a color map for translating scanner output data into device-independent form.

Calibration of a color monitor is accomplished by first measuring the spectrum of each RGB phosphor used in the monitor at full gun intensity and measuring the monitor's full intensity white, produced by a fixed combination of the R, G and B colors. These spectra are then converted to XYZ values, as described above. The transfer function of each gun is determined by measuring the intensity at a spectral peak for each phosphor at a sequence of gun input voltages between zero phosphor intensity and full phosphor intensity. Normally, measurement of output intensities for five to seven different voltages suffices for each phosphor. Fig. 5 exhibits typical gun transfer functions for the R, G and B phosphors. The gun transfer functions may be used to determine gain, offset and gamma values for each gun. Knowledge of the gun chroma and transfer functions can then be used to compute conversion color maps or matrix coefficients. The color transform engine 58 is used to make the pixel data corrections required to correct the color values associated with a given peripheral device. A combination of the colorimeter 11, the CPU 56, the color map module 57 and the color transform engine 58 can thus provide for monitoring, conversion and correction of the color data associated with each peripheral device.

After the color values associated with a given color conversion have been determined, these values may be stored in memory and retrieved for use as needed. These values would only be changed if re-calibration is required. It may be preferable to incorporate the colorimeter 11, color map module 57, color transform engine 58 and CPU 56 in the host computer to allow manual calibration of a host computer system. The colorimeter 11 may be hand held and may be provided with an attachment through an I/O port to the remainder of the computer system shown in Fig. 2. The colorimeter 11, color map module 57, color transform engine 58 and CPU 56 may also be provided as a part of a peripheral device to allow auto-calibration. The colorimeter 11 may also be a stand-alone device, with color values being displayed or stored for later transfer to a color matching system.

In colorimetry, a color-sensing apparatus receives light having a plurality of wavelength components (1) from a self-luminous object or (2) as reflected or transmitted light from an object that is illuminated by a

light source. In this second instance, the wavelength distribution sensed by the color-sensing apparatus depends, in part, on the wavelength distribution of the illuminating light source. In order to eliminate the influence of the illuminating light source, the wavelength distribution of this light source is "divided out" or compensated for, wavelength interval by interval, and the effect of use of a reference light source is factored in, wavelength interval by interval. As a result of this, the wavelength distribution sensed by the color-sensing apparatus after compensation includes only the effect of illumination of any object by the reference light source.

Previously, this compensation was usually implemented by using color filters having large transmission wavelength intervals so that the compensation procedure was not very accurate. The compensation approach afforded by the invention disclosed here allows compensation using wavelength intervals as small as $\Delta\lambda$ = 1.5 nm width, or smaller if desired. This compensation approach also allows compensation for the non-uniform sensitivity, as a function of wavelength, of the individual light sensors and optical and VWF components that are part of the apparatus. The compensation process is carried out electronically here for each separate wavelength interval of width $\Delta\lambda$. The wavelength distribution of the received light is convolved separately with the $\bar{x}(\lambda)$, $\bar{y}(\lambda)$ and $\bar{z}(\lambda)$ color matching functions to convert the received light spectrum into tristimulus values.

The system of Fig. 2 may also be adapted to provide correct color rendering by a peripheral device located in an ambient environment that differs spectrally from the ambient environment of the user workstation. Such conditions occur for shared printers, for example, that may be remotely located in, and accessed over, a network by a printer server. In such instances, the ambient spectrum of the user workstation may be measured by colorimeter 11 and transmitted over a network 67 to the print server. A similar system as in Fig. 2, located within the server, would be used to calibrate the remote printer 63. The contents of the color map module 57 or matrix coefficients used in the server, however, would be computed using the ambient spectrum of the user workstation instead of using the spectrum of the server ambient illumination. The resultant print, would thereby appear correctly colored when viewed in the user environment. Similarly, print colors can be manipulated to appear correct in any given ambient environment by transmitting the target ambient spectrum to the CPU 56 that controls the target printer 63 and conversion map generation. A user operating in an incandescent illumination environment, for example, could produce printed images appearing color correct in sunlight or in fluorescent illumination by forwarding the appropriate target ambient spectrum to the controlling CPU 56. Such spectra may optionally be accessed from a library of spectral values.

A colorimeter, constructed according to the invention, may include a probe 71 that can be placed contiguous to the screen of a color monitor 73 or other self-luminous source, as in Fig. 6, to sense the color(s) present on that part of the monitor screen, using the VWF and photosensor array combination discussed herein. The probe 71 may be moved from one area to another on the screen of the monitor 73 to sense and calibrate different patches of colors displayed on the screen. The probe 71 may also be used to sense a patch or patches of color on a non-luminous object, such as a color printer 77 shown in Fig. 7. The object, a page 75 containing colored portions that the printer 77 has issued, is illuminated by an ambient light source 79, and the probe 71 is then pointed at, and used to sense the wavelength distribution of light emitted by, the source 79 for calibration and (optionally) compensation purposes. The color sensed on an object, such as the screen of the color monitor 73 or on a page 75 issued by a color printer 77 may also be entered into a computer system for use in graphics or computer aided design.

Filter Construction

The variable wavelength filter 17 used in the colorimeters shown in Figs. 1A-1D is described in various embodiments in a related patent application, U.S.S.N. 07/480,172, filed by the inventor hereof on February 14, 1990 and assigned to the assignee of this application. These embodiments of the filter(s) include the following.

(1) A wedge of light filter material whose thickness $h_2(x)$ in the direction of light propagation varies linearly, as shown in Fig. 8, or non-linearly with a spatial coordinate x measured in a selected direction lying in a light-receiving surface of the filter. The filter 81 shown in Fig. 8 is a wedge-shaped stratum of filter material having a first light-receiving surface 83 that receives an incident light beam 85 at an incidence angle θ_1 and having a second light-receiving surface 87 that is spaced apart from the first surface 83, where these two surfaces are both planar and intersect one another at a small, non-zero angle ϕ. One or both of the light-receiving surfaces 83 and 87 may have a coating or other filter layer thereon, of respective thicknesses $h_1(x)$ and $h_3(x)$ as shown. Although Fig. 8 illustrates operation of the filter 81 in a light transmission mode, the filter may also be operated in a light reflection mode. At each spatial position x, the

thickness $h_2(x)$ at that position will promote the transmission (or reflection) of light of wavelength $\lambda = \lambda_c(x) = 2n_2h_2(x)$ ($\lambda = \lambda_c(x) = 4n_2h_2(x)$) and light of certain side band wavelengths that are spaced apart from the wavelength $\lambda_c(x)$, where n_2 is the refractive index of the filter material. In order to suppress or eliminate the side bands, a narrow band variable wavelength light filter as discussed above is used in serial combination with a wider band variable wavelength light filter that passes the central wavelength $\lambda = \lambda_c(x)$ but suppresses the undesired side bands. The serial combination of the narrow band filter and the wider band filter is the variable wavelength filter ("VWF") 17 that is used in Figs. 1A, 1B, 1C and 1D.

The variable distance of separation $h_2(x)$ between the two reflecting surfaces or layers 83 and 87 in Fig. 8 preferably is monotonically increasing or monotonically decreasing with increase of a spatial coordinate x and includes at least one position x_0 for which $h_2(x_0) = m_2\lambda_c/2n_2$, where m_2 is a positive integer and n_2 is the real part of the refractive index for the filter material in the filter stratum 81. Thus, for at least one position x_0 along the filter the total optical path length for transmission through the stratum 81 is an integral multiple of the half wavelength $\lambda_c/2$. The incident light may arrive with a small or modest incidence angle relative to the first light-receiving surface 83. In practice, this incidence angle is usually chosen to be zero degrees. The spatial coordinate x may be a rectilinear variable such as a Cartesian coordinate, may be an angular variable such as a polar or azimuthal coordinate, or may be any other curvilinear spatial coordinate.

Passage of a light beam through a single wedge-shaped stratum of filter material 81 may produce fringes at nearby wavelengths that are commensurate with the central wavelength for which the system is designed. Optionally, one can include a single filter layer on one of the light-receiving surfaces 83 and 87 of the filter stratum 81; or two or more filter layers can be provided at one or both of the light-receiving surfaces 83 and 87 of the stratum 81. These filter layers are chosen to have a relatively narrow wavelength band pass region centered at or near a central wavelength λ_c of interest and to filter out by absorption or reflection any incident light at any fringe wavelengths that might otherwise be present in the transmitted beam 89.

A function h(x) is defined to be monotonically increasing in the coordinate x if $h(x_1) \leq h(x_2)$ whenever $x_1 < x_2$. In the embodiments of the invention discussed herein, it is sufficient if the variable distance of separation h(x) of two surfaces increases (or decreases) monotonically with the spatial coordinate x so that intervals of constant separation h(x) may be interspersed with intervals where h(x) increases strictly monotonically with x ($h(x_1) < h(x_2)$ whenever $x_1 < x_2$). One example of a monotonic, but not strictly monotonic, increasing function is the distance h(x) between a reflecting surface 165 and a sequence of staircase step reflecting surfaces 167-q (q = 1,2,...) illustrated in Fig. 16, discussed below. An example of a strictly monotonically increasing function is the distance h(x) between the two light-receiving surfaces 83 and 87 shown in Fig. 8.

The materials from which the first and second reflecting surfaces 83 and 87 are made should be at least partly transmitting and should absorb at most only a small fraction of the light of wavelength λ_c transmitted through that thickness of material. The materials for the first and second reflecting surfaces 83 and 87 and for the filter stratum 81 may be drawn from glasses, insulators, semiconductor materials such as silicon and germanium, metals such as aluminum, or other materials such as magnesium fluoride, zinc sulfide, calcite, sapphire, and oxides of aluminum, silicon, germanium, calcium, cadmium, titanium, cerium and zirconium. The spacer material for the filter stratum 81 should be transmitting, with a small or infinitesimal absorption for the light of wavelength λ_c and the range of thicknesses $h_2(x)$ used here. The material for the spacer layer may also be vacuum or air, if the layers 83 and 87 serve as reflecting surfaces for the incident light.

Thin film optical filters are well known in the art. They are normally constructed by vacuum deposition of alternating high and low refractive index optic layers on a given substrate, such as glass. The comparative phrases "high refractive index" and "low refractive index" are used relative to one another here. In the visible and near-infrared wavelength regions, a low (high) refractive index material might have an index of refraction $n \leq 1.7$ ($n > 1.7$). In the remainder of the infrared wavelength region, a low (high) refractive index material might have an index of refraction $n \leq 2.5$ ($n > 2.5$).

Light entering a stack of optically transmitting layers is partially reflected at the boundaries of each layer, with a reflectance amplitude determined by the ratio of the refractive indices across the boundary. A reflection that occurs when a beam approaches a higher refractive index medium from within a lower index medium includes a 180 degree phase shift. A reflection that occurs when a beam approaches lower refractive index medium from within a higher index medium has no phase shift at the boundary. The reflected beams recombine constructively or destructively depending on their phase relationship. By constructing the optical filter with multiple thin layers, each having an optical thickness equal to a quarter wavelength of the incident light or an appropriate multiple thereof, the transmittance or the reflectance of the filter can be made high over a limited range about a central wavelength, and at certain fractional multiples

of this wavelength, depending on the ratio of the high and low refractive indices and number of layers.

Outside these spectral zones the reflectance of the multi-layer combination changes abruptly to a low value. The range of reflected wavelengths can be extended by including additional layers of different quarter wavelength thickness, by varying the layer thicknesses, and by maximizing the ratio of refractive indices of successive layers used in the stack. When the filter is constructed of essentially non-absorbing optical materials, wavelengths of light not rejected by reflection are transmitted through the filter, and conversely. Because of this behavior, one can construct thin film optical filters having desired characteristics over a broad wavelength range, including but not limited to short and long wave pass edge filters, narrow and broad band pass filters, and notch filters for both reflection and transmission applications.

Narrow band pass thin film filters are normally constructed using one of two approaches. A first approach combines two edge filters whose transmission ranges or reflection ranges overlap spectrally over a very narrow wavelength band of interest. Sharp edged short wave and long wave pass quarter wave plate filter stacks are well suited for producing narrow transmission bands. Here, the phrase "quarter wave plate" means an optically transparent material (with low absorption at the wavelength λ of interest) having a multiplicative product of thickness h and refractive index $n(\lambda)$ that satisfies $hn(\lambda) = \lambda/4, 3\lambda/4, 5\lambda/4$, etc. The phrase "half wave plate" refers to an optically transparent material for which $hn(\lambda) = \lambda/2, 2\lambda/2, 3\lambda/2$, etc.

A second approach combines two or more half wave plate spacer strata, each sandwiched between two high reflection (typically greater than 80%) multilayer stacks, to form an interferometer similar to a Fabry-Perot interferometer. Light reflected within the spacer strata undergoes multiple beam interference. Transmission (or reflection) of light incident on the filter becomes extremely high only over a narrow band of wavelengths around that for which a spacer stratum optical thickness is an integer multiple of one half the wavelength of interest. In some instances, reflective metal layers, or combinations of thin film reflective coatings and metal reflectors, are substituted for the pure thin film high reflectance coatings described above.

In each of the above narrow pass band filter approaches, the desired wavelength bandpass is accompanied by higher or lower order fringes and side bands. These undesired bands must be suppressed by additional filtering, usually using combinations of broad band pass thin film filters, absorptive filters or different order Fabry-Perot type filters designed to spectrally overlap only at the desired narrow band pass zone. Normally, the combination of narrow band and broad band attenuation filters will require the coating of one or both sides of one or more substrate plates.

Many materials are suitable for thin film deposition of the light-transmitting layers and have low absorption within specific spectral regions. For the visible and near-infrared spectrum, some of the most common high index coating materials include germanium (refractive index $n = 4.0$), silicon ($n = 3.2$), zinc sulfite ($n = 2.35$), titanium dioxide ($n = 2.2\text{-}2.4$), zirconium dioxide ($n = 2.2$), cerium oxide ($n = 2.2$), zinc sulfide ($n = 2.12$) and cadmium oxide ($n = 2.06$). The most common low index coating materials for the visible and near-infrared spectrum include magnesium fluoride ($n = 1.38$), quartz ($n = 1.45$), silicon dioxide ($n = 1.5$) and aluminum oxide ($n = 1.62$). Antimony trioxide and cryolite are typically used as coating materials suited for the 300-400 nm region of the ultraviolet. For the mid- and far-infrared region, germanium ($n = 4.0$) and lead telluride ($n = 5.5$) are good high index materials, and zinc-sulfide ($n = 2.35$) is a good low index material.

There are an unlimited number of combinations of quarter wave and half wave plate layers, and optical materials for producing filters of the varieties already described in treatises such as that of H.A. McLeod, Thin Film Optical Filters, MacMillan, 1986, incorporated by reference herein. This reference is representative of the types and number of designs available in the art.

Continuously variable narrow band pass interference filters, of the type used for the present invention, can be made by varying the thickness of one or more coating layers in the filter stack along a given direction in a plane of the filter stack. In one embodiment, the filter is a wedge defined by two planar layers, so that the center wavelength λ_c of the narrow band pass filter will vary approximately linearly along the length or other selected direction of the filter. Each wedge or otherwise variable thickness filter stratum can be fabricated by moving a mask across the substrate during vacuum deposition of the filter layer on the substrate. The moving mask blocks deposition over areas shadowed by the mask. The desired coating thickness gradient can thus be produced by controlling the ratio of the deposition rate and mask translation rate.

Fig. 9 shows the theoretical transmittance of the second order fringe of a two-cavity Fabry-Perot filter having 85% reflectance metallic layers and a glass spacer stratum ($n = 1.52$) that varies linearly in physical thickness from about $h = 400$ nm to about $h = 1000$ nm along its length. The third, fourth and fifth order pass band transmittances peak at $\lambda = 733$ nm, $\lambda = 550$ nm and $\lambda = 440$ nm wavelengths at the position of the filter where the half wave physical thickness is $h = 812$ nm, 609 nm and 487 nm, respectively. The solid line

transmittance curve and broken line transmittance curve correspond, respectively, to values of the coordinate $x = x_1 = 0.5L$ and $x = x_2 = 0.7L$ at the observation point, where L is the length of the filter. Intermediate peaks occur at intermediate wavelength positions between the two ends of the filter.

As mentioned before, a spectrally broad, high reflectance thin film stack can be substituted for the above metallic layers. The technique can be applied to a layer or layers of any of the above thin film filter types, including broad band pass filters, short wave pass edge filters, long wave pass edge filters, and narrow band pass filters, in transmission mode or reflection mode. Usually, a combination of filter types will be required to provide a single narrow band pass with side band or fringe blocking. Variable thickness layers are preferred in the side band blocking layers as well as in the narrow band pass layers so that in-band filtering and out-of-band filtering complement one another along the variable wavelength direction of the filter. However, the side band blocking filter may use a fixed pass band in some situations. Often more than one substrate will be required to accommodate all filter layers, and the substrates will need to be properly aligned.

If one ignores the variation of the separation distance $h_2(x)$ with x and concentrates on a light beam 85 incident on the filter stratum from above in Fig. 8, the normalized intensity reflection coefficient I_r and the normalized intensity transmission coefficient I_t for the filter stratum 81 alone become, respectively,

$$I_r = \frac{4\,R\,\sin^2(\beta/2)}{(1-R)^2 + 4\,R\,\sin^2(\beta/2)}, \qquad (4)$$

$$I_t = \frac{(1-R)^2}{(1-R)^2 + 4\,R\,\sin^2(\beta/2)}, \qquad (5)$$

$$\beta = 4\pi\, n_2 h_2(x) \cos\theta/\lambda, \qquad (6)$$

where $n_2(\lambda)$ is the refractive index of the filter stratum 81, θ is the incidence angle of the light beam, R is the reflection coefficient for the interface between the filter stratum 81 and the ambient medium. Absorption in the filter stratum 81 is ignored. This configuration produces a sequence of band pass regions separated by wavelength free spectral ranges of $\Delta\lambda = 2nh_2(x)\cos\theta/m$ $(m = 1,2,3,...)$ that depend upon R and fringe order m. These results are found in M. Born and E. Wolf, Principles of Optics, Pergamon Press, Fifth Ed., 1975, pp. 323-329, incorporated herein by reference. Inclusion of the effects of absorption in these equations would produce a small quantitative change in the results. For $R < 1$ and $\theta = 0$, the intensity transmission coefficient I_t is maximized by the choice $\sin\beta/2 = 0$ or

$$h_2(x) = m(\lambda_c/2n_2) \quad (m = 1,2,3,...); \qquad (7)$$

for light of wavelength $\lambda = \lambda_c$. The intensity reflection coefficient I_r is maximized by the choice $\sin\beta/2 = \pm 1$ or

$$h_2(x) = (m-1/2)(\lambda_c/n_2) \quad (m = 1,2,...). \qquad (8)$$

Different wavelengths are emphasized for the transmission and for the reflection situations, for fixed wavelength and thickness $h_2(x)$. These relations are true only for a single stratum. For multi-layer thin films, other considerations apply.

The apparatus shown in Fig. 8 is not, strictly speaking, a Fabry-Perot etalon because the reflecting planar surfaces or layers 83 and 87 are not quite parallel. Closely spaced groups of rays from the incident light beam will pass through substantially identical thicknesses h_2 of the filter stratum 81 and will not rapidly "walk off" the etalon because of the small angle ϕ between the planes of the reflecting surfaces 83 and 87.

Two closely spaced groups of rays that are separated by a distance Δx in the selected direction of the order of 10μm or more may pass through substantially different thicknesses $h_2(x)$ of the filter stratum 81 and thus may manifest different interference effects. Thus, the portion 89 of the light beam 85 transmitted through the filter stratum 81 in Fig. 6 at two positions $x = x_1$ (unbroken lines in Fig. 9) and $x = x_2$ (dotted lines in Fig. 9) may exhibit peaks of transmitted light intensity $I(\lambda;x)$ at different sets of wavelengths, as illustrated in Fig. 9.

Alternatively, if one fixes the incident light beam wavelength λ and observes the transmitted light

intensity $I(\lambda;x)$ as a function of position x along the filter, a graph of this intensity will show a sequence of well separated peaks and intervening valleys. The set of wavelengths for which maxima of transmitted intensity $I(\lambda;x)$ occur will shift approximately continuously, and the magnitudes of these maxima will change approximately continuously, as one varies the position x of observation of the transmitted portion 89 of the beam shown in Fig. 8. Thus, one has in hand a technique for controllably and continuously shifting the peak wavelengths, which technique can be used in spectrophotometric, colorimetric and other applications.

An optical system such as the light filter embodiment shown in Fig. 8 promotes or enhances the intensity of light issuing at a selected fundamental wavelength $\lambda = \lambda_0$ of order $m = 1$ and at other fringe wavelengths $\lambda = \lambda_0/m$ (m an integer ≥ 2). If a higher order fringe is used for the central wavelength ($\lambda_c = \lambda_0/m$ with $m \geq 2$), the rise and fall with respect to wavelength of that fringe signal will be sharper than the corresponding rise and fall for the fundamental ($m = 1$), but the center-to-center wavelength difference $\Delta\lambda$ of adjacent and undesirable fringes will be reduced. If, now, a broad band light filter is also provided for which a representative central wavelength is $\lambda_{bb,c} = \lambda_0/m_{bb}$ for the first or second order ($m_{bb} = 1$ or 2), the attenuation with respect to wavelength will be less sharp but the fringe or side band wavelengths $\lambda = \lambda_0/m_{bb}$ ($m_{bb} \geq 2$) will be spaced apart much further and their presence may be ignored here. Thus, the serial combination of a narrow band pass light filter operating at a central wavelength $\lambda_c = \lambda_0/m$ ($m \geq 2$) and a broad band light filter operating at a central wavelength $\lambda_{bb,c} = \lambda_0/m_{bb}$ with $m_{bb} = 1$ or 2, will produce a single, sharply attenuated, narrow band filter in which the controlling or central wavelength λ_c may be caused to vary by the approaches discussed herein.

Preferably, a light filter according to the invention will be provided with both a narrow band filter and a broad band filter. The broad band pass filter may have a fixed broad band pass or may have a variable broad band pass with a central wavelength that is the same as, and changes with, the central wavelength λ_c of the narrow band pass filter.

(2) The filter may also be operated in the reflection mode, as illustrated in Fig. 10 for a simple etalon with quarter wave plate spacing between the reflective surfaces, if the amplitude reflection coefficient R of the first reflecting surface is low ($R \leq 0.2$). An incident light beam 91 approaches a first reflecting surface 93 of a wedge-shaped or variable thickness light filter stratum 95 at an incidence angle θ_1. The stratum 95 has a thickness $h_2(x)$, measured between two reflecting surfaces 93 and 97 of the device, that varies with a coordinate x measured along some direction lying in the light-receiving surface 93. The incident light beam 91 is partly reflected at the surface 93, producing one of the reflected rays 99 in an array of such rays, and is partly transmitted at a refraction angle θ_2 into the filter stratum 95 in a well known manner. The refracted light beam 101 is partly reflected at the second reflecting surface 97 of the filter stratum 95, producing a reflected light beam 103 that propagates back toward the first reflecting surface 93. The remainder of the refracted light beam 101 is transmitted and emerges as another light beam 105 that is treated here as a rejected light beam. High reflection at the surfaces 93 and 97 can be suppressed, except for a narrow, sharply peaked wavelength pass band, by use of appropriate anti-reflection coatings 107 and 109, respectively, at the surfaces. These coatings are discussed by H.A. McLeod, Thin-Film Optical Filters, op.cit., pp. 71-136.

(3) A narrow band variable wavelength filter 111, operating in the reflection mode, followed or preceded by a wide band variable wavelength filter 113, also operating in the reflection mode, as shown in Fig. 11, where the initial light beam 115 is twice-reflected and received by a photosensor array 117 as shown. The narrow band and wide band variable wavelength filters are constructed as discussed above.

(4) A narrow band variable wavelength filter 121, operating in the transmission mode, followed or preceded by a wide band variable wavelength filter 123, operating in the reflection mode, as shown in Fig. 12, where the initial light beam 125 is once-transmitted and once-reflected and is received by a photosensor array 127 as shown. Alternatively, the transmission mode filter 121 may be wide band and the reflection mode filter 123 may be narrow band. Optionally, an optical prism 129 may be provided between the first light filter 121, the second light filter 123 and the photosensor array 127, as shown in Fig. 12. Preferably, the light beam in Fig. 11 or Fig. 12 should approach the photosensor array at approximately normal incidence.

For a high finesse system, where the finesse $F = \pi R^{1/2}/(1-R) >> 1$, the intensity reflection coefficient I_r will generally be close to 1 except adjacent to wavelengths for which $\sin \beta/2 = 0$, where $I_r = 0$ and the spacer thickness $h_2(x)$ and the light wavelength λ_c are related by Eq. (7). Where the reflection mode is used, then, it may be preferable to use a combination of a high pass edge filter and a low pass edge filter to provide an intensity reflection coefficient I_r that is sharply peaked at a sequence of wavelengths (for fixed spacer stratum thickness) or at a sequence of spacer stratum thicknesses (for constant wavelength), as discussed in connection with Figs. 13A, 13B, 13C and 13D.

The filters shown in Figs. 8, 10, 11 and 12 may be replaced by compound edge filters to produce a

narrow band of reflected (or transmitted) wavelengths, as illustrated in Figs. 13A and 13C, or a narrow band of reflected (or transmitted) wavelengths, as illustrated in Figs. 13B and 13C. In Fig. 13A, the variation of light wave transmission $T_{HP}(\lambda)$ through a high pass edge filter and transmission $T_{LP}(\lambda)$ through a low pass edge filter are each shown as a function of wavelength λ. The low pass transmission filter has a "knee" wavelength $\lambda = \lambda_{e1}$, for which $\lambda \leqq \lambda_1$ insures that $T_{LP}(\lambda)$ is near its maximum value; and for $\lambda > \lambda_{e1}$ and increasing, $T_{LP}(\lambda)$ falls rapidly to approximately zero. The high pass transmission filter has a knee wavelength $\lambda = \lambda_{e2}$, for which $\lambda \geqq \lambda_{e2}$ insures that $T_{HP}(\lambda)$ is near its maximum value; and for $\lambda < \lambda_{e2}$ and decreasing, $T_{HP}(\lambda)$ falls rapidly to approximately zero, as illustrated in Fig. 13A. If the low pass and high pass transmission filters are chosen so that $\lambda_{e1} \cong \lambda_{e2}$, the serial combination of these two filters will provide a composite transmission filter with an issuance $I(\lambda)$ (here, issuance refers to transmittance) that has a sharply peaked, narrow pass band at a central wavelength $\lambda_c = \lambda_{e1} = \lambda_{e2}$, as illustrated in Fig. 13C.

In Fig. 13B, the variation of light wave reflectance $R_L(\lambda)$ from a low end band pass edge filter and reflectance $R_H(\lambda)$ from a high end band pass edge filter are each shown as a function of λ. The low end reflection filter has a knee wavelength $\lambda = \lambda_{e3}$, for which $\lambda < \lambda_{e3}$ insures that $R_L(\lambda)$ is near its maximum value; and for $\lambda > \lambda_{e3}$ and increasing $R_L(\lambda)$ falls rapidly to approximately zero, as shown in Fig. 13B. The high end reflection filter has a knee wavelength at $\lambda = \lambda_{e4}$, for which $\lambda > \lambda_{e4}$ insures that $R_H(\lambda)$ is near its maximum value; and for $\lambda > \lambda_{e4}$ and decreasing $R_H(\lambda)$ falls rapidly to approximately zero, as illustrated in Fig. 13B. If the low end and high end reflection filters are chosen so that $\lambda_{e3} \cong \lambda_{e4}$, the serial combination of these two filters will provide a composite reflection filter with an issuance $I(\lambda)$ (here, issuance refers to reflectance) that has a sharply peaked, narrow pass band at a central wavelength $\lambda_c = \lambda_{e3} = \lambda_{e4}$, as illustrated in Fig. 13C.

In Fig. 13D, the two knee wavelengths λ_{e1} and λ_{e2} from Fig. 13A are spaced further apart, and the result is a broad band pass filter as shown.

In each of the other embodiments discussed below, although the transmission mode of operation is discussed the invention may also be operated in the reflection mode, by analogy to Figs. 10, 11, 12, 13A, 13B, 13C and 13D and the above discussions thereof.

(5) With reference to an embodiment 131 of the filter shown in Fig. 14, if a light beam 133 is passed serially through a high pass edge filter 135 and through a low pass edge filter 137, both variable wavelength filters, in either order, in the embodiment 131, and if the two edge filter knee wavelengths are related as discussed in connection with Figs. 13A and 13B, the desired light beam 139T (transmitted mode) or 139R (reflected mode) that emerges from the edge filter combination will have a band of wavelengths centered approximately at a wavelength $\lambda_c(x)$ that varies monotonically with the coordinate x. For fixed position x, the reflected intensity of the beam 139R (or transmitted intensity of the beam 139T) issuing from the combination of two edge filters shown in Fig. 14 will resemble the intensity shown in Fig. 9.

In another embodiment of the filter, an incident light beam may be directed to a broad band pass filter, constructed with variable central wavelength λ_c according to any of Figs. 8, 10, 11, 12 or 14 or with a fixed broad pass band, and the resulting transmitted or reflected light beam may then be directed to a narrow band wavelength filter with variable central wavelength λ_c that is also constructed in accordance with one of these Figures. The serial arrangement of the broad band pass filter and narrow band pass filter, in either order, would be similar to the serial arrangement of two edge filters shown in Fig. 14. The broad band pass wavelength filter will remove wavelengths from all nearby pass bands from the incident light beam except the desired central band wavelengths so that no fringes appear in the light beam that issues from the combined filters.

(6) Fig. 15 illustrates another embodiment 141 of the filter, which includes a filter stratum 143 of variable thickness, another reflecting surface that is arranged in a staircase configuration and consists of a sequence of planar staircase segment surfaces 145-1, 145-2, 145-3, 145-4 and 145-5, and a staircased filter stratum 147, which may be a vacuum or may be a material medium, positioned between the filter stratum 143 and the reflecting surfaces 145-q. Each staircase segment surface 145-q (q = 1,2,3,4,5) is planar and is spaced apart from and approximately parallel to the plane of a first reflecting surface 149 of stratum 143, with a distance of separation h(x) between a nearest reflecting surface of the stratum 143 and a staircase reflecting surface 145-q that is constant for a small range of the coordinate x. The distance h(x) is increasing monotonically with the spatial coordinate x measured in a selected direction in a light-receiving plane of the stratum 143. Each staircase segment 145-q optionally has a filter layer 151-q (q = 1,2,3,4,5) deposited on or adjacent to the front or back reflecting surface of the staircase segment. The filter stratum 147 behaves as a narrow band pass light filter with variable spacer layer thickness h(x). The stratum 143 serves as a wide band pass filter whose thickness may be constant or may be monotonically increasing with x. In another embodiment, the stratum 143 may serve as a narrow band pass filter and the filter stratum 147 may serve as a broad band pass filter, with each staircase segment 145-q defining a different

broad pass band for the device.

An incident light beam 153 is partly transmitted through the stratum 143 and through the staircased filter stratum 147 and emerges as a transmitted light beam 155-q (q = 2,4 and 5 are shown in Fig. 15) that exhibits interference effects similar to those discussed above in connection with Figs. 8 and 9. The interference effects vary by small increments from one staircase segment to the next; but within a given staircase segment the system behaves as a variable wavelength filter similar to that of Fig. 8. The strata 143 and 147 may be contiguous or may be spaced apart from one another.

(7) In an alternative embodiment 161 of the filter, shown in Fig. 16, a first filter or spacer stratum 163 has a first reflecting surface 165 that is approximately planar and has a second reflecting surface 167-q (q = 1,2,3,4,5) that is formed in a staircase configuration with a monotonically increasing distance of separation $h_1(x)$ between the surfaces 165 and 167-q. A second filter or spacer stratum 177 has a second staircase configuration surface 179-q as a first reflecting surface and has a third staircase configuration surface 181-q as a second reflecting surface, with the distance of separation $h_2(x)$ between two corresponding surfaces 179-q and 181-q being monotonically increasing in x. One of the two filter strata 163 and 177 serves as a narrow band pass filter, and the other stratum serves as a wide band pass filter. An incident light beam 183 passes through both filter strata 163 and 177 and issues from the embodiment 161 as a sequence of light beamlets 185, each with a narrow wavelength range. The filter strata 163 and 177 may be contiguous or may be spaced apart from one another.

A "staircase configuration", as used herein, will refer to a configuration similar to that illustrated in Fig. 15 or Fig. 16, where a sequence of two or more planar reflecting segments is arranged so that each segment is approximately parallel to a given plane (for example, a plane of the strata 143 in Fig. 15), and the distance of separation of the sequence of staircase segments from the given plane increases monotonically as one proceeds consecutively from one segment to the next. The filter stratum 177 in Fig. 16 has two reflecting surfaces 179-q and 181-q, each of which is formed in a staircase configuration.

(8) Normally, a tradeoff must be made between narrowness of the wavelength pass band and transmission. McLeod, in his treatise Thin-Film Optical Filters, op. cit., pp. 270-276, discusses the use of a multi-layer thin film light filter to produce a very narrow, approximately rectangularly shaped optical transmission band with adequate transmission. Fig. 17A illustrates construction of one embodiment of a double half wave ("DHW") thin film filter that will provide such a band. For a fixed wavelength λ_f, a plurality of optically transparent layers of alternating high (H) and low (L) refractive indices n_H and n_L, respectively, are positioned contiguously as shown. High index layers (H) 191, 193, 195, 197, 199, 201, 203 and 205 are each quarter wave plates for the high index n_H. Low index layers (L) 192, 196, 198, 200 and 204 are each quarter wave plates for the low index n_L. Low index layers 194 and 202 are each half wave plates and thus function as Fabry-Perot etalons. Each of these two etalons 194 and 202 has substantially 0 percent reflectance and substantially 100 percent transmittance at the center wavelength $\lambda = \lambda_f$. However, the reflectance quickly rises toward 100 percent on either side of the valley at wavelength $\lambda = \lambda_f$. In a multilayer thin film embodiment of the invention, the two half wave plate layers such as 194 and 202 would each have a thickness that varies monotonically along a selected direction in a plane of the film. The computed transmittance of the thin film configuration shown in Fig. 17A, for the choices of refractive indices $n_H = 2.40$ (TiO_2) and $n_L = 1.45$ (quartz), is shown in Fig. 18A (similar to Fig. 7.14 of McLeod, op. cit.) as a function of the wavelength ratio $g = \lambda_f/\lambda$ (λ variable). The computed transmittance has a moderate width peak with a steeply rising or steeply falling curve at $|1 - \lambda_f/\lambda| \approx 0.05$ and some side band structure for $\lambda > 2\lambda_f$. Fig. 18B illustrates the computed transmittance of a configuration similar to that of Fig. 17A in which the two half wave plate layers are of high refractive index material rather than low refractive index material, another suitable embodiment of the invention.

One embodiment of the filter uses a first multilayer film filter stack with approximately constant reflectance over the wavelength band of interest and a second multilayer thin film filter stack in which the reflectance increases rapidly from a very low value in a narrow wavelength band (positioned at a central wavelength λ_c) to a much higher reflectance value just outside this narrow band. The wavelength λ_c is determined by a thickness $h_2(x)$ of a layer in the second film stack, and the thickness $h_2(x)$ is caused to increase monotonically with increase of a spatial coordinate x in a selected direction in the light-receiving plane of the filter. Serial combination of the first and second thin film filter stacks produces a very narrow band filter, centered at a wavelength λ_c that varies monotonically with position x in the selected direction. These two filter stacks are serially combined with a wide band pass filter (not shown in Fig. 17A) that is transmitting at wavelength $\lambda = \lambda_c$, in order to remove any undesirable side band structure and produce a single narrow band pass filter with a central wavelength λ_c that increases monotonically with change in position in a selected direction in a light-receiving plane of the filter.

With reference to Figs. 17A and 17B, the quarter wave plates 191, 192, 193 serve as a first reflector

211; the plates 195, 196 and 197 serve as a second reflector 213; the plates 199, 200 and 201 serve as a third reflector 215; the plates 203, 204 and 205 serve as a fourth reflector 217; the double thickness plates 194 and 202 serve as half wave plates 212 and 216; and the plate 198 serves as an optical coupler in the embodiment 224 shown in Fig. 17B. The optical coupler is optional and may be deleted here. The combination of the reflectors 211 and 213 and the half wave plate 212 as shown in Fig. 17B provides a special reflector whose light reflectance R as a function of wavelength λ is illustrated in Fig. 19. The reflectance has a high value, near 1.0, over most of the range of the wavelength λ, except for a narrow wavelength interval centered at $\lambda = \lambda_f$, where the reflectance drops sharply to a lower value R_0. The special reflector consisting of the elements 211, 212 and 213 in Fig. 17B, may be used by itself or may be concatenated with other, similar special reflectors, with or without optical couplers 214 interposed, to provide a narrow, sharply peaked transmittance T ($\approx$ 1-R) at the selected wavelength $\lambda = \lambda_f$.

A multi-layer thin film filter that produces the desired narrow, sharp peaks in reflectance or transmittance is not limited to the configuration shown in Fig. 17A. The half wave plates 194 and 212 may consist of high refractive index material, or the positions of the high and low index materials may be exchanged, or both of these changes may be made. Further, each of the four groups of quarter wave plates (191, 192, 193), (195, 196, 197), (199, 200, 201) and (203, 204, 215) shown in Fig. 17A may each be replaced by an odd number (1,3,5,7,9,...) of quarter wave plates of alternating refractive index (high/low) material. An example of this is shown in Fig. 20, in which four quarter wave plates 221, 223, 225 and 227 of low index serve as reflectors, two double thickness plates 222 and 226 of high index serve as half wave plates, and a quarter wave plate 224 of high index serves as an optical coupler, analogous to the arrangement shown in Fig. 17B.

Reduced to its minimum, a multi-layer thin film filter, of the type shown in Figs. 17A, 17B or 20, that produces a narrow, sharp peak in transmittance or reflectance requires N_1 ($\geq$4) quarter wave plates of high (or low) refractive index alternating with N_1-1 (or N_1-2) wave plates of low (or high) refractive index, where N_1-3 of these latter N_1-1 plates are quarter wave plates and the remaining two of these plates are half wave plates. In another embodiment, represented by the wave plates 221, 222 and 223 in Fig. 20, a suitable multi-layer thin film filter requires N_2 ($\geq$2) quarter wave plates of high (or low refractive index alternating with N_2-1 plates of low (or high) refractive index, where one of these N_2-1 plates of low (or high) index is a half wave plate and the remaining N_2-2 such plates are quarter wave plates. In either of these configurations, each half wave plate has variable thickness.

Thin film optical layers are most commonly manufactured by vacuum deposition. In this process, each thin film is condensed from the vapor phase onto the substrate in a vacuum chamber. The substrate is held at a temperature somewhat below the solidification temperature of the film. A variety of techniques are used to vaporize the film material. These include ion bombardment (sputtering), thermal evaporation, electron beam bombardment and other techniques.

The non-constant separation distance h(x) of two substrates, two surfaces of a single substrate, or two surfaces of a single filter layer, should preferably vary continuously, with the exception of the staircase configurations shown in Figs. 15 and 16. The shape function h(x) may vary linearly with x or may vary non-linearly with x, for example as $a + bx^k$ with $k < 1$, $k = 1$ or $k > 1$, as illustrated in the curves 231, 233 and 235, respectively, in Fig. 21.

For $k = 1$ in Fig. 21, a sequence of uniform width wavelength intervals will correspond to uniform widths on the variable wavelength filter. For $k > 1$, these wavelength intervals will have greater width at the low wavelength end than at the high wavelength end of the filter. This configuration would be useful where the light is to be dispersed more broadly at the low wavelength end and will cause the filter to transmit a smaller amount of light per unit length at the low wavelength end of the filter, as compared to the high wavelength end. For $k < 1$ the conditions are reversed, and the filter disperses light less at the low wavelength end. This configuration is useful if, as usually occurs, the photosensors used have reduced sensitivity to light at lower wavelength (for example, at $\lambda \approx 400$ nm) so that more light reception is needed for each photosensor at the low wavelength end. The separation distance h(x) is not limited to functions of the form $a + bx^k$. Exponential, trigonometric, logarithmic and other similar shape functions h(x) may also be used here.

Fig. 22 illustrates the use of the wavelength filter in spectrophotometer apparatus 241 that can be contained on a single semiconductor chip if desired. A spectrophotometer filter 243, fabricated in accordance with any of the embodiments discussed herein, is positioned so that it overlies and is adjacent to a linear array 245 of photosensor elements extending in the selected direction. Each photosensor element has a finite width w that may be of the order of 50 μm or less so that the linear array 245 of photosensor elements has a finite number of such elements present. Each of these photosensor elements receives a transmitted (or reflected) portion of an incident light beam 247 that issues from the filter 243 for a small

interval $x_0 - w/2 \leq x \leq x_0 + w/2$ of the spatial coordinate x, where x_0 is the central value of x in this interval. The wavelength increment per photosensor element is as small as $\Delta\lambda_c$ = 1 nm (nanometer) or as large as one wishes in one embodiment of this invention. Thus, the result of the interference effects will differ from one photosensor element to an adjacent element, and the wavelength(s) corresponding to a peak of transmitted intensity will also vary from one photosensor element to the next. Used in this manner, the linear array 245 of photosensor elements will distinguish between wavelengths on the basis of different peak wavelengths that are present in the narrow wavelength interval or portion of the light beam received by each photosensor element.

Photosensors suitable for the present invention are well known in the art and readily available commercially. The devices are offered in single and two dimensional arrays over a variety of photoelement aperture sizes, spectral sensitivity ranges, signal transfer techniques and package configurations. The linear, area and circular silicon photodiode arrays produced by EG&G Reticon Corp., Sunnyvale, California are representative devices for applications operating over the near ultraviolet to near infrared spectral range. These devices have relatively large photoelement areas, e.g. 50 microns by 100-2500 microns and are well suited for spectroscopic applications. Typical commercial array sizes are 128 to 2048 elements in a linear array, 256 by 256 elements to 64 by 2048 elements in an area array, and 64 to 720 elements in circular arrays. The photoelements are typically processed on a ceramic substrate and housed in a standard dual in-line package that is pin-compatible with integrated circuit sockets. Photoelements made of different materials can be used to extend photosensitivity to, or to alter photosensitivity in, other spectral ranges. For example, HgCdTe and InSb are often used in infrared sensors. Suitable photosensor materials for differing spectral ranges are well known and are discussed in L.A. Masten, "Understanding Optronics", Texas Instruments Learning Center, 1981, Chaps. 3, 4 and 7.

In a simple construction of the spectrophotometer, the filter substrate or a set of filter substrates is mounted to the surface of the photoelement package. To minimize the spacing between the photoelement array and the filter, the filter and sensor may be coupled optically through a fiber face plate. Sensors prefabricated with fiber face plates are offered commercially by EG&G Reticon, op. cit. The fiber face plate and minimized spacing reduce the possibility of bandpass broadening with high numeric aperture light incidence.

The projection of light on the photosensor face may be facilitated by use of a lens or lenses located on the object or image side of the filter substrate or substrates. For example, a projection lens can be mounted between the filter and sensor so that the filter and sensor are separated by some distance as shown in Fig. 22B, discussed below. In this configuration, the active dimension of the filter becomes related to the active dimension of the sensor through the magnification of the lens. In this manner, a relatively small dimension filter can be projected to a relatively large dimension sensor for increased resolution. Alternatively, a large dimension filter can be projected to a small dimension sensor for increased light sensitivity or economy.

Fig. 23 illustrates an embodiment 249 that includes two or more filters 244A and 244B to each receive and transmit a portion of a light beam 247, with each such filter having a non-overlapping or partially overlapping wavelength interval for which that filter is effective, and one or more photosensor arrays 245. This allows a single spectrophotometer to cover an increased wavelength range of any reasonable size with a single instrument, including the visible region and portions of the ultraviolet and infrared regions if desired. Fig. 24 illustrates the partial overlap of two wavelength intervals, denoted $\Delta\lambda$ 244A and $\Delta\lambda$ 244B, for the two adjacent filters 244A and 244B in Fig. 23, the first filter being effective for the coordinate range $x_1 \leq x \leq x_2$ and the second filter being effective for the coordinate range $x_3 \leq x \leq x_4$ where $x_1 < x_2 < x_3 < x_4$.

Fig. 23 illustrates the use of two or more light filters and photosensor arrays in an end-to-end configuration 249. An array of light filters 253 and an array of photosensors 255 can also be combined in a side-by-side configuration or in a two-dimensional array and embodiment 251 as shown in Fig. 25. In the two-dimensional array shown in Fig. 23, a central wavelength $\lambda_c(x,y)$ could vary continuously and monotonically with the coordinate x and could vary monotonically, either continuously or by discrete amounts, with the coordinate y, measured in a direction perpendicular to the x direction.

Figs. 26A and 26B illustrate two embodiments that allow a VWF 271 and a photosensor array 273 to reside on a common substrate 275, with the VWF 271 and the photosensor array being positioned on the same side of the substrate 275 in Fig. 26A and being positioned on opposite sides of the substrate in Fig. 26B. Several sensor processes allow deposition of photosensitive material and driving circuits on glass substrates. One such process is described by Shinji Morozumi, et al., in "Completely Integrated a-Si:H Linear Image Sensor with Poly Si T.F.T Drivers", Extended Abstracts of the 16th (1984 International) Conference on Solid State Devices and Materials, Kobe, 1984, pp. 559-562. The sensor is constructed by depositing poly-silicon thin film transistor switches and amorphous silicon diodes on glass. Photodiode array sensors on glass substrates are offered commercially by Ovonic Imaging Systems, Troy, Michigan, Suwa

Seikosha Co., Ltd., Japan, and others. The ability to deposit both filter and sensor on silicon dioxide (quartz or glass), and further, the ability for one of the multi-component layers of the filter to be silicon dioxide, allows the filter and sensor to share a common substrate, sharing opposite sides of the substrate, or deposited, one over the other, on a common side of the substrate.

In the embodiment of Fig. 26C, a broad band pass VWF 281 is positioned on another light-receiving surface of the substrate 283 from a narrow band pass VWF 285 and a photosensor array 287. Alternatively, a broad band pass filter material may be incorporated with the substrate 283 so that no additional stratum or layer 281 is required, as suggested in Figs. 26A and 26B. In this instance the substrate may be a "colored glass" that transmits light wavelengths only within a pass band of modest width. For example, colored glass of Schott glass type UG1 and UG11 is available from Melles Griot that transmits only in the wavelength bands 275 nm $< \lambda <$ 405 nm and $\lambda >$ 680 nm (for UG1) and 240 nm $< \lambda <$ 395 nm and 660 nm $< \lambda <$ 800 nm (for UG11).

Preferably, the filter shown in any of Figs. 8, 10-17, 21, 22 and 25 includes: (1) a broad band pass filter that attenuates any light of wavelength lying outside a sharply defined pass band including a central wavelength $\lambda = \lambda_c$ and (2) a narrow band pass filter that has a central wavelength $\lambda = \lambda_c$ lying in the narrow pass band, with any other multiples or side bands of this wavelength for which the narrow pass band filter is transmitting being heavily attenuated by the broad band pass filter. The narrow band pass filter and, optionally, the broad band pass filter, will have central wavelengths $\lambda = \lambda_c$ that vary with position x along the filter.

The present invention is intended to cover computer peripheral input and output devices, such as monitors, printers, scanners and networks, and other image input/output devices such as film recorders and digital and analog cameras. Computer workstations, and networks thereof, may be individually color calibrated and may communicate device-independent color data between themselves, using the invention disclosed here. The colorimeter and color calibration system may also be used to match colors or determine the colorant content of paints, inks, body fluids (blood, urine, etc.), dyes, fabrics, plastics and other colored liquids and solids. In these applications, the tri-stimulus data from the colorimeter is used to monitor and/or control two or more color constituents that are combined in the end product. The colorimeter and color calibration system disclosed here may also be constructed as a stand-alone instrument for industrial, commercial, medical and analytical color measurement. Using only one color component, such as brightness or luminance L, the invention may also serve for gray scale calibration or photometry.

Claims

1. A color measurement device (11 or 35) for determining the color composition of a small predetermined area at an arbitrary position on an object (14) having an exposed surface, the device being characterized by:

a variable wavelength light filter (17) having a light-receiving surface to receive incident light from the object having a plurality of wavelengths in a selected wavelength band, and to disperse the beam into a plurality of narrow wavelength pass bands, each with a control wavelength $\lambda_c(x)$ that increases monotonically with increase of a position coordinate x in a selected direction lying in the light-receiving surface;

light restriction means (13, 15) for receiving and admitting light from a restricted portion of the object (14) that defines the small predetermined area and for directing this light to the light-receiving surface of the light filter (17);

an array of photosensors (19), positioned to receive light that issues from the light filter (17) so that each photosensor in the array receives light in a very narrow band of wavelengths having the central wavelength $\lambda_c(x)$ for light received and transmitted at the position x on the light filter, with each photosensor in the array issuing an output signal that represents the cumulative light energy received by that photosensor from the light filter; and

color measurement means (45, 46) for receiving the photosensor output signals and for determining the color or colors present in the incident light based on the relative amounts of light of different wavelengths received at three or more of the photosensors (19).

2. The apparatus of claim 1, wherein said light restriction means is characterized by a window (13) having a first area transparent to light in said selected band of wavelengths, where this first area approximately coincides with said small predetermined area, and having a second area surrounding the first area that is substantially opaque to light in the selected band of wavelengths.

3. The apparatus of claim 2, further characterized by said window (13) including a light-admitting area of controllably variable size.

4. The apparatus of claim 1, further characterized by a mobile housing (31) surrounding and containing said variable wavelength light filter (17), said photosensor array (19) and said light restriction means (13, 15), the housing being adapted for movement in one direction or in two independent directions across at least a portion of said exposed surface of said object (14) and having position sensing means for sensing the present position of said light restriction means on said exposed surface.

5. The apparatus of claim 1, further characterized by a light source (23) that illuminates said small predetermined area of said object (14) with light having wavelengths in said selected band of wavelengths, when said color measurement device is placed at said arbitrary position on or adjacent to said object.

6. The apparatus of claim 5, wherein said photosensor array is characterized by spectrum adjustment means for compensating for the wavelength distribution of light emitted by said light source (23) relative to the wavelength distribution of a selected reference light source.

7. The apparatus of claim 6, wherein said spectrum adjustment means is characterized by:

spectrum memory means for receiving and storing a signal representing the intensity of light received from said light source (23) in a wavelength interval corresponding to each of said photosensors (19), and for storing a signal representing the intensity of light received in each of these wavelength intervals from said reference light source;

compensation means for dividing the light intensity signal from said reference light source for each wavelength interval by the light intensity signal from said light source (23) for the same wavelength interval to form a light intensity ratio for each of the wavelength intervals, and for storing this sequence of light intensity ratios; and

multiplication means for receiving a light intensity signal from said object in the wavelength interval corresponding to each of said photosensors, for multiplying this light intensity signal by the light intensity ratio for that wavelength interval stored in the compensation means, and for issuing the resulting product signals as a sequence of compensated light intensity output signals for the wavelength intervals corresponding to said photosensors.

8. The apparatus of claim 1, further characterized by position indicating means for visibly indicating said arbitrary position of said small predetermined area on said object.

9. The apparatus of claim 1, further characterized exposure adjustment means (41A, 41B) for receiving a cumulative light energy signal from at least one of said photosensors in said photosensor array (19) and for adjusting the exposure time of said photosensor to the light so that the cumulative light energy E received at that photosensor lies in a range given by $E_1 \leqq E \leqq E_2$, where E_1 and E_2 are two predetermined light energy values.

10. The apparatus of claim 1, further characterized by intensity determination means (42) for exposing a selected photosensor in said photosensor array (19) to light that issues from said light filter for a predetermined time interval of length $\tau > 0$, and for dividing the accumulated light energy E received by the selected photosensor during an exposure interval of length τ by τ to produce and issue a photosensor average light intensity signal $I = E/\tau$ as an output signal.

11. The apparatus of claim 10, further characterized by dark current compensation means (42, 44) for determining a dark current contribution E_d to said accumulated light energy E for said exposure time interval length τ, for determining a compensation factor K_d, where E_d and K_d may depend upon said length τ and upon operating temperature T so that the ratio $E_d(\tau,T)/K_d(\tau,T)\tau$ is substantially constant as said length τ is varied, and for forming and issuing a compensated average light intensity signal $I' = (E/\tau) - E_d(\tau,T)/K_d(\tau,T)\tau$ as an output signal.

12. The apparatus of claim 1, further characterized by convolution means (52, 53, 54) having a memory, for storing three color tristimulus functions $\bar{x}(\lambda)$, $\bar{y}(\lambda)$ and $\bar{z}(\lambda)$ that depend upon a central wavelength λ of a light beam received, for receiving said photosensor intensity signals $I(\lambda)$, where each of said photosen-

sor intensity signals depends upon said central wavelength $\lambda = \lambda_c(x)$, and for forming and issuing tri-stimulus convolution integral signals X, Y and Z defined by

$$X = \bar{x}(\lambda)\, I(\lambda)\, d\lambda,$$
$$Y = \bar{y}(\lambda)\, I(\lambda)\, d\lambda,$$
$$Z = \bar{z}(\lambda)\, I(\lambda)\, d\lambda.$$

13. The apparatus of claim 1, further characterized by compensation means (46) for receiving said photosensor output signals, for storing multiplicative compensation factors for each of said photosensor output signals that compensate for non-uniformity of response of each of said photosensors (19) with variation of wavelength of said light that is received from said light filter (17).

14. The apparatus of claim 1, wherein said variable wavelength filter is characterized by:

a first stratum (83, 135, 143 or 163) of filter material having two spaced apart light-receiving surfaces facing one another and that is partly transmitting and partly reflecting for light received of a light-receiving plane thereof and having a wavelength in a selected pass band of wavelengths, where the distance of separation of the two surfaces increases monotonically with displacement of position in the selected direction and for at least one position x the transmissivity or the reflectivity of the first stratum is enhanced for light of a selected wavelength λ_c in the selected pass band; and

a second stratum (85, 137, 147 or 177) of filter material having two spaced apart light-receiving surfaces facing one another with a distance of separation that varies monotonically with displacement in the selected direction, that has enhanced transmissivity or enhanced reflectivity for light having the wavelength λ_c,and that has substantially zero transmissivity or zero reflectivity, as the case may be, for light having a second wavelength approximately equal to a wavelength of any side band of the first stratum of filter material, and that is positioned so that light transmitted through one of the first filter stratum and the second filter stratum is received by the other filter stratum.

15. A color calibration system for monitoring and controlling the spectrum of colors presented by one or more computer peripheral devices (61, 63 or 65) controlled by a computer (56), the system being characterized by:

a variable wavelength light filter (17) having a light-receiving surface to receive incident light from the peripheral device (61, 63 or 65) having a plurality of wavelengths in a selected wavelength band, and to disperse the beam into a plurality of narrow wavelength pass bands, each with a control wavelength $\lambda_c(x)$ that increases monotonically with increase of a position coordinate x in a selected direction lying in the light-receiving surface;

light restriction means (13, 15) for receiving and admitting light from a restricted portion of the peripheral device (61, 63 or 65) that defines the small predetermined area and for directing this light to the light-receiving surface of the light filter (17);

an array of photosensors (19), positioned to receive light that issues from the light filter (17) so that each photosensor in the array receives light in a very narrow band of wavelengths having the central wavelength $\lambda_c(x)$ for light received and transmitted at the position x on the light filter, with each of at least three photosensors in the array issuing an output signal that represents the cumulative light energy received by that photosensor from the light filter;

color measurement means (46) for receiving the photosensor output signals and for determining the color or colors present in the incident light based on the relative amounts of light of different wavelengths received at three or more of the photosensors (19);

light calibration means (45) for receiving the output signal from the photosensor array (19), for comparing the amount of light received in the photosensors in the array with a reference table of light values that should be received by each of the photosensors, or by a linear combination of at least two of the photosensors, and for generating and issuing a sequence of output signals that indicates the difference between, or the ratio R of, the reference table entry for a photosensor, or combination thereof, and the amount of light actually received by that photosensor, or a combination thereof, in the array;

where the light calibration means output signals are received by the computer (56) and, in response thereto, the computer adjusts color data signals that are received by or rendered by the computer peripheral devices (61, 63 or 65), to reduce the differences between the reference table entries and the corresponding output signals from the color measurement means.

16. The apparatus of claim 15, wherein said color measurement means (45) is further characterized by being operatively associated with a remote imaging device and associated remote imaging device light source that produces and renders a color image of said object, and the apparatus includes remote device spectrum adjustment means for compensating for the wavelength distribution of light emitted by the remote imaging device light source relative to the wavelength distribution of a selected light source.

17. The system of claim 15, wherein said light filter (17) is characterized by a variable wavelength light filter having a light-receiving surface (93) to receive incident light (91) from the peripheral device having a plurality of wavelengths in a selected wavelength band, and to disperse the beam into a plurality of narrow wavelength pass bands, each with a central wavelength $\lambda_c(x)$ that changes with changes of a position coordinate x in a selected direction lying in the light-receiving surface.

18. The system of claim 15, further characterized by said central wavelength $\lambda_c(x)$ increasing monotonically with increase of said position coordinate x.

19. The system of claim 15, wherein said apparatus monitors and controls the spectrum of colors presented by first and second computer peripheral devices, the apparatus being further characterized by:

color transform means (58) for receiving said calibration means output signals as input signals for each of a first and a second computer peripheral device (61, 63 or 65), for transforming said calibration means output signals according to said color output signals presented by each of the first peripheral device and the second peripheral device and for issuing the transformed signals as output signals that are received by said computer (56) for color correction purposes.

20. The system of claim 15, wherein said light received Bfrom said computer peripheral device (61, 63 or 65) is initially provided by a light source (23) that illuminates an object produced by or associated with said computer peripheral device, the apparatus being further characterized by:

light source adjustment means (41A, 41B), for receiving said colorimeter output signals, for computing compensated wavelength distribution signals that compensate for the wavelength distribution of the light source used relative to the wavelength distribution of a selected reference light source, and for issuing these compensated wavelength distribution signals as output signals that are received as input signals by said light calibration means (45).

21. The system of claim 15, further characterized by optical compensation means (46) for receiving said photosensor output signals, for storing multiplicative compensation factors for each of said photosensor output signals that compensate for non-uniformity of response of each of said photosensors (19) with variation of wavelength of said light that is received from said light filter (17).

22. The apparatus of claim 15, further characteried by convolution means (52, 53, 54) having a memory, for storing three color tristimulus functions $\bar{x}(\lambda)$, $\bar{y}(\lambda)$ and $\bar{z}(\lambda)$ that depend upon a central wavelength λ of a light beam received, for receiving said photosensor intensity signals $I(\lambda)$, where each of said photosensor intensity signals depends upon said central wavelength $\lambda = \lambda_c(x)$, and for forming and issuing tristimulus convolution integral signals X, Y and Z defined by

$$X = \int \bar{x}(\lambda)\, I(\lambda)\, d\lambda,$$
$$Y = \int \bar{y}(\lambda)\, I(\lambda)\, d\lambda,$$
$$Z = \int \bar{z}(\lambda)\, I(\lambda)\, d\lambda.$$

23. The apparatus of claim 22, further characterized by color mapping means (57, 58), for receiving said tristimulus convolution integral signals, for computing a color transformation map derived from said integral signals, and for transforming image data from a first color system having three color components to a second color system having three color components through the color transformation map.

24. The apparatus of claim 23, further characterized by one of said color systems being drawn from the class of device-dependent and device-independent color systems consisting of red/green/blue, cyan/yellow/magenta, CIE Lab, CIE YUV, CIE LUV and CIE XYZ.

25. The apparatus of claim 22, further characterized by said object being the display screen of a monitor (61) and emitting light having an intensity that can be controllably increased and decreased by changing the value of a monitor control parameter associated with the monitor, the apparatus further comprising:

light intensity control means (41A, 41B) for controllably varying the intensity of light emitted by the monitor (61) by variation of the monitor control parameter among a sequence of predetermined values of the control parameter; and

memory means (46) for receiving and storing at least one of said photosensor signals or at least one of said convolution integral signals X, Y or Z, for each of the sequence of predetermined values of the control parameter.

26. The apparatus of claim 25, further characterized by transfer function means (57, 58) for receiving said memory means contents and for determining at least one of the color gain value, the color offset value or the color gamma value from said photosensor or said integral values X, Y or Z, for each of said sequence of predetermined values of said control parameter.

27. A color calibration system for monitoring and controlling the spectrum of colors presented by one or more computer peripheral devices (61, 63, 65) controlled by a computer (56), the system being characterized by:

at least three individual light filters (17), each filter having wavelength passing characteristics that are similar to one of the wavelength passing characteristic or one of the color matching filter functions or one of the human eye and being positioned to receive incident light and to issue light that has been subjected to filtering action by that filter;

light restriction means for receiving and admitting light from a restricted portion of the peripheral device (61, 63, 65) that defines the small predetermined area and for directing this light to the light filters;

an array of three or more photosensors (19), each photosensor being positioned to receive light that issues from the one of light filters (17), and to issue an output signal that represents the cumulative light energy received by that photosensor from the corresponding light filter;

color measurement means (46) for receiving the photosensor output signals and for determining the color or colors present in the incident light based on the relative amounts of light of different wavelengths received at three or more of the photosensors;

light calibration means (45) for receiving the output signal from the photosensor array, for comparing the amount of light received by the photosensors (19) in the array with a reference table of light values that should be received by each of the photosensors, or by a linear combination of at least two of the photosensors, and for generating and issuing a sequence of output signals that indicates the difference between, or the ratio R of, the reference table entry for a photosensor, or combination thereof, and the amount of light actually received by that photosensor, or a combination thereof, in the array;

where the light calibration means output signals are received by the computer (56) and, in response thereto, the computer adjusts color data signals that are received by or rendered by the computer peripheral devices (61, 63, 65), to reduce the differences between the reference table entries and the corresponding output signals from the color measurement means.

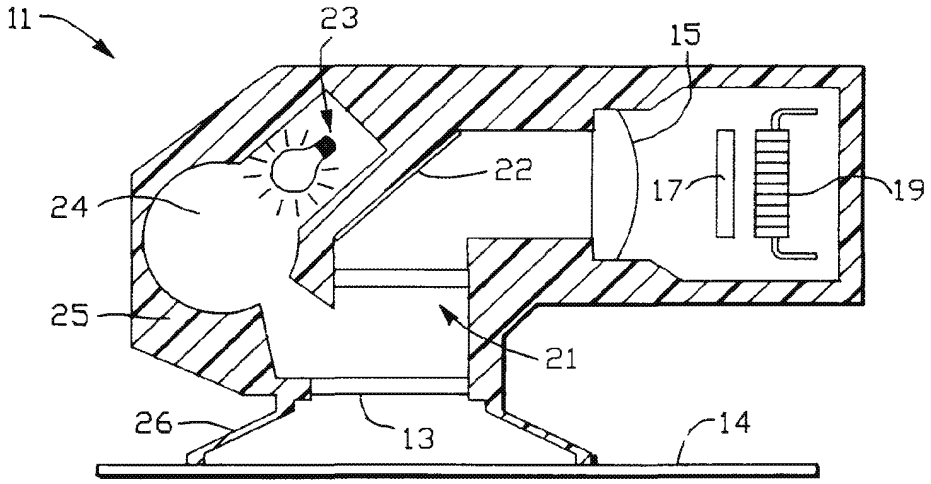


FIG.-1A

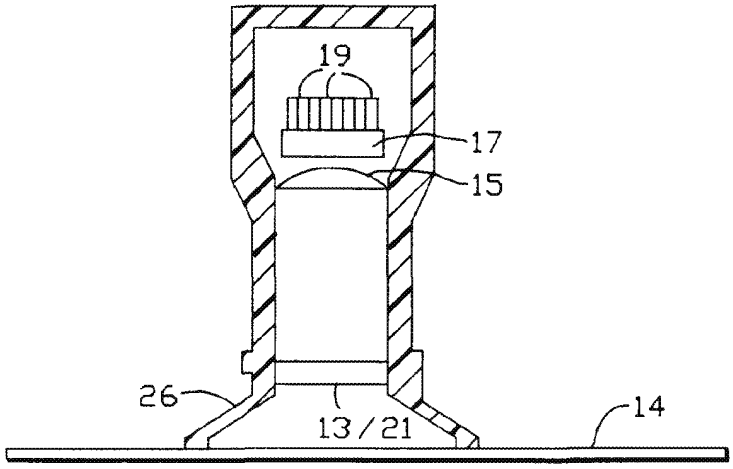


FIG.-1B

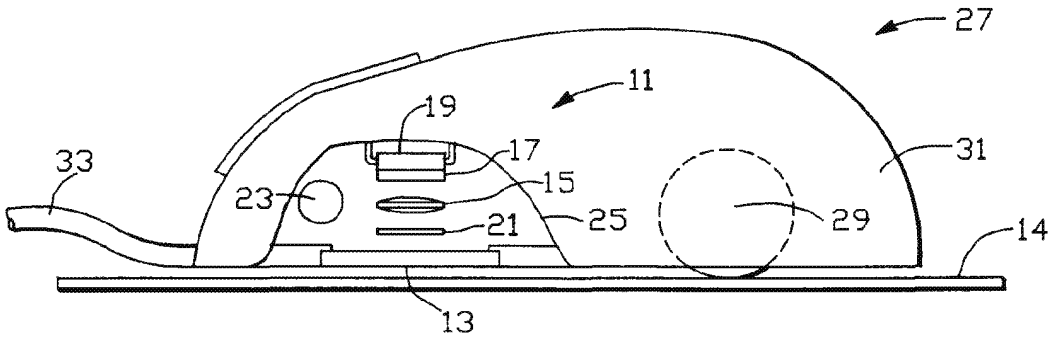


FIG.-1C

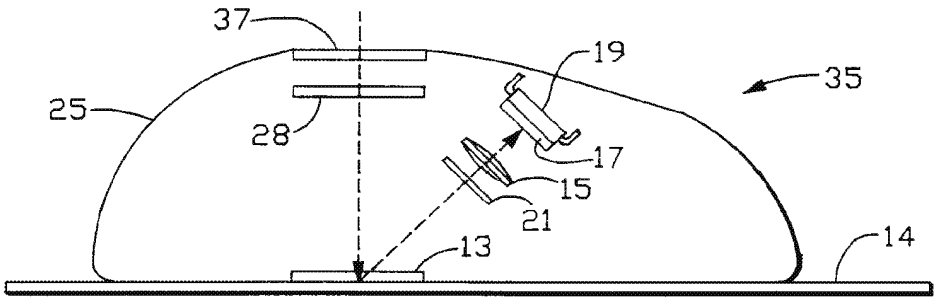


FIG.-1D

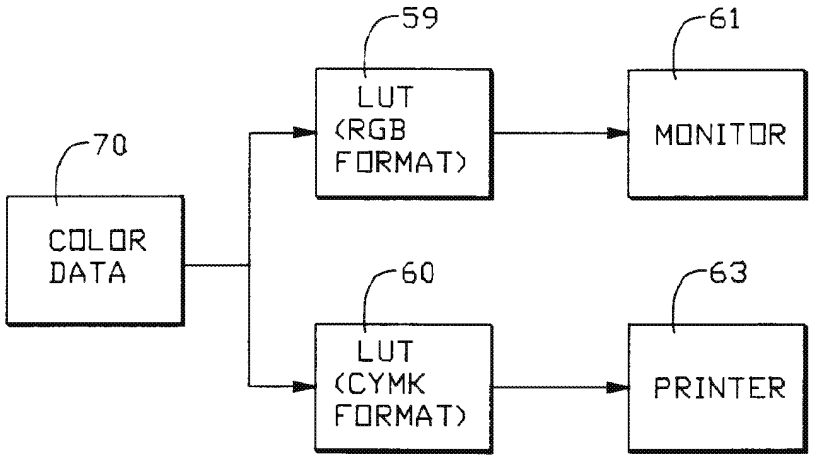


FIG.-4

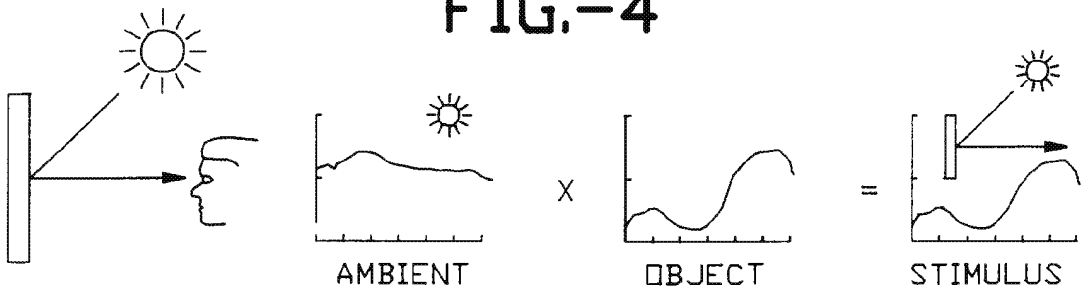


FIG.-3A

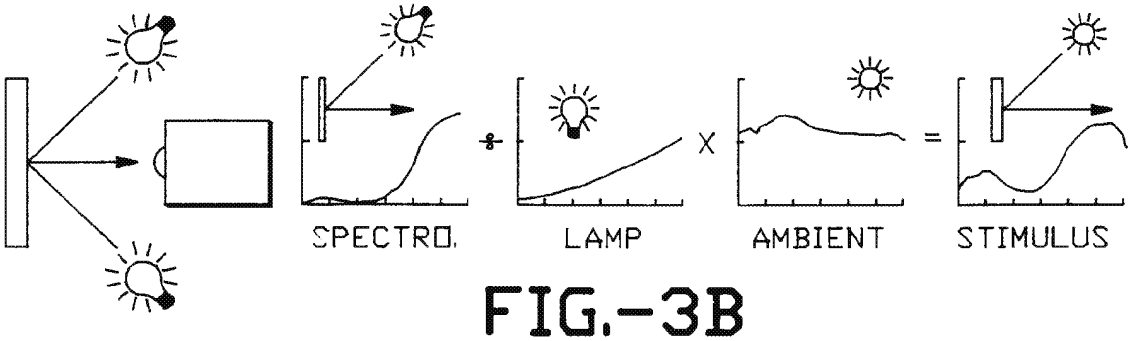


FIG.-3B

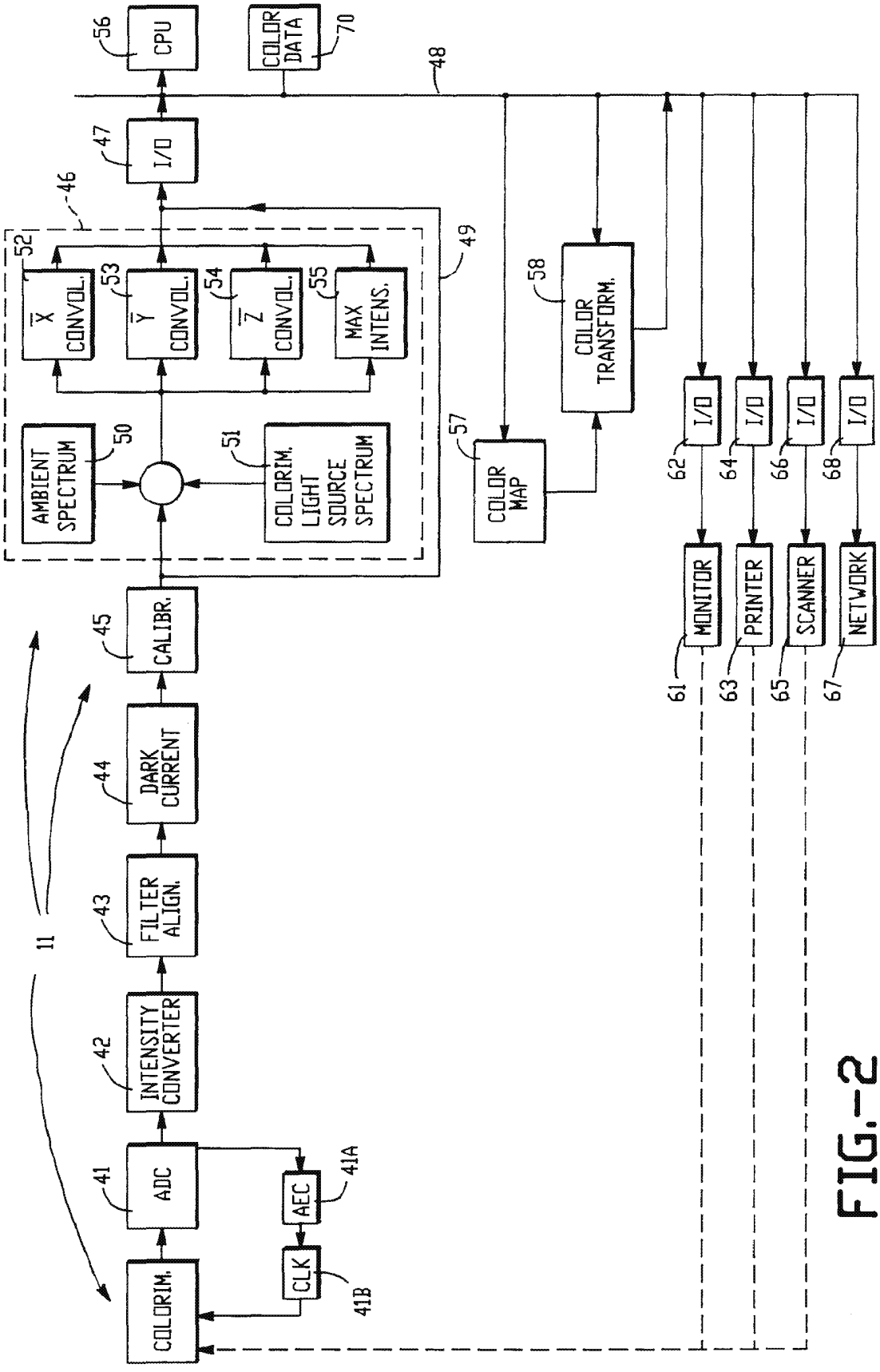

COLORIM.
ADC
INTENSITY CONVERTER
FILTER ALIGN.
DARK CURRENT
CALIBR.
AMBIENT SPECTRUM
COLORIM. LIGHT SOURCE SPECTRUM
X CONVOL.
Y CONVOL.
Z CONVOL.
MAX INTENS.
I/O
CPU
COLOR DATA
COLOR MAP
COLOR TRANSFORM.
MONITOR
PRINTER
SCANNER
NETWORK
AEC
CLK
11
41
41A
41B
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
61
62
63
64
65
66
67
68
70

FIG.-2

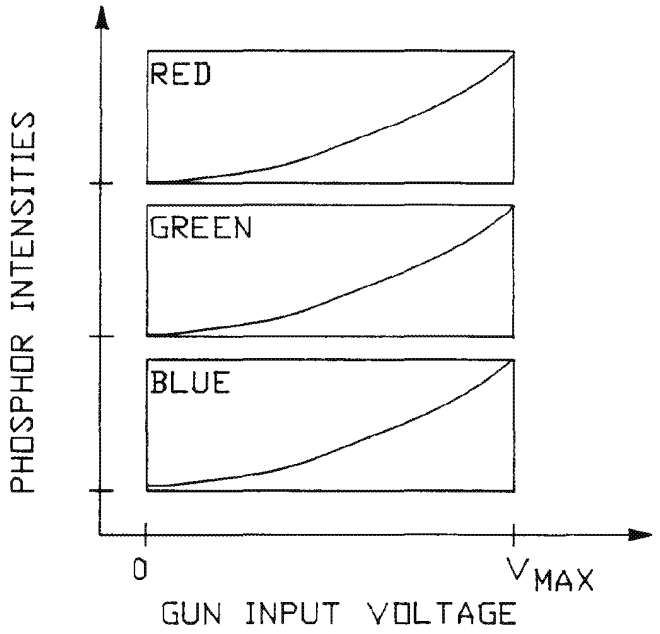


FIG.-5

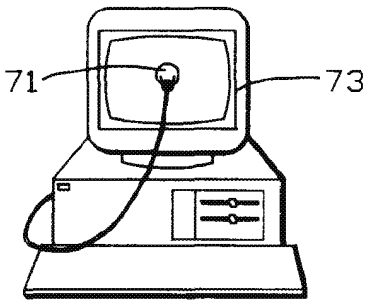


FIG.-6

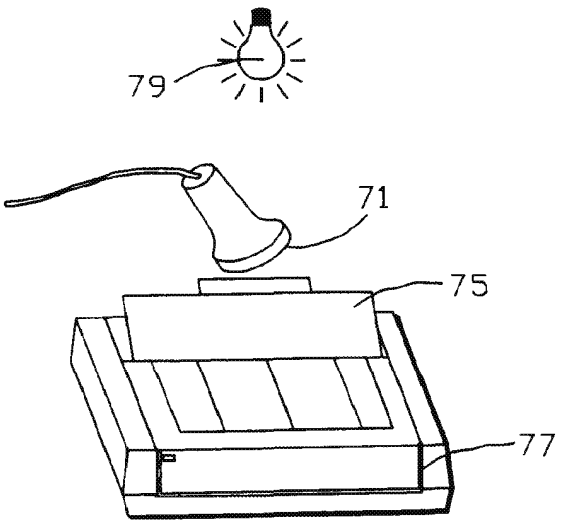


FIG.-7

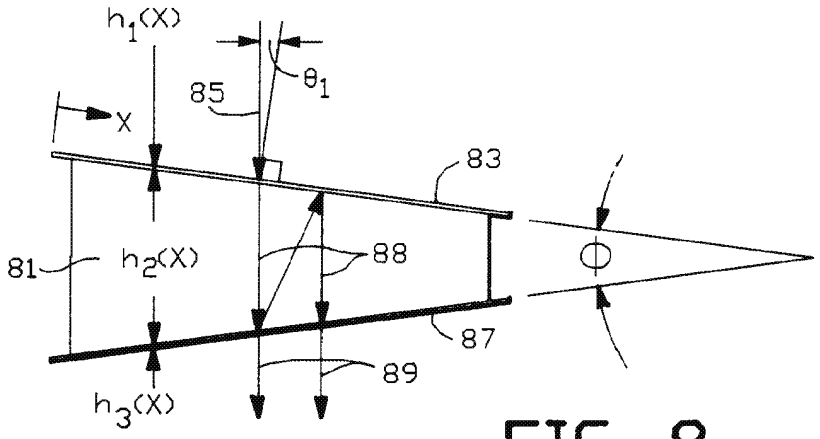


FIG.-8

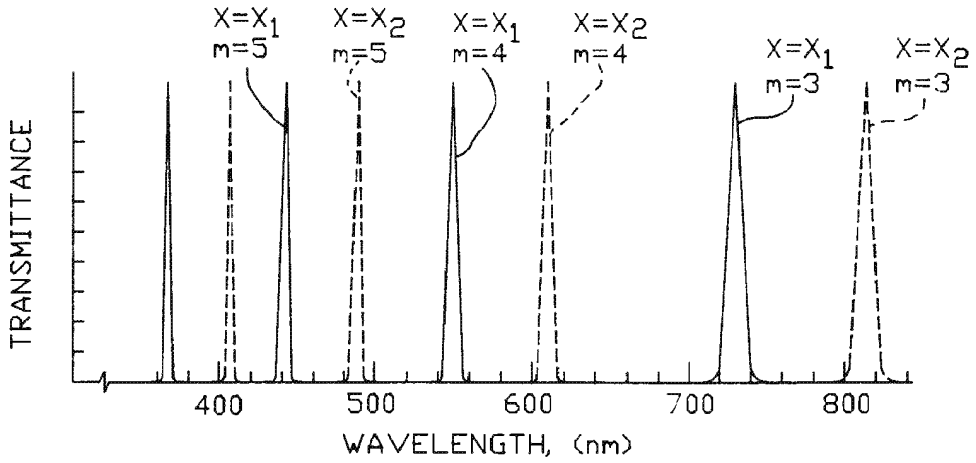


FIG.-9

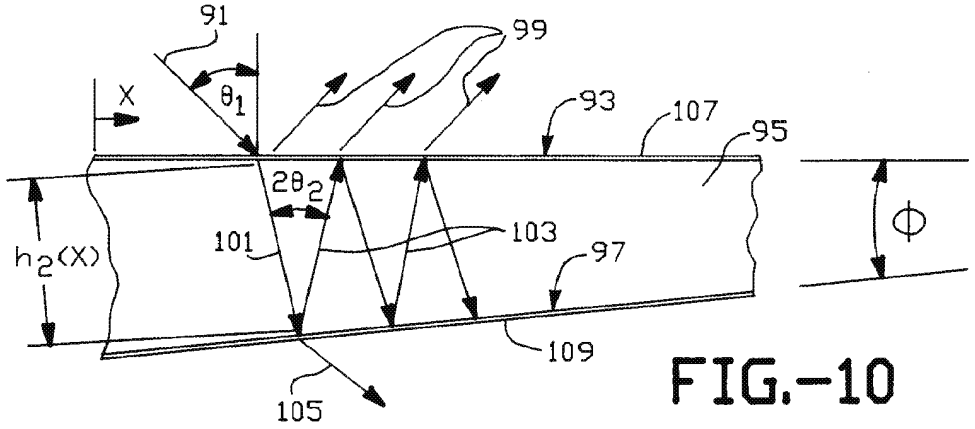


FIG.-10

SPECTRAL_0000201

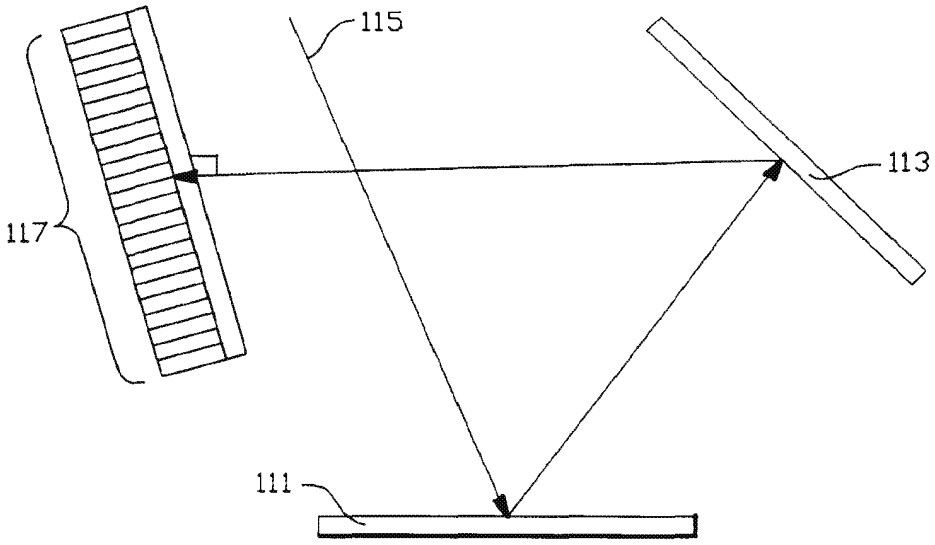


FIG.-11

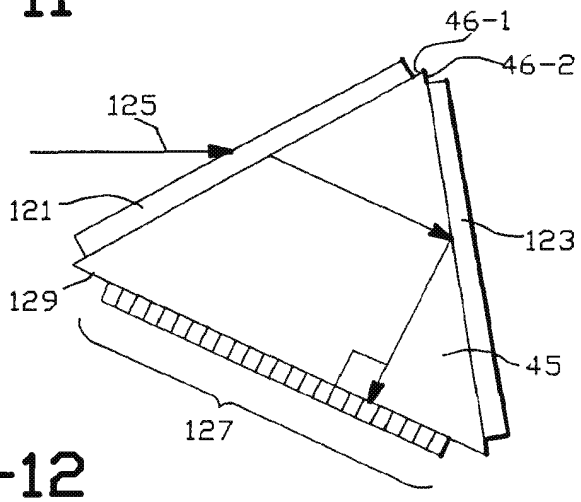


FIG.-12

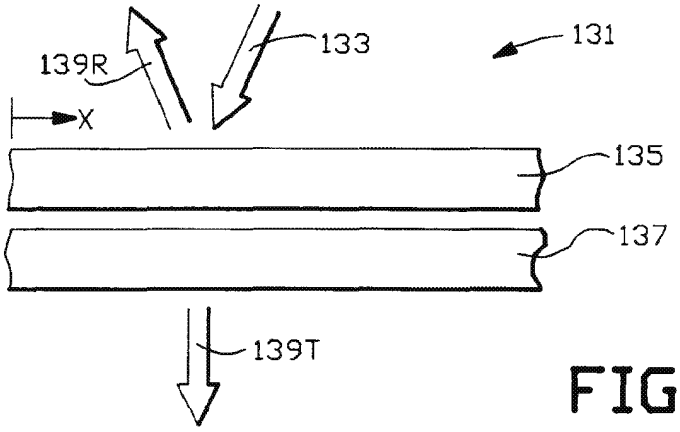


FIG.-14

SPECTRAL_0000202

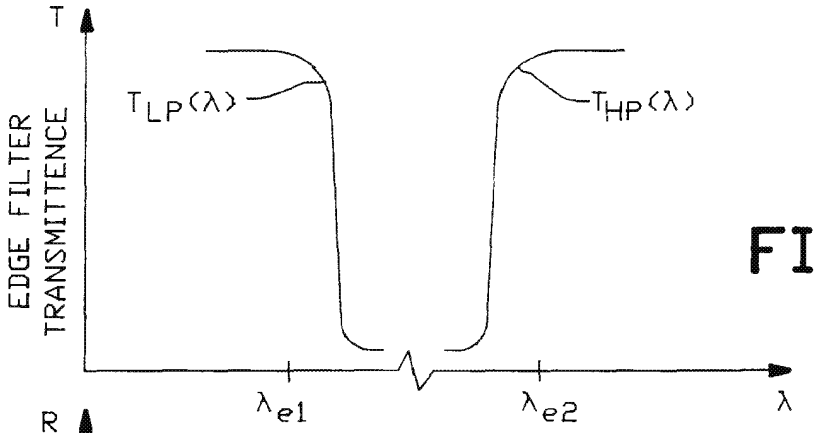


FIG.-13A

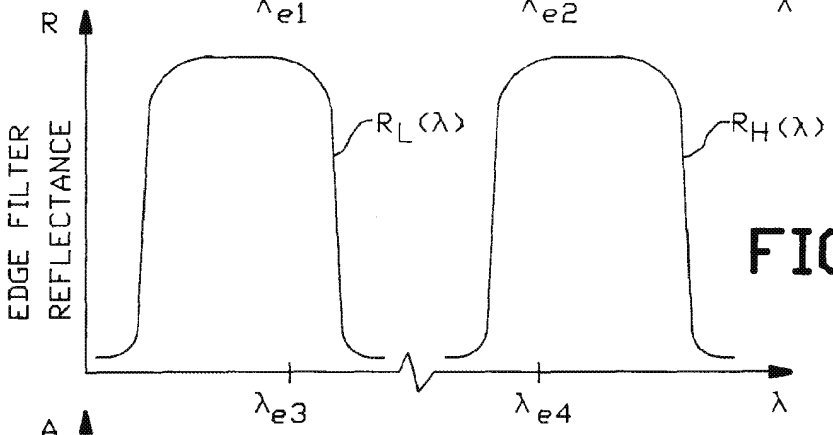


FIG.-13B

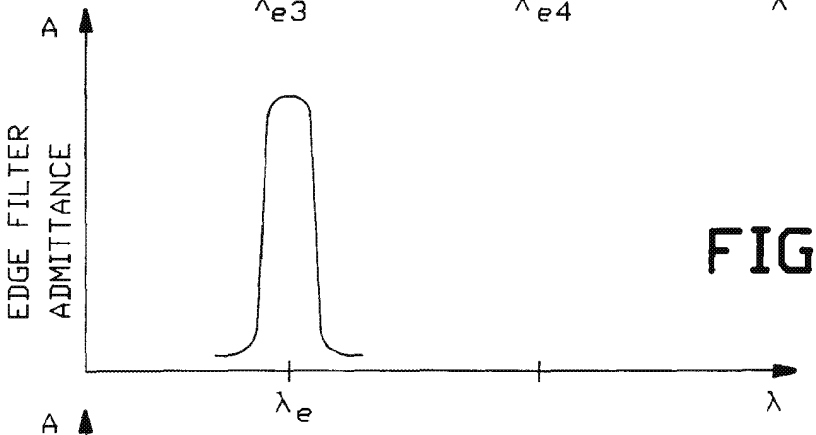


FIG.-13C

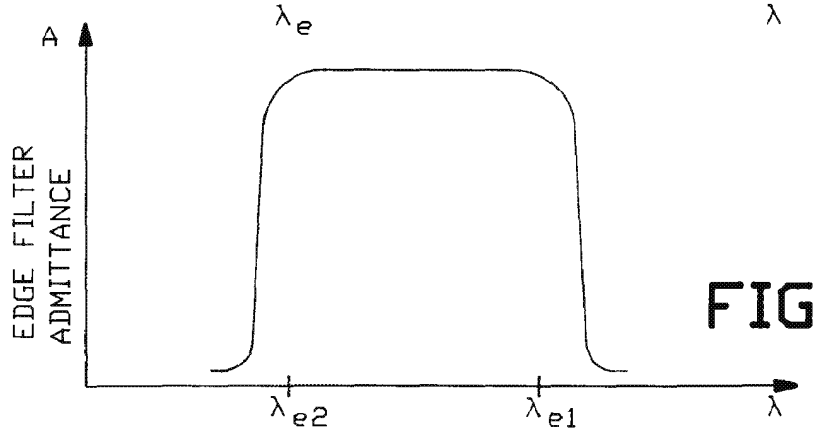


FIG.-13D

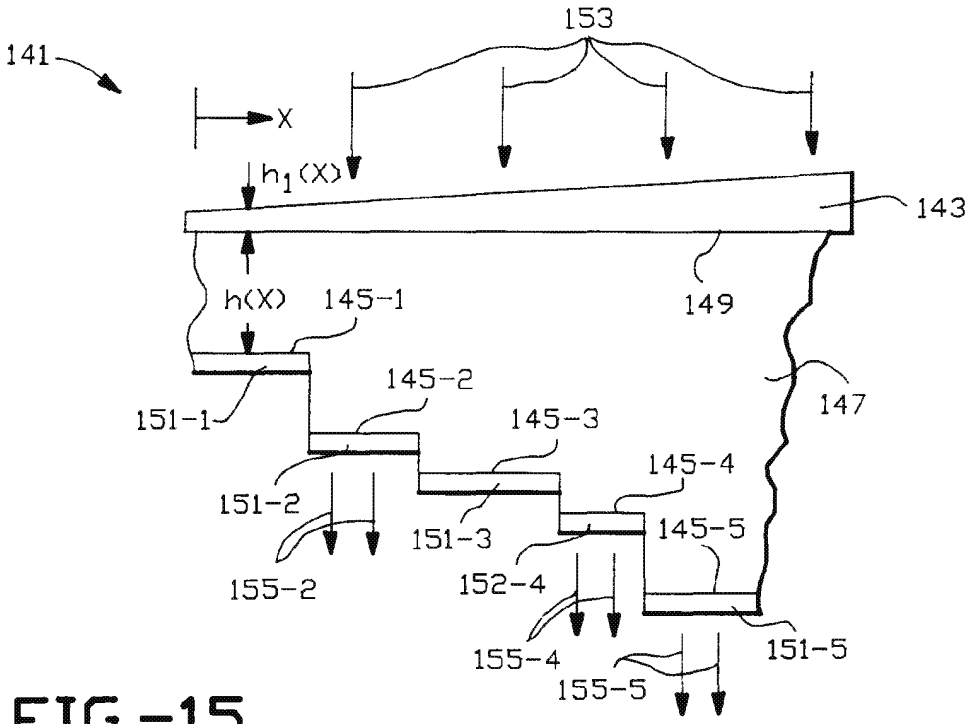

141
153
X
$h_1(X)$
143
h(X)
145-1
149
147
151-1
145-2
145-3
145-4
151-2
151-3
145-5
155-2
152-4
151-5
155-4
155-5

FIG.-15

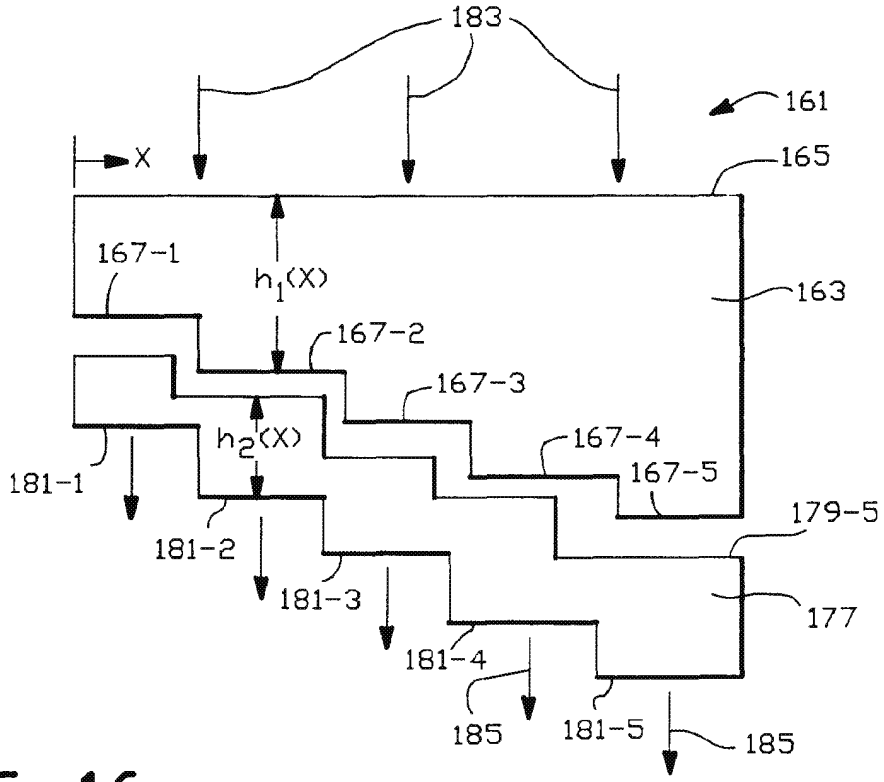

183
161
X
165
167-1
$h_1(X)$
163
167-2
167-3
$h_2(X)$
167-4
181-1
167-5
179-5
181-2
181-3
177
181-4
185
181-5
185

FIG.-16

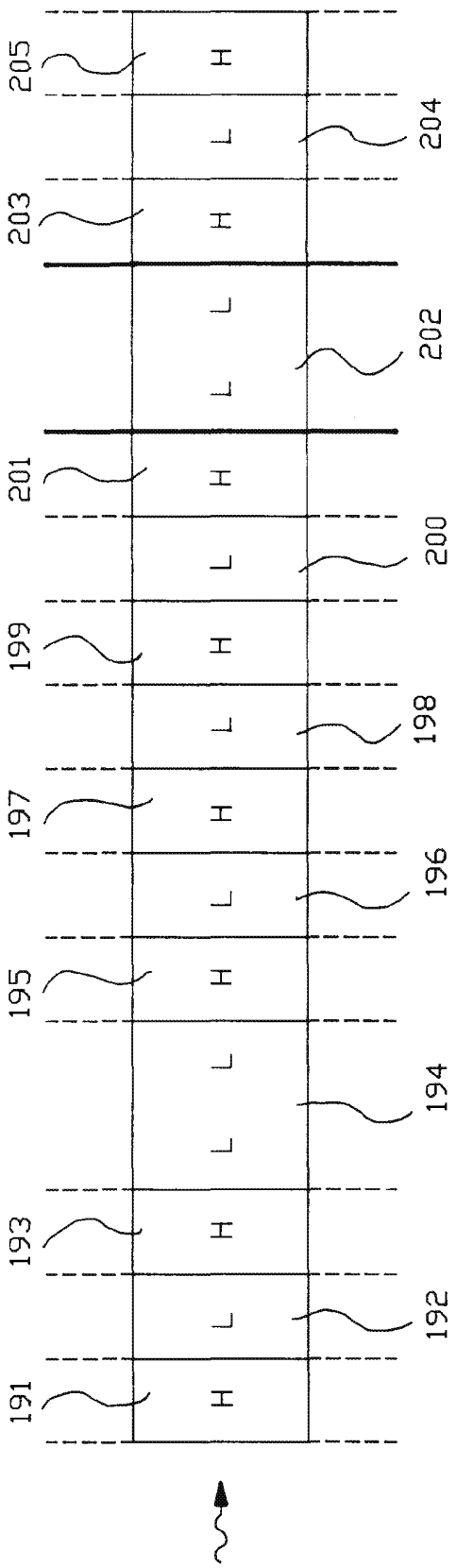


FIG.-17A

205

SPECTRAL_0000205

206

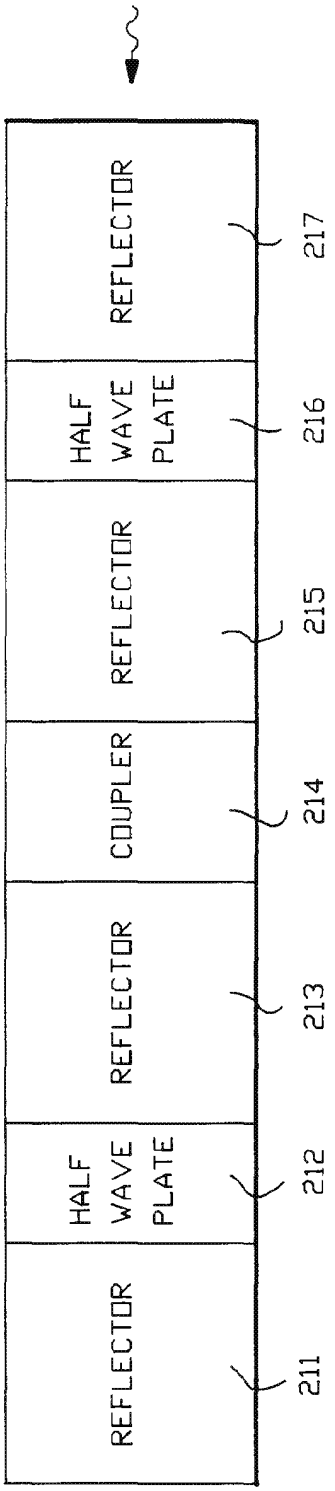


FIG.-17B

SPECTRAL_0000206

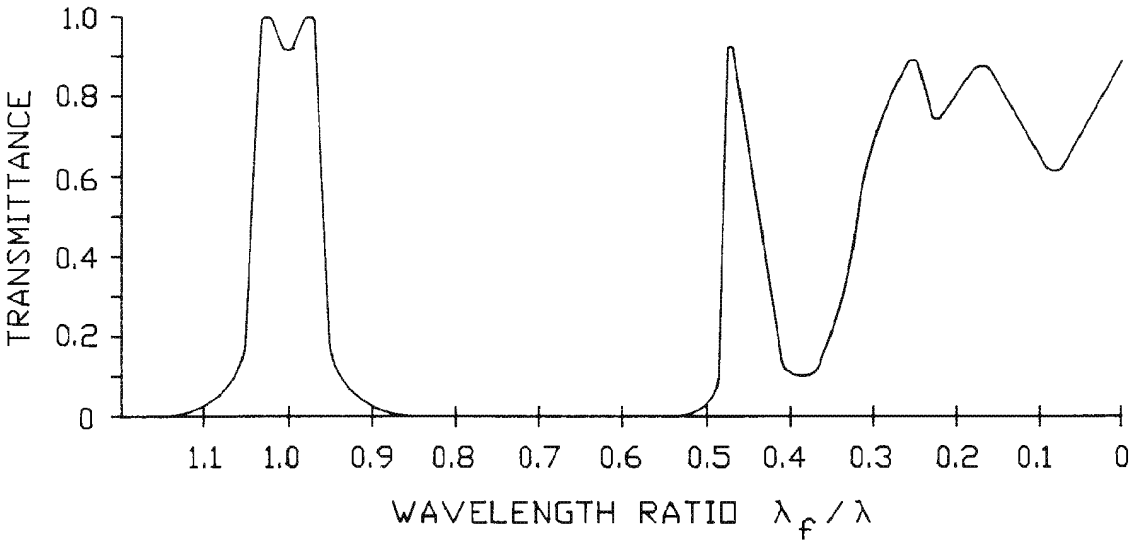


FIG.-18A

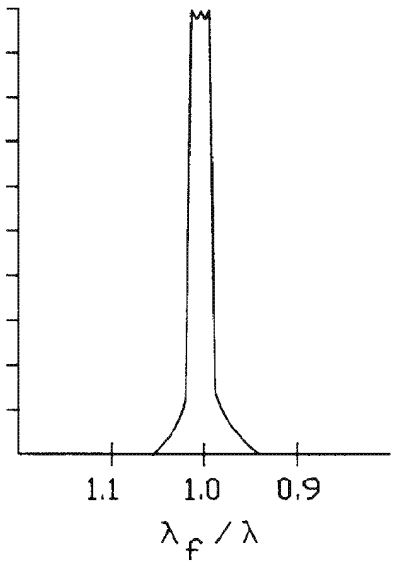


FIG.-18B

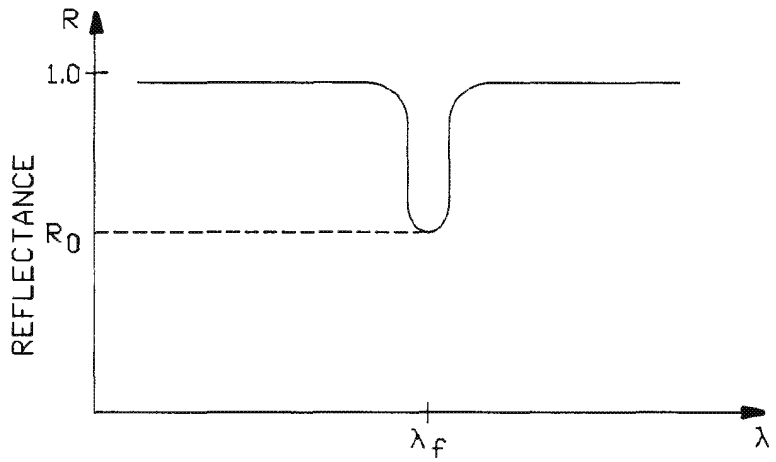


FIG.-19

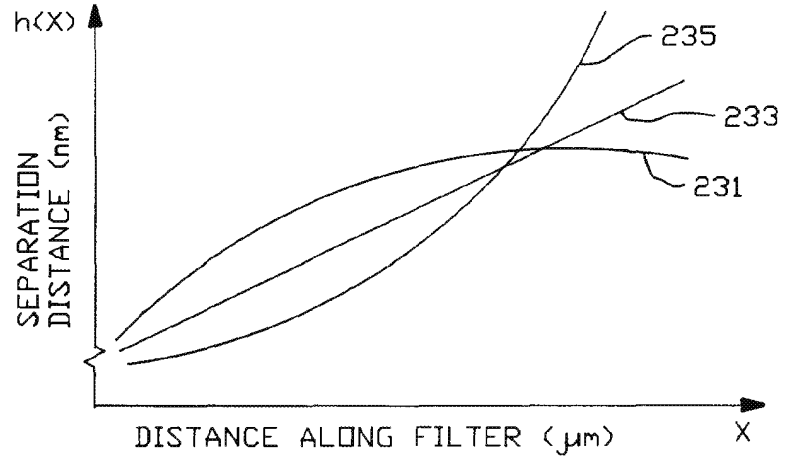


FIG.-21

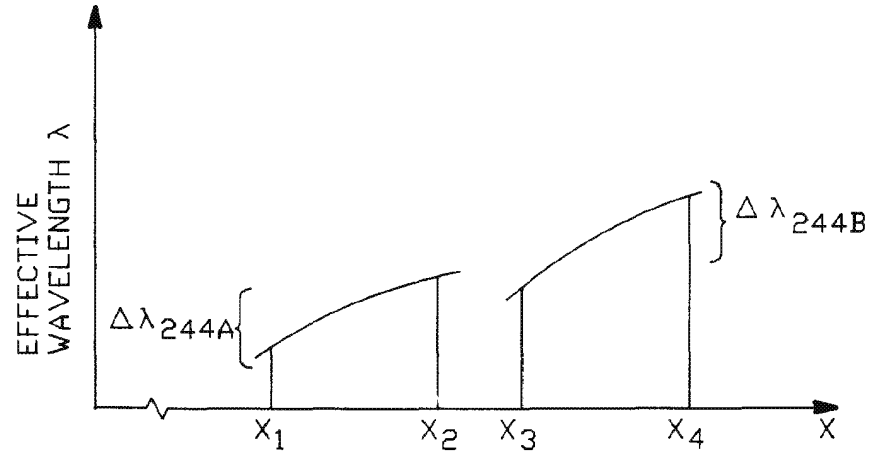


FIG.-24

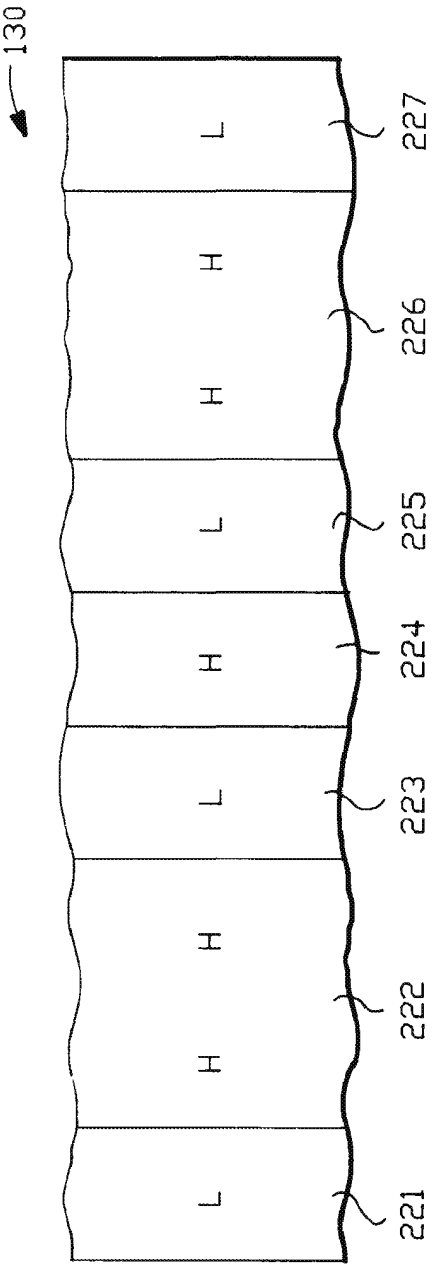


FIG.-20

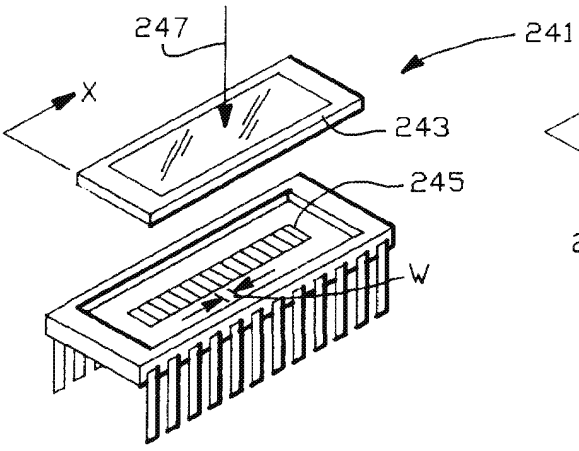


FIG.-22

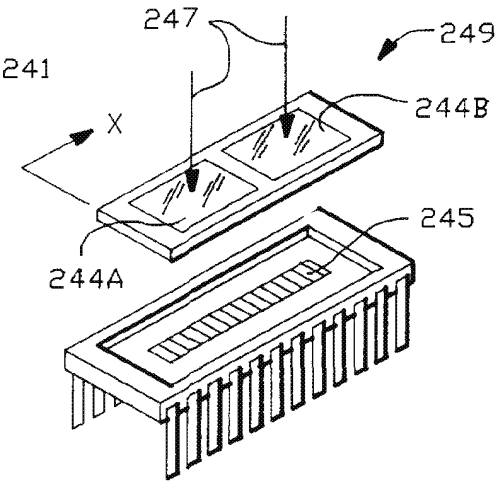


FIG.-23

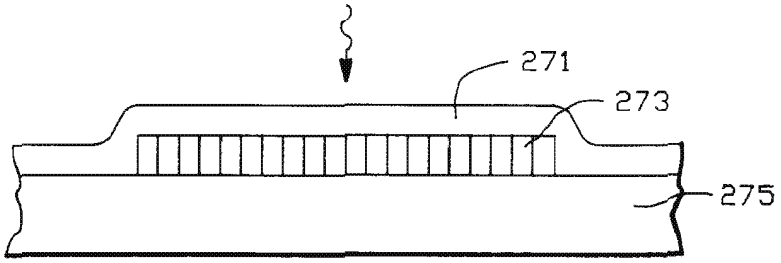


FIG.-26A

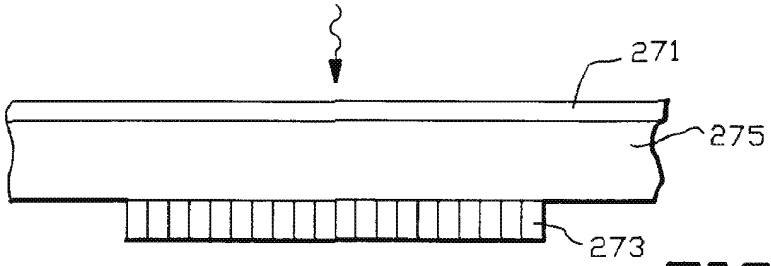


FIG.-26B

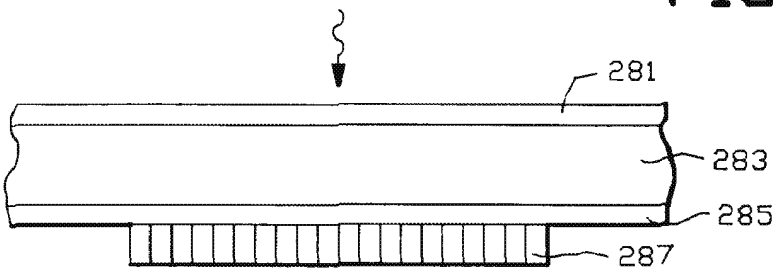


FIG.-26C

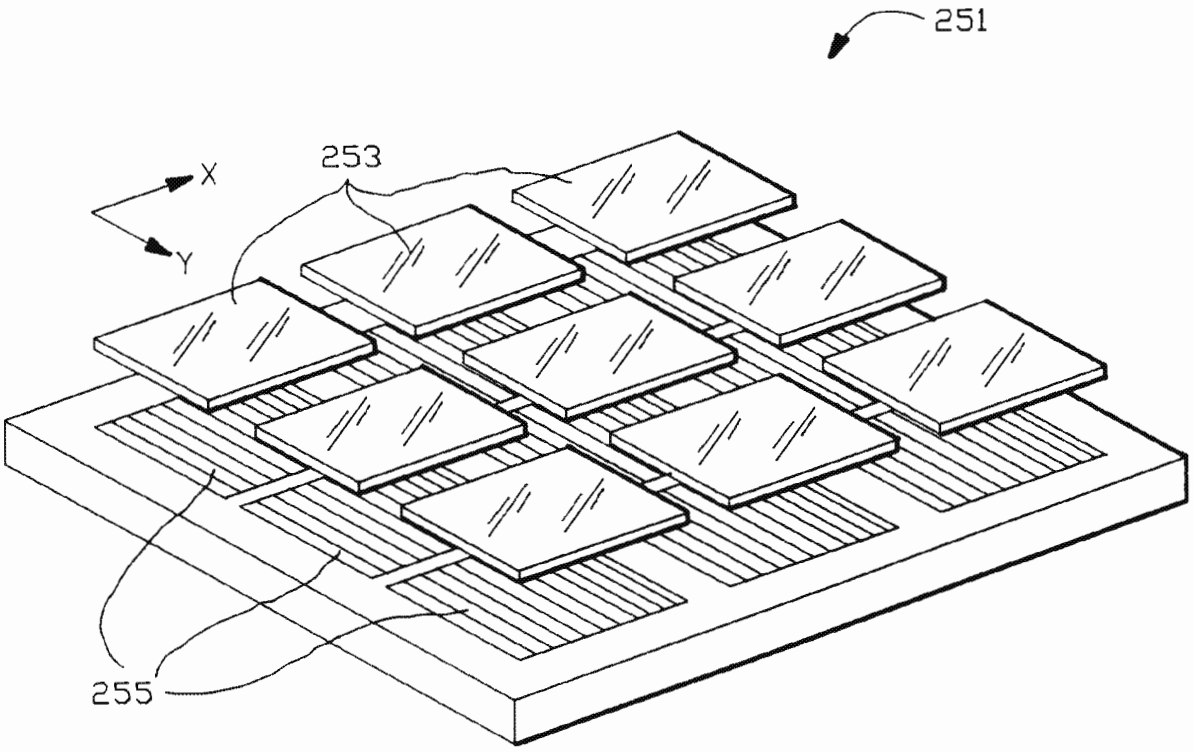


FIG.-25

European Patent Office

EUROPEAN SEARCH REPORT

Application Number EP 91117745.9

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	US - A - 4 870 268 (VINCENT) * Column 6, line 20 - column 16, line 14; fig. 1-21 *	1,14, 15,17, 19,27	G 01 J 3/51
A	US - A - 4 902 136 (MUELLER et al.) * Column 4, line 4 - column 6, line 60; fig. 1-3 *	1,14, 15,17	
A	EP - A - 0 011 377 (TOBIAS) * Claim 1; fig. 1,2 *	1,4,5	
A	PATENT ABSTRACTS OF JAPAN, unexamined applications, P field, vol. 13, no. 475, October 27, 1989 THE PATENT OFFICE JAPANESE GOVERNEMENT page 101 P 950 * Kokai-no. 1-187 419 (HITACHI)	1,4,7, 12,15, 16,19, 22-25	
D,A	WO - A - 89/05 465 (SANTA BARBARA RESEARCH) * Claims 1-20; fig. 1-4 *	14,17	TECHNICAL FIELDS SEARCHED (Int. Cl.5) G 01 J 3/00 G 01 N 21/00
D,A	US - A - 3 552 826 (GARNET et al.) * Column 3, line 4 - column 4, line 10; fig. 2 *	14,17	
D,A	US - A - 3 498 693 (FEIN et al.) * Column 3, line 41 - column 4, line 43; fig. 1-3 *	14,17	
D,A	US - A - 4 822 998 (YOKOTA et al.)	1,14, 17	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	20-12-1991	BAUER

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)

SPECTRAL_0000212

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 91117745.9

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
	* Column 4, line 32 - column 7, line 20; fig. 1-12 *		
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	20-12-1991	BAUER

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)

SPECTRAL_0000213

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 493 707 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **91121105.0**

(51) Int. Cl.5: **G03B 17/26**

(22) Date of filing: **09.12.91**

(30) Priority: **31.12.90 US 636335**

(43) Date of publication of application:
08.07.92 Bulletin 92/28

(84) Designated Contracting States:
DE FR GB IT

(71) Applicant: **EASTMAN KODAK COMPANY**
343 State Street
Rochester, New York 14650-2201(US)

(72) Inventor: **DiPietro, Matthew, c/o EASTMAN KODAK COMPANY**
Patent Department, 343 State Street
Rochester, New York 14650-2201(US)

(74) Representative: **Blickle, K. Werner, Dipl.-Ing. et al**
KODAK AKTIENGESELLSCHAFT Postfach 600345
W-7000 Stuttgart-Wangen 60(DE)

(54) Light lock.

(57) A light lock (34) is formed by a channel (36) with spaced walls (38,40) with the channel being open at one side. A light absorbing material (44) is positioned in the channel and has bristles (48) projecting toward the opening. A flange (50) projects through the opening toward the material but is spaced from the bristles. The channel can be formed on one portion of a magazine, cassette or the like, for light tight materials such as photographic film, and the flange can be formed on another portion of such a magazine or cassette.

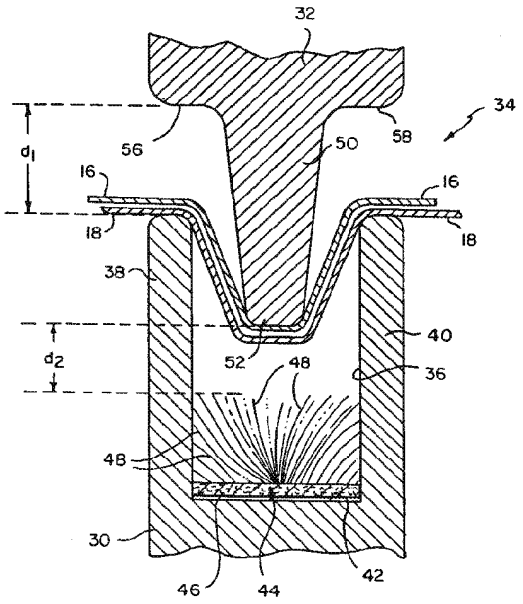


FIG. 2

EP 0 493 707 A2

This invention relates to an improved light lock that is especially suitable for effectively blocking light from entering a magazine, cassette or the like for photographic films.

Magazines, cassettes and the like for undeveloped photographic film commonly have light locks that are designed to exclude light from film in the magazine or cassette. One example of a light lock for a cartridge is found in US-A 4,634,071, issued January 6, 1987, entitled "Light Tight Film Cartridge With Floating Light Seal". The seal of this patent comprises a pair of elongate sealing elements disposed adjacent opposite surfaces of a film strip. The sealing elements include a layer of black velvet-like light sealing material formed with a plurality of small, thin, flexible filaments closely spaced together and projecting outwardly into engagement with the film strip. Light locks of this kind rely on contact between the bristles of the sealing element and the film strip to exclude light from the interior of the magazine. Such contact increases wear on the bristles and encourages the bristles to break loose and adhere to the film.

US-A 4,264,821, issued April 28, 1981, is directed to a cassette for an x-ray film. The cassette has a body part and a cover part that are hinged together so that the cassette can be opened to receive a sheet of x-ray film and closed to contain the sheet in a light tight manner. The cassette is provided with light seals around the edges comprising two parallel lips on one part of the cassette which receive a lip on the other part of the cassette when the cassette is closed.

Another x-ray film cassette is disclosed in US-A 4,110,624, which issued on August 29, 1978. In this patent, a light trap has two parts formed of molded spongy, opaque plastic material comprising a female part of generally U-shaped configuration and a male part that fits within the female part and engages the bottom of the female part. The material forming the light trap also acts as a spring for a catch that holds the parts together and is effective to cause the catch to be retained in the closed position. Thus, there is a force urging the parts of the light trap away from each other.

Thus, in some of the prior light locks, fibers of light lock elements are urged into contact with the film to effect the light lock. Others are void of fibers, and this may enable light to be reflected off the walls forming the light lock and ultimately find its way into the cassette to expose the film. Still others rely on compression of the parts forming the light lock which results in a force urging the parts of the light lock apart.

It is an object of the invention to provide an improved light lock which utilizes bristles for absorbing light but the bristles are not contacted by opposing portions of the light lock, which minimizes wear on bristles in a light lock and reduce the likelihood that bristles will break loose from the light lock, and which does not create forces that oppose closing of the parts of a magazine. This object is accomplished by a light lock having a channel with first and second spaced walls and a base extending between the walls, a light absorbing material being located on the base, characterized in that the material has bristles projecting away from the base, and a flange projects toward the material on the base, the flange being spaced from the bristles and from the first and second walls.

In the detailed description of the preferred embodiment of the invention presented below, reference is made to the accompanying drawings, in which:

Figure 1 is a perspective view of a magazine adapted to receive a package containing a stack of x-ray films or the like; and

Figure 2 is an enlarged, cross-section view taken along line 2-2 in Figure 1, illustrating a light lock of the invention incorporated into the magazine.

Referring initially to Figure 1, a magazine generally designated 10 is adapted to hold one or more sheets of undeveloped film F in a light tight condition within the magazine. By way of example, a stack of film sheets can be provided to the magazine in a light tight package, a portion of which is shown at 12. Packaging material surrounds the unexposed film sheets, and an end portion 14 of the package extends outside the magazine when the package is initially loaded into the magazine. End portion 14 comprises upper and lower sheets 16,18 (Fig. 2) that are secured together by, for example, a heat seal line 20 (Fig. 1) so that the package portion outside the magazine is light tight. The side edges of the package can also be heat sealed together, or the upper and lower sheets can be formed integrally as a tube so that the side edges need not be sealed together.

The magazine 10 comprises two portions that jointly define a space within the magazine for receiving the sheets of film. More specifically, the magazine has a base 22 and a cover 24 which are illustrated as being rectangular in shape and substantially the same size. The base and cover can be secured together along one side edge by a hinge 26 in a conventional manner. This enables the cover 24 to be raised, as shown in phantom in Figure 1, for loading a package of film sheets into the magazine and for removing the packaging material from within the magazine.

The particular magazine 10 illustrated is of the kind which is adapted to remove the packaging material from the sheets after the package is loaded in the magazine and the magazine closed.

Thus, the magazine is provided with a crank or handle 28 that is connected to a spindle 29 inside the magazine. The end portion of the package opposite from the end portion 14 is attached to the spindle when the package is loaded into the magazine and while the cover is opened. Then the cover is closed and latched in the closed position. Next, the package portion 14 is cut between the heat seal line 20 and the magazine, and the handle 28 is rotated to wind the packaging material onto the spindle and thereby remove it from the sheets of film within the magazine. This can be accomplished in a daylight environment.

Base 22 and cover 24 have side edge walls 30,32 that extend entirely around the magazine and define a portion of the space within the magazine that receives the sheets of film. As best illustrated in Figure 2, a light lock generally designated 34 is formed between the edge walls 30,32. More specifically, the light lock 34 comprises a U-shaped channel 36 formed in edge wall 30. Channel 36 comprises two spaced parallel walls 38,40 and a base 42. The upper edges of walls 38,40 preferably are rounded, as illustrated in Figure 2, and the channel has an opening at the top that is opposite the base 42.

A light absorbing material generally designated 44 is provided in the channel between walls 38,40. More specifically, the light absorbing material comprises a support 46 of foam plastic material, or other suitable materials, and a plurality of fibers or bristles 48. Bristles 48 are anchored in the support and project upwardly away from the support toward the opening at the top of the channel 36. However, as shown in Figure 2, the bristles do not extend to the open end of the channel. Preferably, the bristles are relatively long, black in color, and extend across the channel from wall 38 to wall 40 so that they absorb any light that might find its way into the channel and prevent light from being reflected by the channel into the interior of the magazine.

The bristles preferably are anchored to the support in a way which minimizes the chance that bristles might break away from the support and migrate to the film in the magazine. For example, the bristles can be secured to the support by a heat sealing or sonic sealing step.

As indicated in Fig. 2, walls 30,32 are substantially the same width. Wall 32 has a flange 50 that extends from the central portion of the wall and projects downwardly from the wall into the space defined by channel 36. Flange 50 is spaced from both of the walls 38,40 of the channel and from the base 42 of the channel. The flange has a lower or tip end portion 52 that is spaced from the upper ends of the bristles 48.

Side edge wall 32 has shoulders 56,58 that extend alongside the flange 50. When the magazine is closed, these shoulders are spaced from the upper edges of walls 38,40 of the channel by a distance designated d1. Similarly, the tip end 52 of the flange is spaced from the upper ends of the fibers 48 by a distance designated d2. Distances d1, d2 may be, for example, approximately 0.125 inches. This spacing is more than enough to enable the upper and lower sheets 16,18 of the packaging material to pass over the upper edges of walls 38,40 and below the tip end 52 of the flange without contacting either the bristles 48 or the shoulders 56,58, as illustrated in Figure 2. Thus, the packaging material does not present a resistance to the closing of the magazine even though the packaging material is deflected as shown in Fig. 2 when the magazine is closed. Such a resistance occurs in prior magazines and impedes closing movement of the magazine and movement of the packaging material through the light lock.

As will be observed from Figure 2, anytime the cover 32 is in the closed position, light cannot travel from outside the magazine to the inside thereof, except along a tortuous path leading to the bristles 48 of the light absorbing material. More specifically, light from outside the magazine would have to pass between the shoulder 58 and the upper edge of wall 40, then downwardly between wall 40 and the flange 50, and then the light would enter the bristles 48 of the light absorbing material. The bristles are effective to absorb the light and prevent it being reflected upwardly between wall 38 and flange 50 and then between the shoulder 36 and wall 38 into the magazine. Also the light lock deflects the sheets 16,18 as shown in Fig. 2 so that light cannot pass between the sheets and reach the film. At the same time, the elements of the light lock are spaced apart so that the light lock minimizes the force required to close the cover 32. The light lock can be provided along only one side edge of the magazine, if desired, but it is preferred that the light lock be provided along all four side edges of the magazine.

The light lock is simple in construction, easy to manufacture and effective to block light from entering the magazine. Also, it does not provide a resistance to closing of the cover, nor does it provide a force tending to open the cover. There is no contact between the bristles 48 and the flange 50, which is normally required with prior art light locks, nor is there contact between the bristles and the sheets 16,18 of the package as the package is pulled through the light lock. Because the bristles are not contacted, they do not wear out, and the bristles are not likely to break off and find their way into the magazine. Also, the absence of contact between the bristles and the sheets 16,18 results in less resistance to pulling the sheets through the light lock.

Some dirt and other foreign material may enter the bristles over a period of time without adversely effecting the light lock. Weep holes (not shown) can be provided from the absorbent material 44 to the exterior of the magazine to provide a path for any liquid that might accidentally enter the light lock. If desired, light absorbing material 44 also can be provided at other locations, such as on the inside surface of walls 38,40, or on the flange 50 or shoulders 56,58.

Claims

1. A light lock (34) having a channel (36) with first and second spaced walls (38,40) and a base (42) extending between the walls, the channel having an opening opposite the base, and a light absorbing material (44) located on the base, characterized in that the material has bristles (48) projecting away from the base and toward the opening, and a flange (50) projecting through the opening toward the material on the base, the flange being spaced from the bristles and from the first and second walls.

2. A light lock as set forth in claim 1 wherein the bristles extend substantially across the channel between the spaced walls.

3. A magazine (10) for holding one or more sheets of undeveloped film in a light tight condition, the magazine having a base portion (22) and a cover portion (24) for enclosing the film within the magazine, and a light lock (34) around the film in the magazine, the light lock having a U-shaped channel (36) on one of the portions of the magazine and a flange (50) on the other of the portions of the magazine, the channel having first and second spaced walls (38,40) and having an opening facing the flange, the walls being spaced apart at the opening by a distance greater than the width of the flange so that the flange can be received within the opening between the walls without touching the walls, characterized in that a plurality of elongate, light absorbing bristles (48) are provided in the channel between the walls and spaced from the flange.

SPECTRAL_0000217

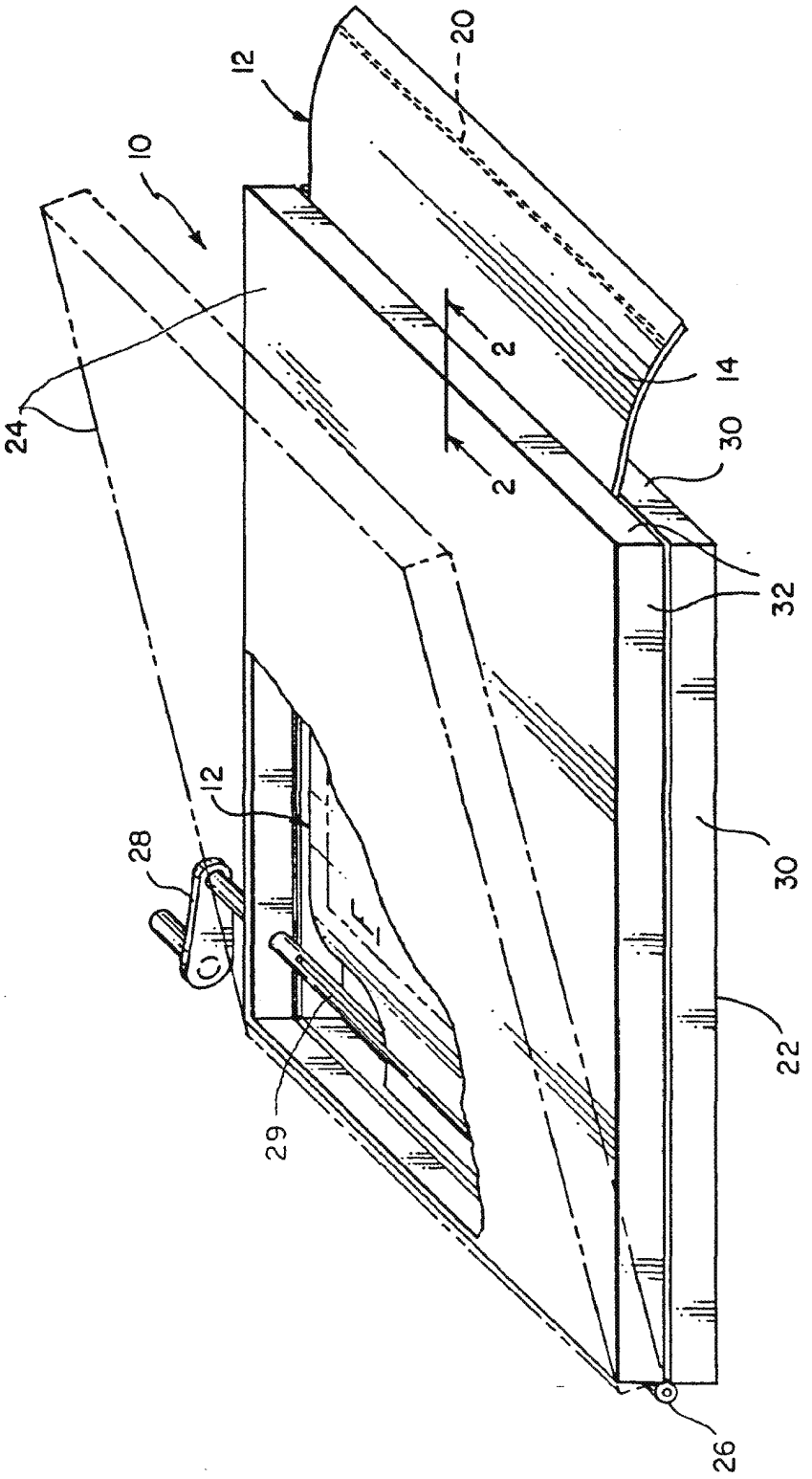


FIG. 1

SPECTRAL_0000218

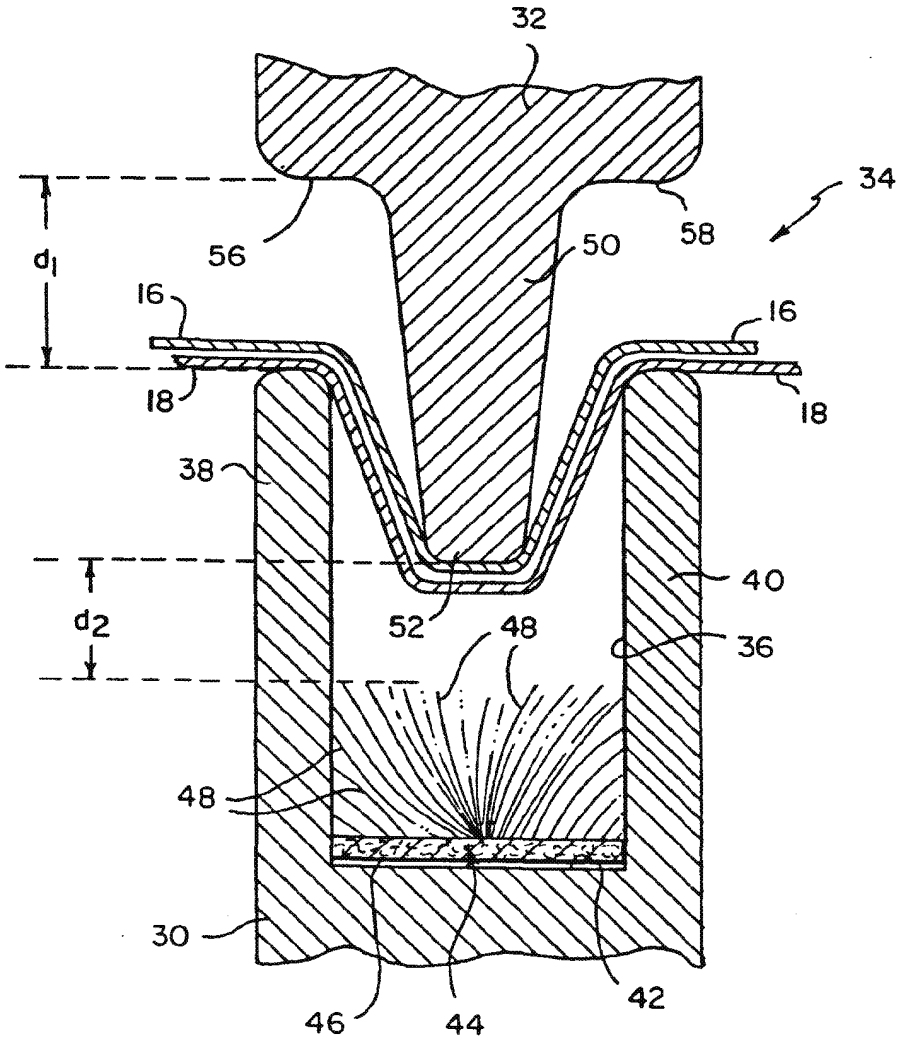


FIG. 2

SPECTRAL_0000219

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 718 622 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
26.06.1996 Bulletin 1996/26

(51) Int Cl.[6]: **G01N 21/76**, C12M 1/34

(21) Application number: **95301079.0**

(22) Date of filing: **21.02.1995**

(84) Designated Contracting States:
BE CH DE DK FR GB IT LI

(30) Priority: **20.12.1994 JP 316667/94**

(71) Applicant: **BIOSENSOR LABORATORIES CO., LTD.**
Toshima-ku, Tokyo (JP)

(72) Inventors:
- **Honzawa, Katsu**
  Hamamatsu-shi, Shizuoka-ken (JP)
- **Atsumi, Kazuhiro**
  Hamamatsu-shi, Shizuoka-ken (JP)
- **Shimomura, Fumihiko**
  Hamamatsu-shi, Shizuoka-ken (JP)
- **Masuko, Masayuki**
  Hamamatsu-shi, Shizuoka-ken (JP)
- **Hayakawa, Tsuyoshi**
  Hamamatsu-shi, Shizuoka-ken (JP)

(74) Representative: **West, Alan Harry et al**
R.G.C. Jenkins & Co.
26 Caxton Street
London SW1H 0RJ (GB)

(54) Apparatus and method for measuring chemiluminescence

(57) The present invention provides a chemiluminescence measuring apparatus which is small in size and high in operability and has a function of performing accurate quantitative analysis and the like. The apparatus comprises at least a box-like housing having an opening, a cover provided on a top surface of the housing and having a through hole for introducing a first detachable vessel into the inner space of the housing, a holder having a concave portion and enclosing the opening of the housing, the hollow chamber having an opening at a side wall thereof and partially housing a second vessel, a photo-sensing unit for detecting luminescence from the second vessel via the through hole of the hollow chamber, and a shutter mechanism for intermittently cutting optical path between the photo-sensing unit and the second vessel. Particularly, the shutter mechanism includes a rotatable hollow chamber partially housing the vessel, provided in the hollow chamber and having an opening at a side wall thereof, and a driving motor rotating the rotatable hollow chamber.

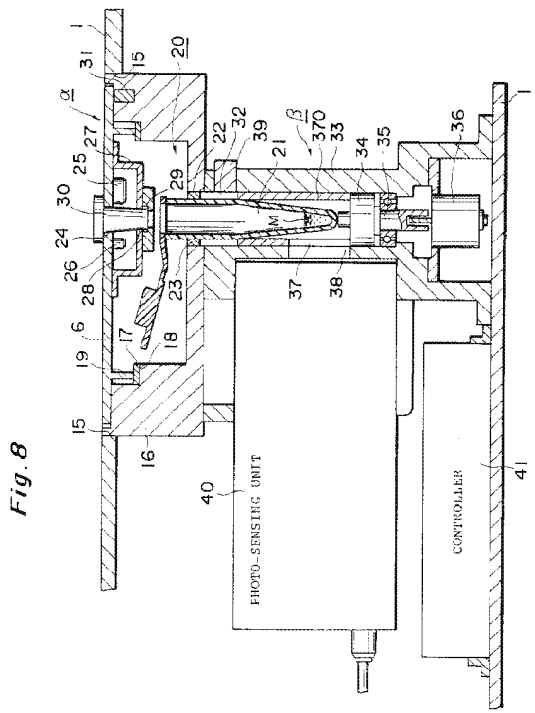


Fig. 8

EP 0 718 622 A2

Printed by Jouve, 75001 PARIS (FR)

SPECTRAL_0000220

Description

The present invention relates to an apparatus and a method for quantitatively analyzing the composition and the like of a sample solution by measuring luminescence such as bioluminescence or chemiluminescence caused when the sample solution and a luminous reagent are mixed with each other.

Consider a case wherein a luminous reagent is mixed with a sample solution obtained by mixing organism cells or a sample solution containing chemical substances, and the sample solution is quantitatively analyzed by measuring luminescence from the sample solution which is caused by this mixing operation. Generally, in this case, when this luminescence continues for a long period of time (i.e., the duration of the luminescence is long), a researcher or the like can perform measurement after one mixes the luminous reagent with the sample solution outside the chemiluminescence measuring apparatus and stores the solution mixture in the apparatus. However, when a sample solution which causes luminescence for only a short duration is to be measured, accurate quantitative analysis cannot be realized by the luminescence measuring apparatus if a measuring operation is performed according to the above procedure. The apparatuses for measuring the sample solution which causes luminescence for only a short duration are described, for example, in Laid-Open Japanese Patent Applications No. 4-29040 and No. 1-22994, and Laid-Open Japanese Utility model Application No. 63-161351.

Objects of the present invention relates to provide luminescence measuring apparatus which is small in size and high in operability, and has a function of performing accurate quantitative analysis and the like. Further, the present invention relates to a method for performing accurate quantitative analysis being superior to the conventional technique.

In order to achieve the above objects, an luminescence measuring apparatus of the present invention comprises a first holding mechanism provided at a cover so as to hold a first vessel as a sample syringe containing luminous reagent, and a second holding mechanism provided in a box-like housing so as to hold a second vessel containing a sample solution independently. The detailed structure of the apparatus is shown in Figs. 1, 2 and 8. The apparatus of the present invention comprises: a box-like housing 1 having an opening 15 at a top surface thereof; a cover 6 provided at the top surface of the housing 1, the cover 6 having a through hole 24 that allows to introduce a distal end of a sample syringe 42 as first vessel containing a luminous reagent into internal of the housing 1; a hollow chamber 33 being disposed in the housing 1 and having an opening 38 at a side wall thereof, the hollow chamber 33 having an inner space that allows to partially house a micro-sample tube 21 as a second vessel containing a sample solution; a holder 16 being disposed in the housing 1 and enclosing the opening 15 of the housing 1, the holder 16 having a concave portion 200 for forming a first space 20 defined with the cover 6 and having a through hole 22 connecting the first space 20 to the inner space of the hollow chamber 33 at a bottom surface of the concave portion 200; a photo-sensing unit 40 being disposed in the housing 1, for detecting luminescence from the second vessel 21 via the opening 38 of the hollow chamber 33; and a shutter mechanism for optically shielding luminescence from the micro-sample tube 21. The housing 1, the cover 6, the hollow chamber 33 and the holder 16 are made of one of nontransparent materials.

In particular, the shutter mechanism comprises: a rotatable hollow chamber 370 made of a nontransparent material, as the second holding mechanism, and holding the micro-sample tube 21 at a predetermined position while partially housing the micro-sample tube 21 therein, the rotatable hollow chamber 370 being disposed in the hollow chamber 33 and having an opening 37 at a side wall thereof; and a driving motor 36 as a rotator for rotating the rotatable hollow chamber 370, the driving motor 36 being disposed in the hollow chamber 33 while holding the rotatable hollow chamber 370.

In other words, the shutter mechanism includes a rotating cylindrical member 370 which houses the vessel 21, is housed in the hollow chamber 33 in the dark box portion (housing 1), and has an opening 37 at a side wall thereof, an optical path between the vessel 21 and the photo-sensing unit 40 (including a photomultiplier) is opened when the opening 37 of the rotating cylindrical member 37 coincides with the opening 38 of the hollow chamber 33 upon rotation of the rotating cylindrical member 370, and the opening 38 of the hollow chamber 33 is closed when the openings 37, 38 of the rotating cylindrical member 370 and the hollow chamber 33 do not coincide with each other, thereby realizing a shutter function.

As shown in Fig. 2, the first holding mechanism of the present invention comprises a guide member 27 is disposed in the first space 20 and contacting the cover 6 for defining a second space 201 with the cover 6, the guide member 27 having a through hole 28 that allows to introduce the distal end of the sample syringe 42 into the micro-sample tube 21. For detecting existence or absence of the micro-sample tube 21, in the second space 201 a photodiode 26 and a laser-emitting diode 25 are provided. Further, an O-ring 29, as a stopper for holding the sample syringe 42 at a predetermined position, is disposed in the first space 20 and has a through hole 290 that allows to partially introduce the distal end of the sample-syringe 42 into the micro-sample tube 21. A diameter (W3) of the through hole 290 of the O-ring 29 being smaller than that (W2) of the through hole 28 of the guide member 27, and the diameter (W2) of the through hole 28 of the guide member 27 is smaller than that (W1) of through hole 24 of the cover 6.

The apparatus of the present invention further comprises a first optical sensor 39 for detecting existence or

absence of the micro-sample tube 21 and a second optical sensor 31 for detecting open or close of the cover 6. The first optical sensor 39 is mounted with passing through the side wall of the hollow chamber 33, and the second optical sensor 31 is disposed at a portion facing to the cover 6, especially the portion is included in the concave portion 200 of the holder 16.

A seal member 23 mounted at the opening 22 of the holder 16 functions so as to seal the inner space of the hollow chamber 33 and functions so as to support the micro-sample tube 21.

The apparatus of the present invention further comprises a chip guide 30 being a hollow member that allows to detachably be mounted in the through holes 24, 28 of the cover 6 and the guide member 27, and a chip member 43 attached to a predetermined portion of the sample syringe 42, thereby a method for measuring chemiluminescence comprises in steps of: disposing the micro-sample tube 21 containing a sample solution in a black box-like housing 1 that includes a through hole 24 positioned at a top surface thereof; thereafter, introducing the distal end of the sample syringe 42 on which the chip member 43 is mounted into inner space of the micro-sample tube 21 via the detachable chip guide 30 mounted in the through hole 24 of the housing 1. Since the chip guide 30 is a hollow member made of a nontransparent material and the chip member 43 is made of a nontransparent material, the chip guide 30 and chip member 43 seal the housing 1 when the chip guide 30 is in contact with the chip member 43 by introducing the distal end of the sample syringe 42 via the chip guide 30 into internal of the housing 1.

Thereafter, the method of the present invention performs sealing internal of the housing 1 by being in tightly contact with the chip member 43 to the chip guide 30, and detecting luminescence from the micro-sample tube 21 in which the luminous regent is infused, by a photomultiplier. In particular, the method of the present invention performs detecting step while optically shutting luminescence from the micro-sample tube 21 at a predetermined time interval.

As described above, the apparatus of the present invention has a structure which is attachable and detachable for the sample syringe 42 and which is also attachable and detachable for the micro-sample tube 21.

If a sample solution may scatter and adhere to the tank, the tube, and other mechanisms, or microorganisms may propagate in sample solution deposits to serve as contaminant sources, such contaminant sources interfere with luminescence from the above solution mixture or become unnecessary luminescence sources, thereby interfering with accurate quantitative analysis. The apparatus of the invention prevents propagation of undesirable various germs.

Although, the previous luminous reagent must be completely removed when a luminous reagent different in type from a previous luminous reagent is to be used, the invention does not necessitate the above cleaning.

Further, since the apparatus of the present invention does not necessitate to move the first or second vessel, the apparatus does not has an automatic injecting mechanism. Therefore, the overall chemiluminescence measuring apparatus is small in size and superior in terms of operability and installation.

In performing quantitative analysis, the vessel in which a sample solution is stored is housed in the hollow chamber in the dark box portion (housing made of nontransparent material), and the chip member is mounted on the chip guide to set the sample solution in a perfectly dark state. The optical path between the second vessel and the photo-sensing unit is opened by the shutter mechanism to allow detection of luminescence from the sample solution. A luminous reagent stored in the chip member in advance is poured (injected) into the second vessel by operating the reagent syringe. As a result, luminescence from the sample solution is detected. In addition, a change in luminescence over time can be measured in real time by displaying the luminescence on a monitor or the like on the basis of a signal output from the photo-sensing unit.

The present invention will become more fully understood from the detailed description given hereinbelow and the accompanying drawings which are given by way of illustration only, and thus are not to be considered as limiting the present invention.

Further scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art form this detailed description.

Fig. 1 is a sectional view showing a structure of an apparatus for measuring chemiluminescence according to an embodiment of the present invention.

Fig. 2 is a sectional view showing the structure of the cover of the apparatus in Fig. 1.

Fig. 3 is a side view showing the outer shape of a chemiluminescence measuring apparatus according to an embodiment of the present invention.

Fig. 4 is a top view showing the structure of a main portion arranged on the upper portion of the apparatus of the embodiment.

Fig. 5 is a front view showing the structure of a main portion arranged on the front portion of the apparatus of the embodiment.

Fig. 6 is a sectional view showing the structure of a shutter mechanism of the embodiment.

Fig. 7 is a block diagram showing the control system of an embodiment of the present invention.

Fig. 8 is a sectional view showing the internal structure of the apparatus of the embodiment.

Fig. 9 is a view for explaining an operation in per-

forming quantitative analysis by using the apparatus of the embodiment in correspondence with Fig. 8.

A chemiluminescence measuring apparatus of an embodiment will be described below with reference to the accompanying drawings. The outer structure of the apparatus will be described first with reference to Figs. 3 to 5. Fig. 3 is a side view of the apparatus. Fig. 4 is a top view showing the structure of a main portion arranged on the upper portion of the apparatus. Fig. 5 is a plan view showing the structure of a main portion arranged on the front surface of the apparatus.

Referring to Fig. 3, a power switch 2 for turning on/off a commercial AC power supply is arranged on a side surface of a box-like housing 1. An input/output port is arranged on the rear surface of the housing 1. The input/output port includes an electrical outlet 3 for connection of a power cable, a connector 4 conforming to the RS232C standard and designed to exchange data with an external computer system and various measuring devices (not shown), an external connector (coaxial connector) 5 for outputting a detection signal, which is generated in a measuring operation (to be described later), to the above computer system and various measuring devices, and the like.

A cover 6 is swingably provided on the top surface of the housing 1 by a hinge mechanism 160.

In addition, as shown in Fig. 4, a mount portion α (a concave portion of the holder 16) on which a micro-sample tube 21 (to be described later) is to be mounted is arranged on an upper portion A of the housing 1. As shown in Fig. 4, the mount portion α is exposed to the outside when the cover 6 is opened, and is shielded from the outside when the cover 6 is closed. A printer 7 is incorporated in the upper portion A of the housing 1. The printer 7 prints, for example, a plot or numerical values to indicate a change in the above detection signal over time.

Furthermore, as shown in Fig. 5, an operation panel is arranged on an inclined surface B of the front surface of the housing 1. The operation panel includes a control key 8 for instructing a measuring mechanism (to be described later) incorporated in the housing 1 to execute measurement, a cancel key 9 for stopping the execution halfway, function keys 10, 11, and 12 for selecting various measuring operations (measurement menu), a liquid crystal display 13, a multifunction key 14, and the like.

Next, Fig. 6 shows the structure of a shutter mechanism of an embodiment of the invention. The apparatus for measuring chemiluminescence comprises a box-like housing 1 as a casing for the shutter mechanism and photo-sensing mechanism. The housing 1 is made of nontransparent material and has an opening 15 at a top surface thereof. The cover 6 is provided at the top surface of the housing 1 and is in contact with the guide member 27 having a through hole. Further, a projection 19 is provided on inside surface of the cover 6, and the O-ring as a stopper having a through hole is provided on the surface of the guide member 27 as shown in Fig. 6. Since the through hole of the O-ring 29 has a smaller diameter than the cover 6, the O-ring 29 holds the guide member 30 which is a tapered hollow member (W1>W3), thereby the luminous reagent syringe 42 is held at a predetermined position.

As shown in Fig. 6, the hollow chamber 33 is disposed in the housing 1 and has the opening 38 at a side wall thereof, therefore an inner space of the hollow chamber 33 allows to partially house a micro-sample tube 21 containing a sample solution. The holder 16 is disposed on the hollow chamber 33 and has the through hole 22 connecting the inner space (the hollow space 32) of the hollow chamber 33 to the space 20. The distal end of the luminous reagent syringe 42 is partially inserted the inner space of the hollow chamber 33 via the through hole of the holder 16. The rotating cylindrical hollow chamber 370 having an opening 37 and the driving motor 36 rotating the chamber 370 are disposed in the hollow chamber 33, and the optical path between the photo-sensing unit 40 and the luminous reagent syringe 42 is opened when the position of the opening 38 and the position of the opening 37 coincide with each other. Fig. 7 shows a control system of the embodiment of the present invention. The controller 41 is supplied power from the power supply 200 and observes the signals from sensors 26, 31 and 39 as sensor devices. For example, if the controller 41 is received signals from the photodiode 26, the guide member 30 will be exist; if the controller 41 is received signals from the optical sensor 31, the cover 6 will be closed; if the controller 41 is received signals from the optical sensor 39, the luminous reagent syringe 42 will be exist; if the controller is not received signals from one or more of the sensors 26, 31 and 39, the controller 41 displays a predetermined massage on the liquid crystal display 13 in I/O unit 100. On the other hand, the controller 41 performs measuring of chemiluminescence after receiving the instruction signals from various input keys 8, 9, 10, 11 and 12 of I/O unit 100. In measuring of chemiluminescence, the controller 41 outputs the instruction signals to move the driving motor 36, thereby the shutter mechanism intermittently shuts the optical path between the photo-sensing unit 40 and the micro-sample tube 21. After the photo-sensing unit 40 intermittently receives luminescence from the micro-sample tube 21 by the photomultiplier 401, the controller 41 outputs signals as measuring result to I/O unit 100 in order to display the data on the liquid crystal display 13 or print the data by the printer 7.

The arrangement of the measuring mechanism arranged in the apparatus will be described next with reference to Fig. 8. Note that the measuring mechanism is constituted by a plurality of portions having different functions, and includes the mount portion a (the concave portion of the holder 16) on which the micro-sample tube 21 is to be mounted, and an photo-sensing mechanism β for detecting luminescence from a sample solution in the micro-sample tube 21.

The arrangement of the mount portion α will be described. A substantially cup-like holder 16 is fixed in a hole 15 formed in the upper portion of the housing 1, and the concave portion of the holder 16 is opened/closed by the cover 6 provided on one end of the holder 16 by the hinge mechanism 160. A stepped portion 18 on which a seal member 17 consisting of a rubber material or the like having relatively high viscosity is pasted is formed on the inner wall of the holder 16. When the cover 6 is closed, a projection 19 extending from the inner side of the cover 6 is engaged with the stepped portion 18 and brought into slight contact with the seal member 17, thereby preventing leakage of external light into an internal space 20 of the holder 16 via the above engaging portion.

A through hole 22 in which the micro-sample tube 21 is to be fitted is formed in the bottom surface of the holder 16. The micro-sample tube 21 is an elongated vessel consisting of a transparent or semitransparent material, which transmits light. That is, the micro-sample tube 21 is an elongated vessel in the form of a test tube, which is used to store a sample solution or the like in a chemical experiment.

A through hole 24 is formed in the cover 6 to oppose the through hole 22 while the concave portion of the holder 16 is closed by the cover 6. In addition, an optical sensor constituted by a light-emitting diode (LED) 25 and a photodiode (PD) 26 which oppose each other is mounted at the entrance of the through hole 24. When light emitted from the light-emitting diode 25 is blocked upon mounting of the chip guide and the chip in the through hole 24, the photodiode 26 detects the passage of an object and outputs a passage detection signal.

A cup-like guide member 27 is fixed on the inner side of the cover 6, and a through hole 28 is formed in the bottom end of the guide member 27 to oppose the through hole 24. An O-ring 29 is fitted on the inner peripheral portion of the through hole 28. A conical chip guide 30 is mounted in the through holes 24 and 28 and the O-ring 29.

The chip guide 30 consists of a material which does not allow transmission of light, and has a hollow portion in which a chip member 43 to be mounted on the distal end of the luminous reagent syringe 42 is inserted. With this arrangement, the hollow portion of the chip guide 30 opposes the upper opening of the micro-sample tube 21 while the concave portion of the holder 16 is closed by the cover 6. In addition, the chip guide 30 is stably held in the through holes 24 and 28 owing to the clamping force of the O-ring 29. The chip guide 30 is detachably mounted in the through holes 24 and 28 so that the chip guide 30 can be replaced with a new chip guide 30 or properly cleaned to be reused.

An optical sensor 31 is mounted on one end of the holder 16. The optical sensor 31 optically detects the open/closed state of the cover 6 and outputs a open/closed state detection signal. This signal is supplied to the controller 41 (to be described later) together with a passage detection signal from the photodiode 26.

The arrangement of the photo-sensing mechanism β will be described next. The photo-sensing mechanism β includes a cylindrical hollow chamber 33 having a cylindrical space 32 communicating with the space 20 via the through hole 22 formed in the bottom surface of the holder 16. A rotating cylindrical member 370 (including a metal column member 34) having a space for housing the micro-sample tube 21 is arranged in the hollow chamber 33. Note that the space of the cylindrical hollow chamber 33 is designed to have a volume large enough to prevent contact of the side wall of the micro-sample tube 21.

A lower end (the metal column member 34) of the rotating cylindrical hollow chamber 370 located in the cylindrical hollow chamber 33 to be rotatable in the circumferential direction is supported by a bearing mechanism 35, and the bearing mechanism 35 is coupled to the driving shaft of a driving motor 36 mounted on the lower portion of the cylindrical hollow chamber 33. With this arrangement, the rotating cylindrical hollow chamber 370 is driven by the driving motor 36 to be rotated in the circumferential direction within the hollow space 32 of the cylindrical hollow chamber 33 without being brought into contact with the side wall of the micro-sample tube 21. Note that the lower end of the micro-sample tube 21 is brought into contact with the bottom portion of the hollow chamber 32 with a small area. However, since this contact surface corresponds to the rotation center of the rotating cylindrical hollow chamber 370, the micro-sample tube 21 does not move upon rotation of the rotating cylindrical hollow chamber 370.

Openings 37 and 38 are respectively formed at one side of the side wall of the rotating cylindrical hollow chamber 370 and one side of the side wall of the cylindrical hollow chamber 33 to oppose the lower side portion of the micro-sample tube 21. When the rotating cylindrical hollow chamber 370 is rotated, the openings 37 and 38 coincide with each other only at a predetermined timing, but the opening 38 of the cylindrical hollow chamber 33 is closed by the side wall of the rotating cylindrical hollow chamber 370 at other timings.

An optical sensor 39 is mounted on one side of the cylindrical hollow chamber 33. The optical sensor 39 optically detects insertion of the micro-sample tube 21 and supplies the insertion detection signal to the controller 41. The cylindrical hollow chamber 33 has a sealed structure for preventing leakage of external light into the hollow space 32. That is, the cylindrical hollow chamber 33, the cover 6, the holder 16, and the like realize a so-called dark box for preventing leakage of external light into the hollow space 32 in which the micro-sample tube 21 is housed.

A high-sensitivity photo-sensing unit 40 is fixed on the outer side of the cylindrical hollow chamber 33 such that the imaging plane opposes the opening 38. The photo-sensing unit 40 has a photomultiplier 401 for photomultiplying and detecting feeble light received via the

SPECTRAL_0000224

opening 38. For example, a sensor R-647-04 or R5610 available from Hamamatsu Photonics can be used as the sensor device in the photo-sensing unit 40. A signal output from the photo-sensing unit 40 is transmitted to the controller 41 incorporating a microcomputer system.

The coupling portion between the cylindrical hollow chamber 33 and the photo-sensing unit 40 is also sealed to prevent incidence of external light. Further, in the coupling portion, a filter for transmitting light of a predetermined wavelength.

An operation procedure in the use of the above apparatus will be described next with reference to Figs. 8 and 9. The same reference numerals in Fig. 9 denote the same or corresponding parts as in Fig. 8. Fig. 9 shows a state wherein the chip member 43 mounted on the distal end of the luminous reagent syringe 42 is inserted in the hollow portion of the chip guide 30.

A typical operation procedure will be described below. First of all, the cover 6 is opened, and the micro-sample tube 21 containing a sample solution M is inserted in the hollow space 32 of the cylindrical hollow chamber 33 via the through hole 22 of the holder 16.

The cover 6 is then closed, and the chip guide 30 is mounted in the through holes 24 and 28. Note that the chip guide 30 may be mounted in the through holes 24 and 28 in advance.

Subsequently, as shown in Fig. 9, the chip member 43 mounted on the distal end of the luminous reagent syringe 42 is inserted in the hollow portion of the chip guide 30. In this case, the chip member 43 has a cassette-like structure in which a predetermined type of luminous reagent is stored in advance. In addition, the chip member 43 has a conical outer shape to be fitted in the hollow portion of the chip guide 30. Furthermore, the chip guide 30 consists of a material which does not transmit light. By operating an operation rod 44 of the luminous reagent syringe 42, the luminous reagent can be injected from the distal end portion of the chip guide 30 into the micro-sample tube 21. As shown in Fig. 9, when the chip member 43 is mounted, the hollow portion of the chip guide 30 is sealed, thereby preventing incidence of external light into the hollow space 32 of the cylindrical hollow chamber 33 via the hollow portion of the chip guide 30.

The operation state is displayed on the liquid crystal display 13 on the basis of the above passage detection signal, open/closed state detection signal, and insertion detection signal. With this display 13, the user confirms that no external light leaks into the hollow space 32 of the cylindrical hollow chamber 33, and instructs to start the photo-sensing mechanism β.

In accordance with this instruction, the driving motor 36 is operated to rotate the rotating cylindrical hollow chamber 370. When the openings 37 and 38 coincide with each other, the photo-sensing unit 40 starts detection. If these openings 37 and 38 do not coincide with each other, a so-called shutter is closed.

In addition, by transferring a signal output from the photo-sensing unit 40 to the liquid crystal panel via the controller 41, a change over time can be displayed in real time.

In such an observed state, the operation rod 44 of the luminous reagent syringe 42 is operated to pour a predetermined amount of luminous reagent stored therein into the micro-sample tube 21, and light emitted from the sample solution M is detected.

As described above, according to the present invention, even with a very simple apparatus structure, luminescence from the sample solution M can be reliably imaged so that a high-precision quantitative analysis can be performed. In addition, monitor observation can be performed before the luminous reagent is mixed with the sample solution M, and luminescence can be observed in real time immediately after the mixing operation. Therefore, even a sample solution M which causes luminescence for only a short duration can be easily measured. Furthermore, measurement can be performed while the mixing amount of luminous reagent is properly adjusted. In particular, fine adjustment of the mixing amount can be performed by the above monitor observation. Contamination and the like caused by the sample solution M can be completely and easily prevented by only replacing or cleaning the micro-sample tube 21, the chip guide 30, and the chip member 43 as needed. As described above, this embodiment can provide many excellent effects in the field of quantitative analysis.

For example, this embodiment can be applied to quantitative analysis of ATP (adenosine triphosphate) which exists in an organism cell and serves to store and carry energy. The ATP amount in a living cell is constant. However, the ATP amount in a dead cell decreases because of autolysis. Therefore, a dead cell count can be analyzed by imaging luminescence of a specific wavelength which occurs when a predetermined type of luminous reagent is mixed with a sample solution M containing cells, and measuring the luminescence amount (or count). In addition, in measuring such an ATP amount, a luciferin-luciferase reaction represented by luminescence from a firefly may be used. More specifically, when luciferin is coupled with ATP in the presence of luciferase and magnesium to produce AMP (adenosine monophosphate) upon dephosphorization, the luciferin is excited to cause luminescence having a peak intensity at 562 nm. By measuring this luminescence, quantitative analysis of ATP can be performed.

By measuring ATP using this embodiment, measurement of a viable cell count in food such as milk or juice, a sterility test on an injection, measurement of a bacterium count in urine in a clinical bacterium test, or the like can be easily performed.

As has been described above, according to the present invention, the problems posed in the prior art can be completely solved.

The detachable chip guide is mounted on the upper portion of the dark box housing, and the chip member

is mounted on this chip guide, thereby setting the inner space of the housing in a perfectly dark state. In this dark state, only luminescence from a sample solution is detected by the photo-sensing unit. With this simple structure, a reduction in the size of the apparatus can be easily realized.

In addition, since the chip guide and the chip member can be replaced with new ones, contamination of the apparatus can be prevented, and the luminous reagent can be easily replaced with a desired luminous reagent to realize a quick measurement process. As is apparent, since the problem of contamination by deposits can be solved, accurate, high-precision measurement can be realized. Since the amount of luminous reagent mixed with a sample solution can be finely adjusted by operating the reagent syringe, delicate quantitative analysis or the like can be easily performed.

As described above, the present invention exhibits excellent effects with the structure disclosed in the specification.

Claims

1. An apparatus for measuring chemiluminescence, said apparatus comprising:

   a housing having an opening at a top surface thereof;
   a cover provided at the top surface of said housing, said cover having a through hole that allows to introduce a distal end of a first vessel containing a luminous reagent from external of said housing into internal of said housing;
   a hollow chamber disposed in said housing and having an opening at a side wall thereof, said hollow chamber having an inner space that allows to partially house a second vessel which is disposed in said housing and which contains a sample solution;
   a holder disposed in said housing and enclosing the opening of said housing, said holder having a concave portion for forming a first space defined with said cover and having a through hole connecting the first space to the inner space of said hollow chamber at a bottom surface of the concave portion;
   a photo-sensing unit disposed in said housing, for detecting luminescence from the second vessel in said housing via the opening of said hollow chamber;
   a shutter mechanism for optically shielding luminescence from the second vessel in said housing; and
   a stopper for holding the first vessel at a predetermined position, said stopper being disposed in the first space and having a through hole that allows to partially introduce the distal end of the first vessel into the second vessel in said housing, a diameter of the through hole of said stopper being smaller than that of the through hole of said cover.

2. An apparatus according to claim 1, further comprising:

   a guide member disposed in the first space and contacting said cover for defining a second space with said cover, said guide member having a through hole that allows to introduce the distal end of the first vessel into the second vessel;
   a photodiode provided in the second space; and
   a laser emitting diode provided in the second space.

3. An apparatus according to claim 1, wherein said housing, said cover, said hollow chamber, said holder and said guide member are made of non-transparent material.

4. An apparatus according to claim 1, further comprising a first optical sensor for detecting existence or absence of the second vessel, said first optical sensor being mounted with passing through the side wall of said hollow chamber.

5. An apparatus according to claim 1, further comprising a second optical sensor for detecting open or close of said cover, said second optical sensor being disposed at a portion facing to said cover, the portion included in the concave portion of said holder.

6. An apparatus according to claim 1, further comprising a seal member for supporting the second vessel, said seal member being mounted at the opening of said holder.

7. An apparatus according to claim 2, further comprising:

   a chip guide being a hollow member that allows to detachably be mounted in the through holes of said cover and said guide member; and
   a chip member attached to a predetermined portion of the first vessel, whereby
   said chip guide and chip member seal said housing when said chip guide is in contact with said chip member by introducing the distal end of the first vessel via said chip guide into internal of said housing.

8. An apparatus according to claim 1, wherein said shutter mechanism comprises:

a rotatable hollow chamber for holding the second vessel at a predetermined position while partially housing the second vessel therein, said rotatable hollow chamber being disposed in said hollow chamber and having an opening at a side wall thereof; and

a rotator for rotating said rotatable hollow chamber, said rotator disposed in said hollow chamber while holding said rotatable hollow chamber.

9. An apparatus according to claim 8, wherein said rotatable hollow chamber is made of nontransparent material.

10. An apparatus for measuring chemiluminescence, said apparatus comprising:

a housing having an opening at a top surface thereof;

a cover provided at the top surface of said housing, said cover having a through hole that allows to introduce a distal end of a first vessel containing a luminous reagent from external of said housing into internal of said housing;

a hollow chamber disposed in said housing and having an opening at a side wall thereof, said hollow chamber having an inner space that allows to partially house a second vessel which is disposed in said housing and which contains a sample solution;

a holder disposed in said housing and enclosing the opening of said housing, said holder having a concave portion for forming a first space defined with said cover and having a through hole connecting the first space to the inner space of said hollow chamber at a bottom surface of the concave portion; and

a shutter mechanism for optically shielding luminescence from the second vessel in said housing, said shutter mechanism including:

a rotatable hollow chamber for holding the second vessel at a predetermined position while partially housing the second vessel therein, said rotatable hollow chamber disposed in said hollow chamber and having an opening at a side wall thereof; and

a rotator for rotating said rotatable hollow chamber, said rotator being disposed in said hollow chamber while holding said rotatable hollow chamber; and

a photo-sensing unit disposed in said housing, for detecting luminescence from the second vessel in said housing via the openings of said hollow chamber and said rotatable hollow chamber.

11. An apparatus according to claim 10, further comprising a stopper for holding the first vessel at a predetermined position, said stopper being disposed in the first space and having a through hole that allows to partially introduce the distal end of the first vessel into the second vessel, a diameter of the through hole of said stopper being smaller than that of the through hole of said cover.

12. An apparatus according to claim 10, further comprising:

a guide member disposed in the first space and contacting said cover for defining a second space with said cover, said guide member having a through hole that allows to introduce the distal end of the first vessel into the second vessel;

a photodiode provided in the second space; and

a laser emitting diode provided in the second space.

13. An apparatus according to claim 12, wherein said housing, said cover, said hollow chamber, said holder and said rotatable hollow chamber are made of nontransparent material.

14. An apparatus according to claim 10, further comprising a first optical sensor for detecting existence or absence of the second vessel, said first optical sensor mounted with passing through the side wall of said hollow chamber.

15. An apparatus according to claim 10, further comprising a second optical sensor for detecting open or close of said cover, said second optical sensor being disposed at a portion facing to said cover, the portion included in the concave portion of said holder.

16. An apparatus according to claim 10, further comprising a seal member for supporting the second vessel, said seal member being mounted at the opening of said holder.

17. An apparatus according to claim 12, further comprising:

a chip guide being supported by the through hole of said cover and said guide member; and

a chip member attached to a predetermined portion of the first vessel, whereby

said chip guide and chip member seal said housing when said chip guide is in contact with said chip member by introducing the distal end of the first vessel into internal of said housing.

18. A method for measuring chemiluminescence, said

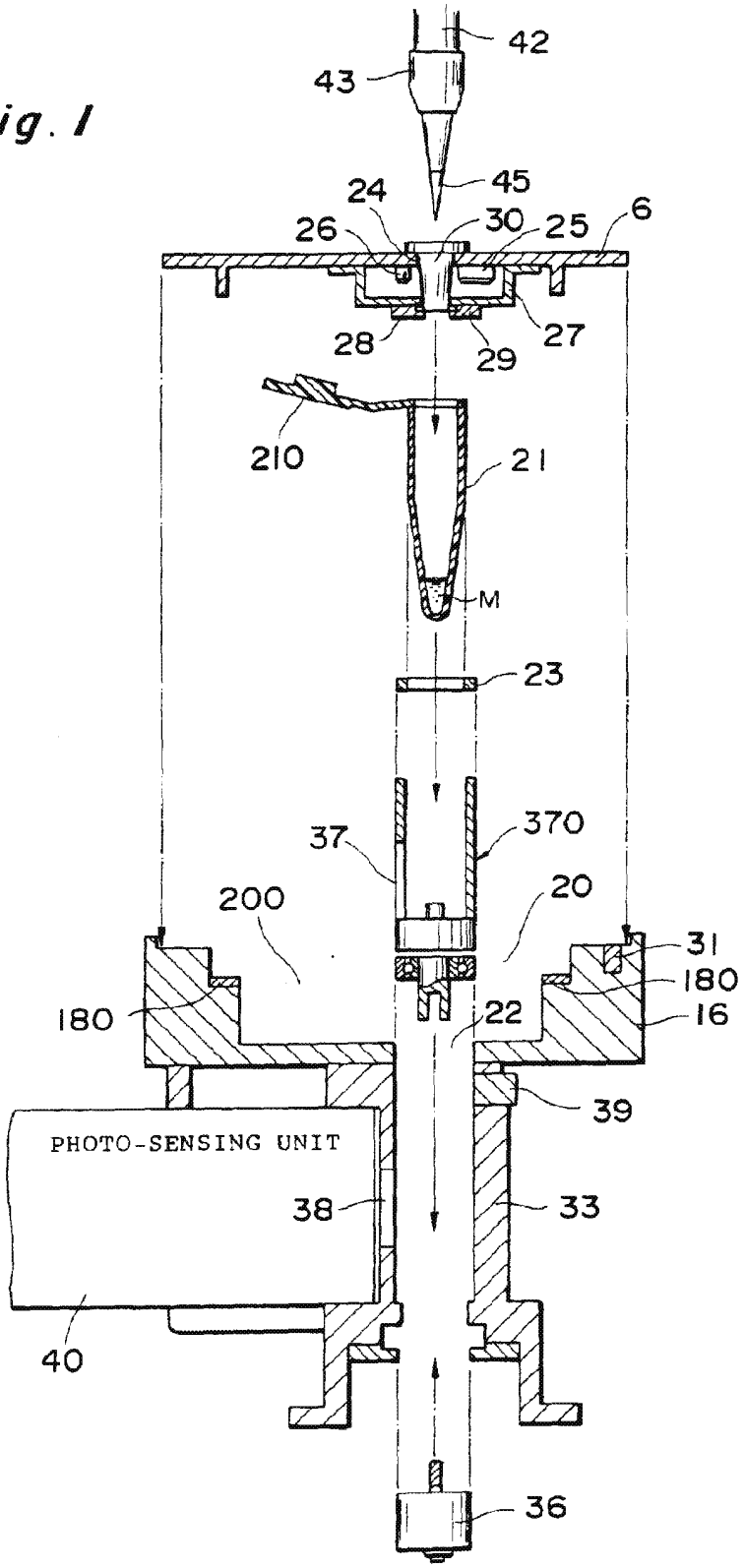
method comprising in steps of:

disposing a second vessel containing a sample solution in a box-like housing that has a through hole positioned at a top surface thereof;
introducing a distal end of a sample syringe as a first vessel on which a chip member is mounted and which contains a luminous reagent into inner space of said second vessel via a chip guide detachably mounted in said through hole of said housing, wherein said chip guide is a hollow member made of nontransparent material and said chip member is made of nontransparent material;
sealing internal of said housing by being in tightly contact with said chip member to said chip guide; and
detecting luminescence from the second vessel in which the luminous regent is infused from the first vessel, by a photomultiplier.

19. A method according to claim 18, wherein said detecting step is carried out while optically shutting luminescence from the second vessel at a predetermined time interval.

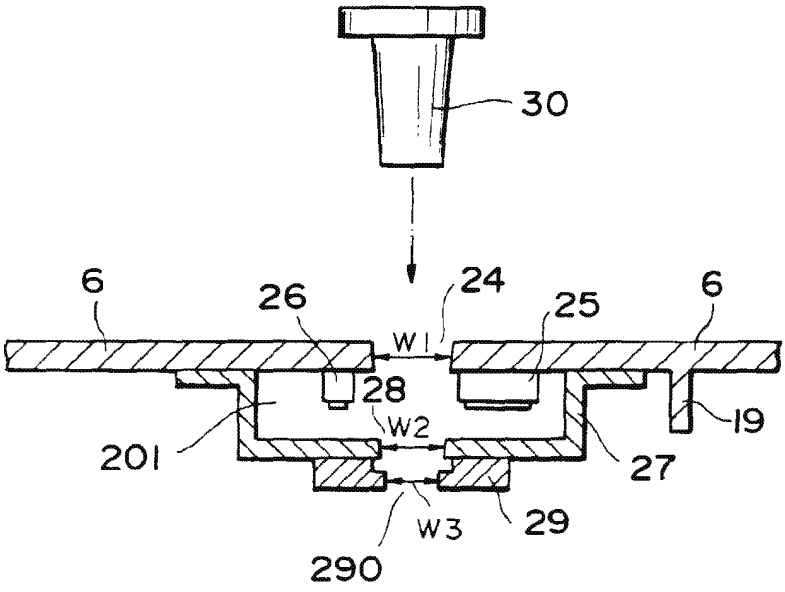
SPECTRAL_0000228

Fig. 1



SPECTRAL_0000229

Fig. 2



SPECTRAL_0000230

Fig. 3

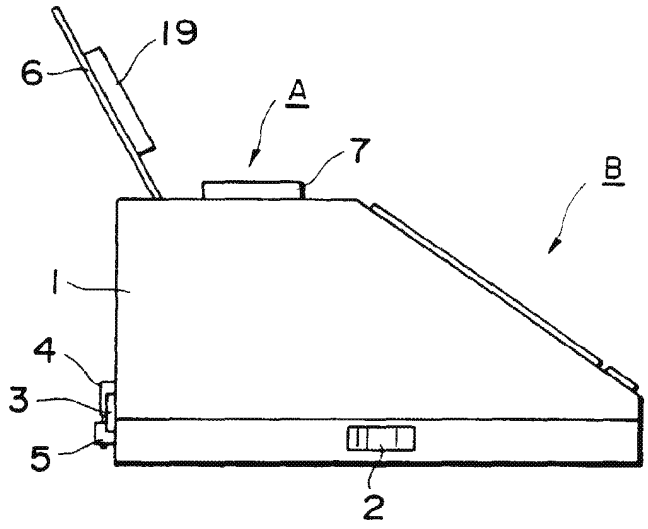


Fig. 4

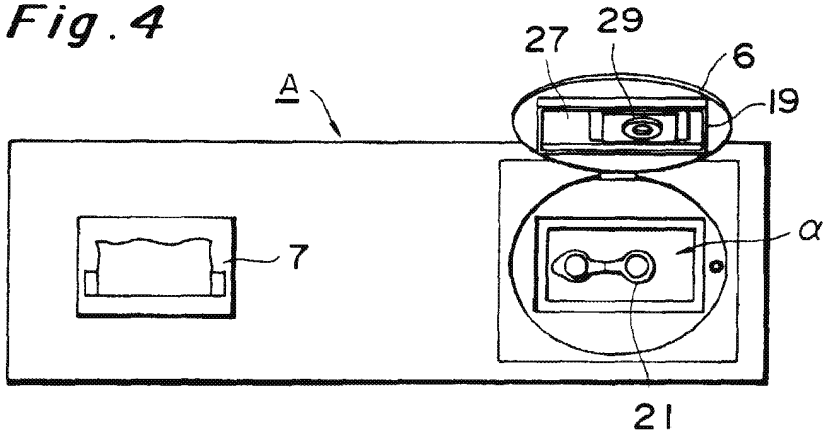
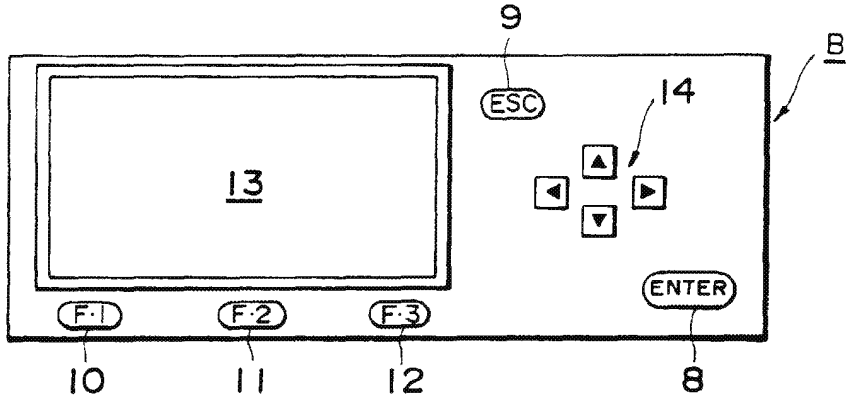
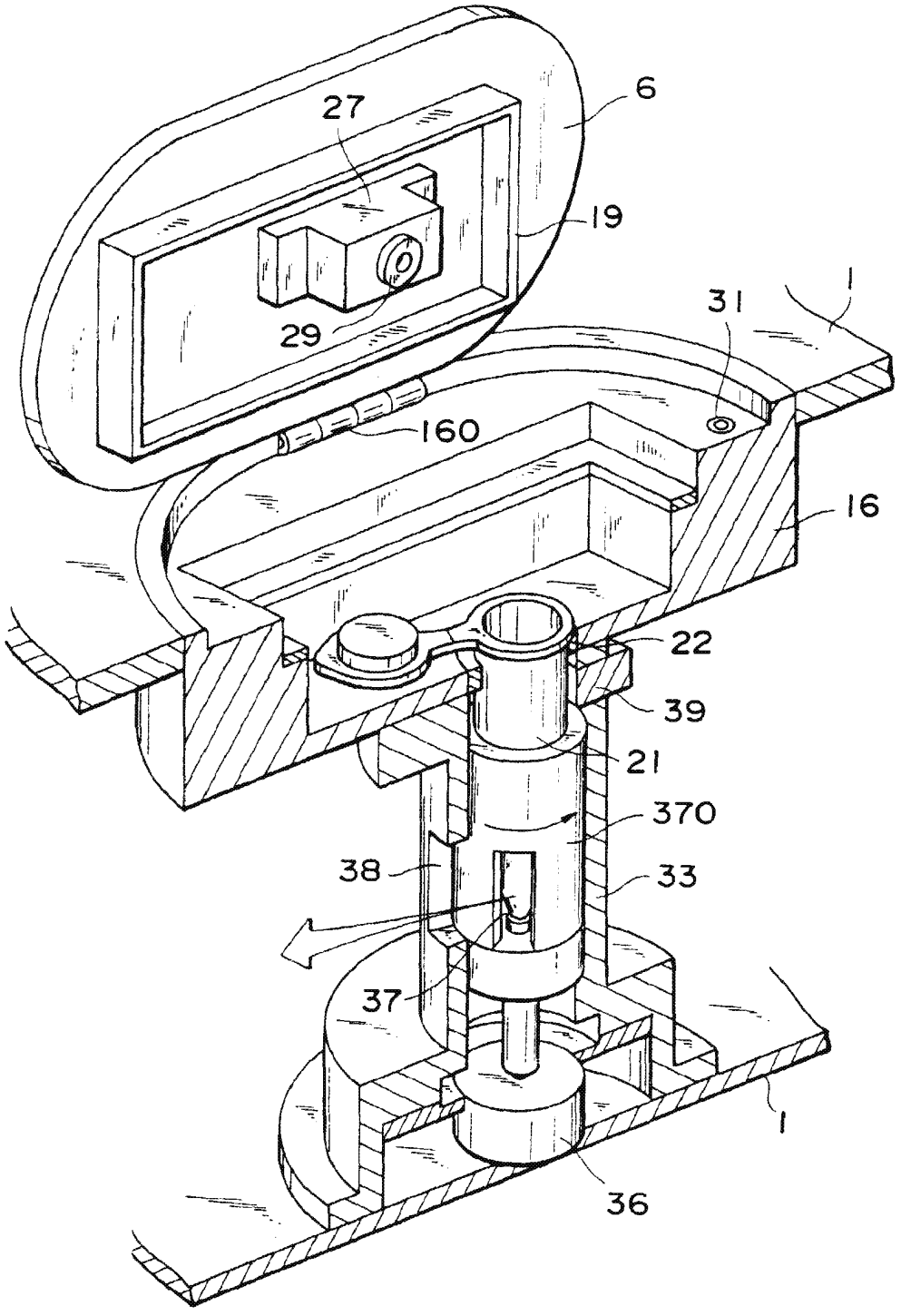


Fig. 5



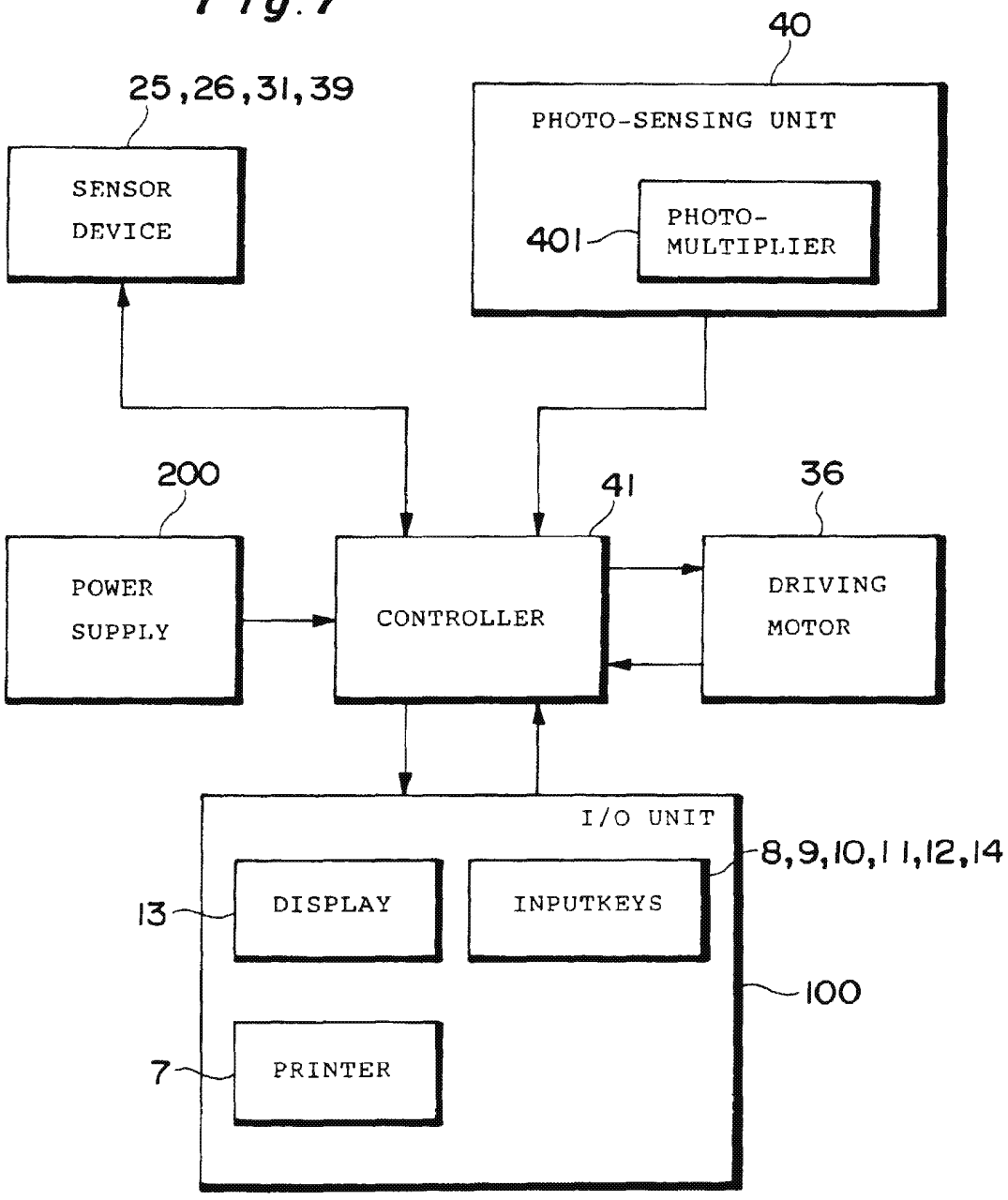
SPECTRAL_0000231

Fig. 6



SPECTRAL_0000232

Fig. 7



SPECTRAL_0000233

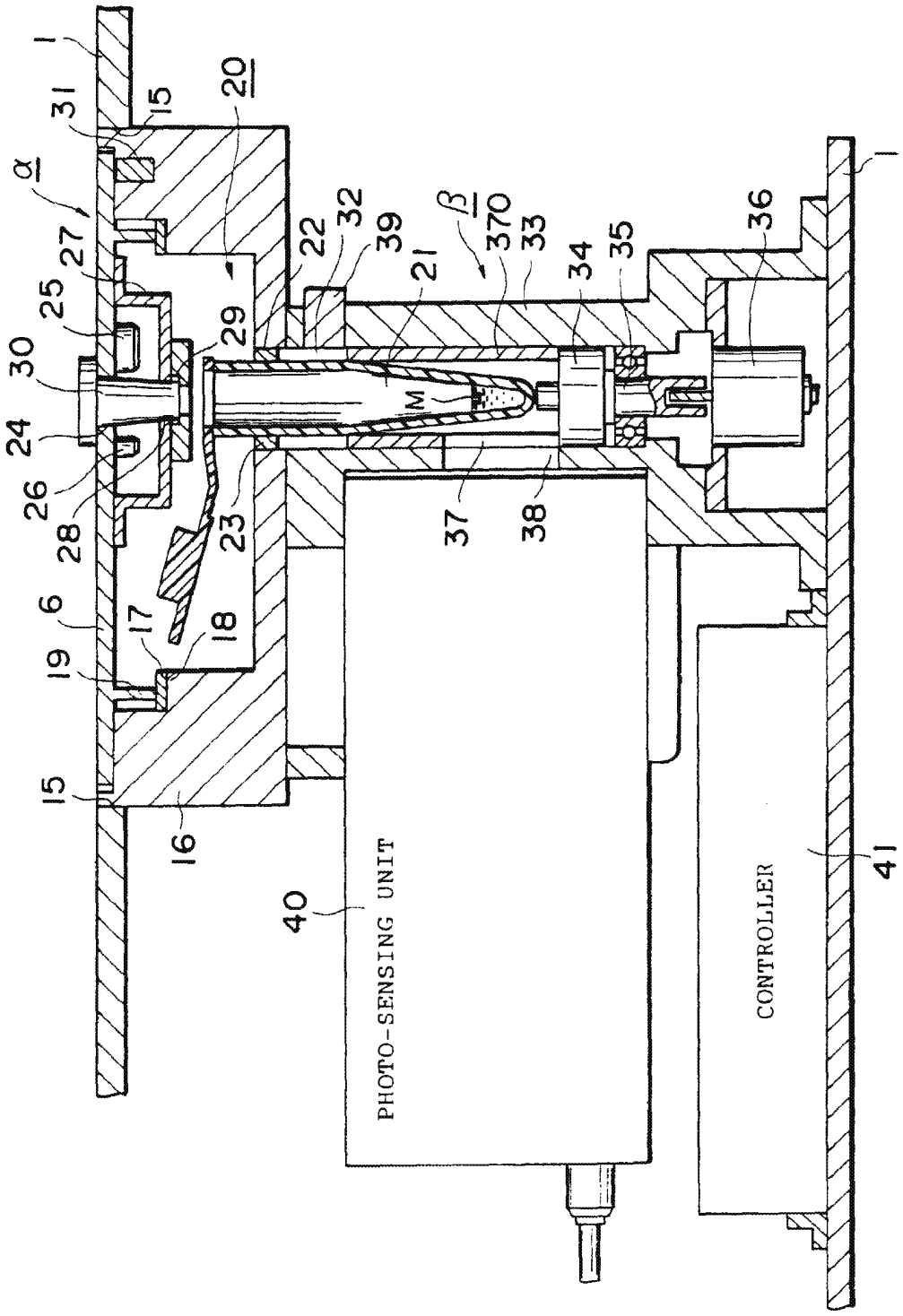
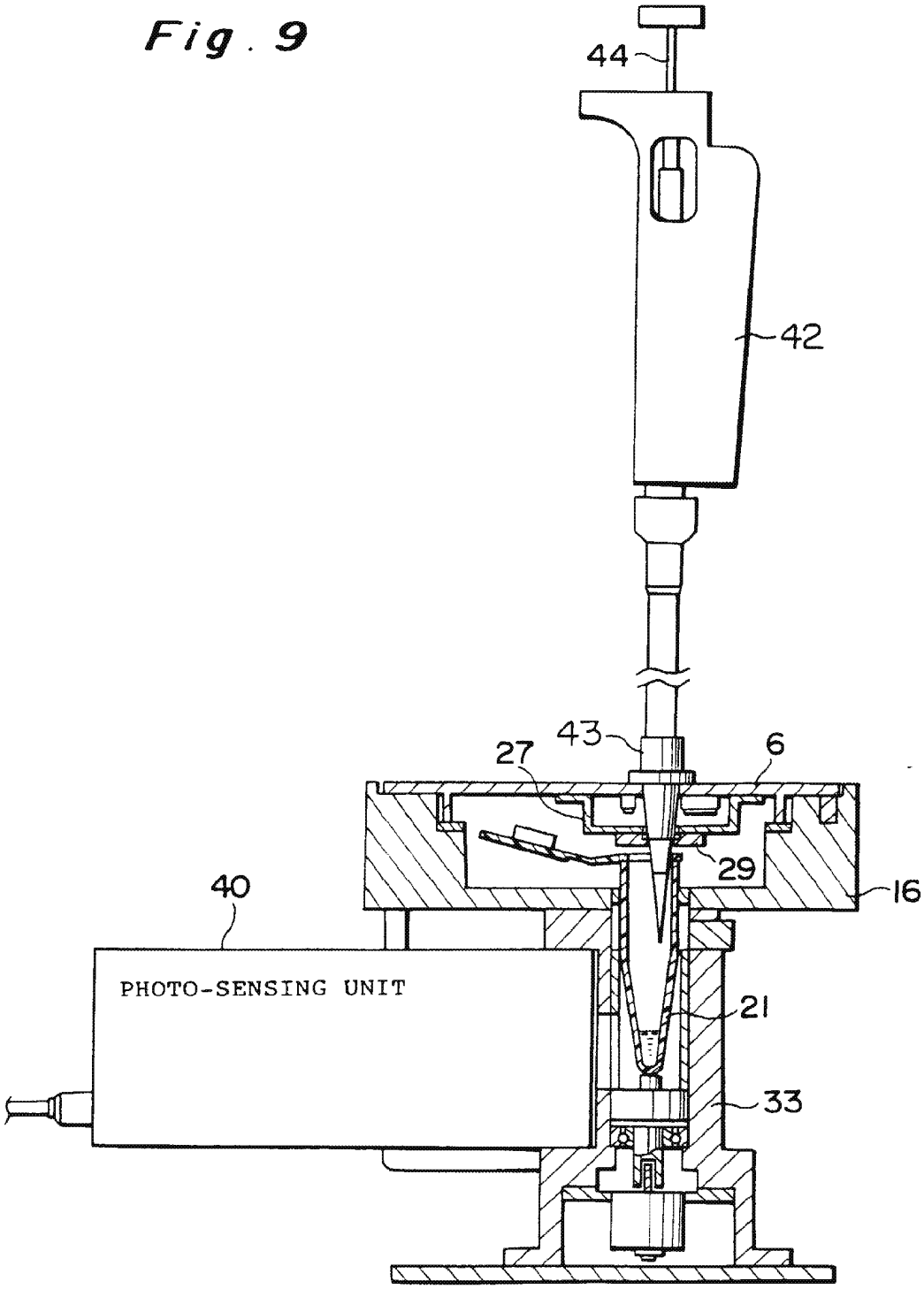


Fig. 8

Fig. 9



(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 478 423 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
26.07.2006 Bulletin 2006/30

(21) Application number: **03713630.6**

(22) Date of filing: **19.02.2003**

(51) Int Cl.:
A61M 16/01 *(2006.01)*

(86) International application number:
PCT/US2003/005493

(87) International publication number:
WO 2003/070308 (28.08.2003 Gazette 2003/35)

(54) **MULTIPLE OUTPUT ANESTHESIA SYSTEM**

NARKOSESYSTEM MIT MEHREREN AUSGÄNGEN

SYSTEME D'ANESTHESIE A SORTIES MULTIPLES

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PT SE SI SK TR

(30) Priority: **20.02.2002 US 81040**
31.05.2002 US 385397 P

(43) Date of publication of application:
24.11.2004 Bulletin 2004/48

(73) Proprietor: **Xenogen Corporation**
Alameda, CA 94501 (US)

(72) Inventors:
- **DALGETTY, Richard, George**
  Oakland, CA 94619 (US)
- **BATES, Brian, Robert**
  Lafayette, CA 94549 (US)
- **NELSON, Michael, Bo**
  San Francisco, CA 94123 (US)

(74) Representative: **Alton, Andrew**
Urquhart-Dykes & Lord LLP
Tower North Central
Merrion Way
Leeds LS2 8PA (GB)

(56) References cited:
EP-A- 0 097 060 **US-A- 5 964 220**

- **DEE HORNE, BRYAN OGDEN, JIM HOUTS, ART HALL: "A Nonrebreathing Anesthetic Delivery System for Mice" LAB ANIMAL MAGAZINE, vol. 27, no. 7, 4 August 1997 (1997-08-04), page 32 XP002243950**
- **MICHAEL S. RAND: "Handling, Restraint, and Techniques of Laboratory Rodents" May 2001 (2001-05) , DEPARTMENT OF ANIMAL CARE, UNIVERSITY OF ARIZONA XP002243951 Page 5 "Methods of Delivery of Inhalant Agents to Rodents" Page 6, second passage**
- **MULDER J B: "A Unit for Inhalation Anesthesia of Small Laboratory Animals" ANESTHESIA AND ANELGESIA, vol. 52, no. 3, 1973, pages 369-372, XP009012162**

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Printed by Jouve, 75001 PARIS (FR)

Description

FIELD OF THE INVENTION:

[0001] The present invention relates generally to anesthesia delivery systems. In particular, the present invention relates to anesthesia systems for use with living specimen imaging applications.

BACKGROUND OF THE INVENTION:

[0002] One new and specialized type of imaging involves the capture of low intensity light - often on the order of only tens to hundreds of photons - from a light-emitting sample. The low intensity light source may be emitted from any of a variety of light-emitting sources within a living specimen, e.g., luciferase expressing cells within a mammalian specimen. The source of the light indicates portions of the sample, such as traced molecules in a particular portion of a laboratory mouse, where an activity of interest may be taking place. Some specialized in-vivo imaging applications may include analysis of one or more representations of emissions from internal portions of a specimen superimposed on a photographic representation of the specimen. The photographic representation provides the user with a pictorial reference of the specimen. The luminescence representation indicates portions of the specimen where an activity of interest may be taking place.

[0003] Obtaining the luminescence representation may involve image capture over an extended period of time, e.g., minutes. The living specimen is typically anesthetized during this time to prevent movement that may compromise image capture. Current imaging systems employ anesthesia delivery systems that do not consistently and reliably anesthetize specimens or deliver anesthesia gases. These conventional systems are miniaturized relatives of anesthesia systems used in hospitals and the like. Systems of this nature are designed for a single recipient. However, many imaging systems as described above may require gas delivery to multiple small mammals. So far, scaling to multiple recipients, and via lower flow rates associated with the smaller recipients has been largely unsuccessful.

[0004] More specifically, the conventional anesthesia delivery systems rely on a single general upstream flow control for the entire delivery system and downstream on/off switches for each outlet. The result is an anesthesia system that does not reliably deliver gas to each outlet. Commonly, anesthesia gas does not arrive at each interface with a consistent pressure or flow rate. For example, when one outlet is turned on/off, the remaining interconnected outlets do not maintain consistent gas output. As a result, anesthesia gas for one or more of the mammals may be interrupted, fluctuate dramatically - or significantly diminished. Insufficient anesthesia gas supply may result in unintentional consciousness for a mammal, and unexpected locomotion that compromises imaging.

[0005] In addition, an induction chamber is typically used to anesthetize the living specimen before being placed in the imaging box. A laboratory technician or researcher places one or more conscious living specimens in the induction chamber. A combination of anesthetizing gas and oxygen is then supplied to the induction chamber. The specimen remains in the induction chamber until it loses consciousness, or is similarly sedated, and is then transported by the laboratory technician into the imaging box. Transporting living specimens in and out of the induction chamber may allow the anesthesia gas to escape into the ambient surroundings. Preferably, the amount of anesthesia gas that escapes is minimized.

[0006] Conventional induction chambers rely on a purge system to manage anesthesia gas escape. The purge system forces high-pressure oxygen into the induction chamber before the door or user access is opened. A passive exhaust port leading from the induction chamber interior receives the high-pressure purge oxygen and any gases present in the induction chamber before the purge. One problem with purge systems is that the high burst of oxygen, and/or removal of all anesthetizing gas, frequently awakens any living specimens in the induction chamber. When a single living specimen is in the induction chamber, this is clearly defeating to the intended induction chamber purpose. Lab technicians however often work with multiple living specimens at a single time and purging the induction chamber to remove one specimen may then lead to more than one specimen awakening. In view of the foregoing, improved anesthesia delivery systems would be desirable.

[0007] US-5,964,220 describes a respitory assistance apparatus which includes a plurality of outputs, each of which can assist one patient. A plurality of links connect a respiratory gas source to one of the outputs of the apparatus. A plurality of links connect a controlled valve fed with respiratory gas to one of the outputs of the apparatus. Each of the outputs of the apparatus receives one link and a flow regulator is mounted on at least some of the links.

[0008] The present invention relates to improved anesthesia delivery systems that consistently and reliably deliver anesthesia gas to multiple gas outlets. The systems are particularly useful for anesthetizing multiple mammals and living specimens to be imaged by a low-light level imaging system. The anesthesia delivery systems are suitable for use with conventional oxygen sources, and convert the high pressures associated with a conventional oxygen source to lower pressures suitable for use with small mammals and suitable for combination with an anesthesia gas at low flow rates. The systems include an anesthesia gas source that combines anesthesia gas with the oxygen. The combination of anesthesia gas and oxygen is supplied to one or more multiple outlets.

[0009] According to the present invention, there is provided a gas delivery system capable of delivering an an-

esthesia gas to a plurality of gas outlets, the system comprising an oxygen inlet that receives oxygen from an oxygen source, a pressure regulator having an inlet that receives oxygen from the oxygen inlet and having an outlet that provides oxygen at a lower pressure, an anesthesia gas source having an inlet coupled to receive low pressure oxygen from the outlet of the pressure regulator and capable of adding anesthesia gas to the low pressure oxygen to produce a combination of anesthesia gas and low pressure oxygen, a first gas delivery outlet coupled to a gas delivery device having one or more living specimen interfaces and capable of providing the combination of anesthesia gas and oxygen to the one or more living specimen interfaces and a second gas delivery outlet coupled to an induction chamber and capable of providing the combination of anesthesia gas and oxygen to the induction chamber.

[0010] One outlet of the anesthesia delivery system may lead to an induction chamber used to sedate a living specimen, prior to insertion in an imaging box or chamber. Another outlet may lead to a manifold or gas delivery device having multiple specimen interfaces, each of which is capable providing anesthesia gas to a specimen associated therewith. The manifold or gas delivery device may be placed within the imaging box or chamber and used to maintain a sedated state of the specimen. In one embodiment, the induction chamber includes a scavenger system to collect anesthesia gas that escapes from the induction chamber. In another embodiment, the gas delivery device includes a scavenger system that collects anesthesia gas locally, which is advantageous when the gas delivery device is employed in an imaging box for extended periods.

[0011] The present invention may also provide independent control of gases to each of the multiple outlets, thereby enabling reliable gas flow to each gas outlet. This is in contrast to prior art systems comprising general flow control and binary on/off switches for each outlet that do not reliably allow controlled flow to each outlet. In a specific embodiment, the present invention includes a dedicated flowmeter and flow control disposed between an anesthesia source and each gas output.

[0012] These and other features of the present invention will be described in more detail below in the detailed description of the invention and in conjunction with the following figures.

[0013] The present invention is illustrated by way of example, and not by way of limitation, in the figures of the accompanying drawings and in which like reference numerals refer to similar elements and in which:

Fig. 1 is a perspective view of an imaging system.
Fig. 2 shows an exemplary functional illustration of an anesthesia delivery system in accordance with one embodiment of the present invention.
Figs. 3A and 3B illustrate front and rear views, respectively, of an exemplary anesthesia delivery console in accordance with one embodiment of the present invention.
Fig. 4A illustrates an induction chamber.
FIG. 4B illustrates a top prospective view of the front of the induction chamber of FIG. 1.
FIG. 4C illustrates a top prospective view of the back of induction chamber of FIG. 4B.
FIG. 4D illustrates a top prospective view of the door underside for the induction chamber of FIG. 4B.
FIG. 4E illustrates a side cross sectional view of induction chamber of FIG. 4B taken through the lateral midpoint.
Fig. 5A illustrates a gas delivery device.
Fig. 5B illustrates a top cutaway view of the gas delivery device of Fig. 5A showing its internal channel taken through the midsection of each specimen interface.
FIG. 6 illustrates a process flow for using the induction chamber of FIG. 1.

[0014] In the following detailed description of the present invention, numerous specific embodiments are set forth in order to provide a thorough understanding of the invention. However, as will be apparent to those skilled in the art, the present invention may be practiced without these specific details or by using alternate elements or processes. In other instances well known processes, components, and designs have not been described in detail so as not to unnecessarily obscure aspects of the present invention.

[0015] In one aspect, the present invention can be used with imaging systems for capturing an image of a low intensity light source. Fig. 1 illustrates an imaging system 10 configured to capture photographic and luminescence images. Imaging system 10 may be used for imaging a low intensity light source, such as luminescence from luciferase-expressing cells, fluorescence from fluorescing molecules, and the like. The low intensity light source maybe emitted from any of a variety of light-emitting samples which may include, for example, animals containing light-emitting molecules, e.g., various mammalian subjects such as mice containing luciferase expressing cells.

[0016] Imaging system 10 comprises an imaging box 12 having a door and walls that define an interior cavity that is adapted to receive a light-emitting sample in which low intensity light, e.g., luciferase-based luminescence, is to be detected. Imaging box 12 is often referred to as "light-tight", e.g., it seals out essentially all of the external light from the ambient room from entering the box 12, and may include one or more seals that prevent light passage into the box when the door is closed. The seals may also be effective to prevent anesthesia gases used within box 12 from escaping into the ambient room. Imaging box 12 is suitable for imaging including the capture of low intensity light on the order of individual photons, for example.

[0017] Imaging box 12 includes an upper housing 16 adapted to receive a camera. A high sensitivity camera

20, e.g., an intensified or a charge-coupled device (CCD) camera, is mounted on top of upper housing 16 and positioned above imaging box 12. CCD camera 20 is capable of capturing luminescent and photographic (i.e., reflection based images) images of a sample placed within imaging box 12. CCD camera 20 is cooled by a suitable source such as a refrigeration device 22 that cycles a cryogenic fluid through the CCD camera via conduits 24. A suitable refrigeration device is the "CRYOTIGER" compressor, which can be obtained from IGC-APD Cryogenics Inc., Allentown, PA. Other methods, such as liquid nitrogen, may be used to cool CCD camera 20.

[0018] Imaging system 10 also includes an anesthesia delivery system (Figs. 2-5). The anesthesia delivery system includes console 52 (Figs. 3A and 3B), induction chamber 54 (Figs. 4A-4E), and a gas delivery device 56 (Figs. 5A and 5B). Gas delivery device 56 may be placed within box 12 and includes multiple specimen interfaces for communicating anesthesia gas to one or more living specimens. For example, box 12 typically includes a stage that supports one or more specimens to be imaged, and gas delivery device 56 may be place on the stage in proximity to the living specimens. Conduit 62 allows gaseous communication between gas delivery device 56 and console 52. A light-sealed hole 27 is included in a side wall of box 12 to allow a gas conduit 62 to pass therethrough while device 56 is in box 12. Conduit 62 may comprise tubing or a suitable hose. For example, 9.5 mm (3/8 inch) OD 6.4mm (¼ inch) ID 90 durometer viton rubber tubing is suitable for use as conduit 62.

[0019] Oxygen delivery conduit 58, such as a rubber tube or hose, is operably coupled to an oxygen inlet of main console 52 and an outlet of an oxygen supply source. For example, the oxygen supply source may be a high pressure oxygen cylinder or conventional medium pressure wall outlet. Conduits 60 and 62 are coupled to outlets of main console 52 and coupled to inlet of induction chamber 54 and gas delivery device 56, respectively.

[0020] An image-processing unit 26 optionally interfaces between camera 20 and a computer 28 through cables 30 and 32 respectively. Computer 28, which may be of any suitable type, comprises a main unit 36 that typically contains hardware including a processor, memory components such as random-access memory (RAM) and read-only memory (ROM), and disk drive components (e.g., hard drive, CD, floppy drive, etc.). Computer 28 also includes a display 38 and input devices such as a keyboard 40 and input mouse 42. Computer 28 is in communication with various components in imaging box 12 via cable 34. To provide communication and control for these components, computer 28 includes suitable processing hardware and software configured to provide output for controlling any of the devices in imaging box 12. The processing hardware and software may include an I/O card, control logic for controlling any of the components of imaging system 10, and a suitable graphical user interface that facilitates user interaction with imaging system 10. Components controlled by computer 28 may include camera 20, motors responsible for camera 20 focus, motors responsible for position control of a platform supporting the living specimens, the camera lens, f-stop, etc.

[0021] Computer 28 may also include suitable processing hardware and software for camera 20 such as additional imaging hardware and software, calibration software, and image processing logic for processing information obtained by camera 20. The logic in computer 28 may take the form of software, hardware or a combination thereof. Computer 28 also communicates with a display 38 for presenting imaging information to the user. For example, the display 38 may be a monitor, which presents an image measurement graphical user interface (GUI) that allows a user to view imaging results and also acts an interface to control the imaging system 10.

II. Anesthesia Delivery System

[0022] The present invention employs an anesthesia delivery system that reliably delivers anesthesia gases and oxygen to multiple gas outlets. Figs. 2-5 illustrate different components of an anesthesia delivery system 50 in accordance with one embodiment of the present invention.

[0023] Fig. 2 shows an exemplary functional illustration of anesthesia delivery system 50 in accordance with a specific embodiment of the present invention. For illustrative purposes, working-pressure oxygen flow arrows 80 and combined anesthesia gas and oxygen flow arrows 84 are provided to help illustrate the flow of gases through system 50 when all control valves are open and gases flow freely.

[0024] Oxygen delivery conduit 58 is coupled to an oxygen inlet 59 of console 52. Oxygen conduit 58 delivers oxygen at a pressure determined by the outlet pressure of an oxygen source that conduit 58 is coupled to. For example, if the oxygen source corresponds to a wall supply, oxygen pressure in conduit 58 is typically between about 45 and about 55 psi. Alternately, if a pressurized cylinder is used as the oxygen source, oxygen pressure is supplied according to the outlet pressure of the tank (up to 2000 psi). Within system 50, oxygen may perform one or more of the following tasks: act as a carrier for an anesthesia gas, life sustainment for a specimen associated with the anesthesia delivery system, a purge gas for induction chamber 54, and a meter for flowmeter measurement. The oxygen inlet 59 of main console 52 includes oxygen on/off valve 66, which allows a user to turn on/off oxygen provided by conduit 58. Oxygen passed through oxygen on/off valve 66 flows to relief valve 63, which limits the pressure seen in system 50 despite the oxygen source outlet pressure. In a specific embodiment, pressure relief valve 63 releases from about 55 to about 95 psi, however, it is understood that the relief pressure of pressure relief valve 63 may adjusted accordingly to the requirements of system 50. From relief valve 63, oxygen flows to purge valve 120 and/or

pressure regulator 68.

[0025] Pressure regulator 68 includes an inlet that receives oxygen from oxygen inlet 59. Pressure regulator 68 reduces the pressure of oxygen as received at its inlet, and outputs oxygen from a regulator outlet with a lower pressure. In one embodiment, pressure regulator 68 outputs oxygen in the range of about 0.5 to about 5 psi gauge. In a specific embodiment, pressure regulator 68 outputs oxygen at about 1 psi gauge. A pressure regulator model number 8286 as provided by Porter Instruments of Hatfield, Pa may be suitable for use as pressure regulator 68.

[0026] Oxygen output from pressure regular 68 travels via conduit 67 to vaporizer 70. Disposed between pressure regulator 68 and vaporizer 70 is a pressure relief valve 72 . In some cases, vaporizer 70 may fail if it sees too high a high-pressure. Pressure relief valve 72 thus protects vaporizer 70 from pressure regulator 68 failure and thereby increases safety of the anesthesia delivery system 50 by maximizing the allowable pressure encountered by vaporizer 70. In a specific embodiment, pressure relief valve 72 releases at 5 psi, however, it is understood that the relief pressure of pressure relief valve 72 may adjusted accordingly to design specifics of vaporizer 70.

[0027] The present invention includes an anesthesia gas source or similar device that provides a controllable level of an anesthesia gas or agent. As the term is used herein, an anesthesia gas refers to any gas or agent that is used to induce any level of anesthetic state, unconsciousness, lack of awareness, or local or general insensibility to pain for a specimen interacting with gas delivery system 50. Vaporizer 70 is an anesthesia gas source that adds an anesthesia gas to low-pressure oxygen and includes an inlet that receives low-pressure oxygen from an outlet of pressure regulator 68. The output of vaporizer 70 typically comprises a controlled and variable gas mixture of life sustaining gases and anesthetizing gases. In a specific embodiment, vaporizer 70 adds isoflurane to low pressure oxygen received from pressure regulator 68 by passing the oxygen across a vaporizer that evaporates isoflurane. In this case, the low-pressure oxygen acts as a carrier for the anesthesia gas, which is added to the oxygen according to the physical characteristics of the anesthesia liquid and its temperature. Vaporizer 70 may employ one or more variable bypass, flow over, temperature compensated, and/or agent-specific vaporization techniques. Although the present invention will now be described with respect to vaporizer 70 adding only a single anesthesia gas, isoflurane, it is understood that an anesthesia gas source of the present invention may add multiple anesthesia gases, as one of skill in the art will appreciate. A VIP 3100 Calibrated Vaporizor as provided by MDS Matrix of Orchard Park, N.Y. may be suitable for use as vaporizer 70.

[0028] The output 84 of vaporizer 70 is a combination of low-pressure oxygen and anesthesia gas. Vaporizer 70 includes one or more output conduits that lead to separate gas delivery outlets, each of which is capable of providing oxygen and anesthesia gas from console 52. As shown, console 52 includes two gas delivery outlets: a first gas delivery outlet 81 for servicing a gas delivery device having specimen interfaces for communicating gases to one or more living specimens, and a second delivery outlet 71 that services induction chamber 54.

[0029] Console 52 includes an on/off valve, flow control valve and flowmeter for each gas delivery outlet that delivers oxygen and anesthesia gas from console 52. More specifically, a first on/off valve 72 allows a user to turn on/off low-pressure oxygen and anesthesia gas provided by output port 71. Conduit 60 provides gaseous commmication between output port 71 and induction chamber 54. Flow control 75 is disposed between on/off valve 72 and output port 71, and allows a user to variably control and set the flow rate of low-pressure oxygen and anesthesia gas supplied from output port 71. Flowmeter 74 is disposed before output port 71 and after flow control 75 and measures the flow rate of gases that pass there through. In one embodiment, flowmeter 74 includes a conventional output, that visually indicates flowrate to a user. After low-pressure oxygen and anesthesia gas pass through on/off valve 72, flow control 75, flowmeter 74, and output port 71, the gases are supplied to induction chamber 54.

[0030] Induction chamber 54 receives low-pressure oxygen and anesthesia gas from conduit 60. Induction chamber 54 allows a user to anesthetize a living specimen that fits within induction chamber 54, and will be described in further detail with respect to Figs. 4A-4E. An exhaust port 132 (Fig. 4A) is included in the side of induction chamber 54 and is coupled to conduit 77. Exhaust port 132 and conduit 77 withdraw gases from induction chamber 54 and provide them to filter 78. In one embodiment, filter 78 is a charcoal filter that removes unused isoflurane that passes therethrough. A filter model number 80120 F/Air Cannister as provided by A.M. Bickford of Wales Center, NY is suitable for use as filter 78. Filter 78 outputs primarily oxygen. As shown, filter 78 outputs oxygen into the ambient room.

[0031] Console 52 includes a second outlet that provides oxygen and anesthesia gas supply to gas delivery device 56 (Figs. 5A and 5B). More specifically, on/off valve 82 allows a user to turn on/off low-pressure oxygen and anesthesia gas provided by output port 81. Conduit 62 provides gaseous communication between output port 81 and gas delivery device 56. Flow control 85 is disposed between on/off valve 82 and output port 81, and allows a user to variably control and set the flow rate of low-pressure oxygen and anesthesia gas supplied from output port 81. Flowmeter 84 is disposed before output port 81 and after flow control 85 and measures the flow rate of gases that pass therethrough. In one embodiment, flowmeter 84 is a conventional mechanical flowmeter, such as a conventional rotometer, and may comprise one or more of the following components: a needle valve, an indicator float, user control knobs, and valve stops. In another embodiment, an electronic flow-

meter is employed and flow rate is indicated on a monitor screen or similar electronic output device. After low-pressure oxygen and anesthesia gas pass through on/off valve 82, flow control 85, flowmeter 84, and output port 81, the gases are supplied to gas delivery device 56.

[0032] Thus, each gas delivery outlet of system 50 comprises its own flow control. As opposed to conventional anesthesia delivery systems in which anesthesia gas delivery is controlled by a general flow control that roughly adapts the amount of oxygen received from an oxygen supply source, disposing a dedicated flow control and flowmeter for each outlet according to system 50 allowsprecise and independent control of gases to induction chamber 54 and gas delivery device 56. In particular, system 50 allows independent and specific control of anesthesia gases to each outlet - after generation of the anesthesia gas by vaporizer 70 - thereby allowing a user to variably tailor the amount of anesthesia gas provided to a specimen at any given time. In a specific embodiment, flowmeter 75 comprises a control knob that allows a user to vary flow rate from about 0 L/min to about 5 L/min through outlet port 71. In another specific embodiment, flowmeter 85 comprises a control knob that allows a user to vary flow rate from about 0 L/min to about 2 L/min through outlet port 81.

[0033] Gas delivery device 56 receives low-pressure oxygen and anesthesia gas from conduit 62. Gas delivery device 56, which will be described in further detail with respect to Figs. 5A and 5B, includes a number of specimen interfaces, each of which is capable of accommodating a living specimen and providing oxygen and anesthesia gas to the specimen. The anesthesia gas may be used to anesthetize a living specimen, or if the specimen is already anesthetized - to maintain a desired anesthetic state.

[0034] Anesthesia gas introduced into imaging box 12 via gas delivery device 56 may be collected and exhausted to alleviate the buildup of anesthesia gases in the box. Imaging box 12 includes a sealed exhaust port 86 that allows collected anesthesia gases to be removed from within imaging box 12. Conduit 95 is coupled on its opposite ends to exhaust port 86 and vacuum port 97, which leads to a vacuum pump 88. Vacuum pump 88 applies a negative pressure through conduit 95 sufficient to draw anesthesia gases from within box 12. In one embodiment, vacuum pump 88 draws and collects anesthesia gases generally from the box 12 interior. In another embodiment, gas delivery device 56 includes a scavenging system that locally removes gases introduced by the specimen interface and not used by one or more living specimens (explained in greater detail with respect to Figs. 5A and 5B). Either way, vacuum pump 88 and conduit 95 collect unused anesthesia gases from box 12. Flowmeter 91 allows a user to determine the flow rate of gases drawn through vacuum pump 88. In another embodiment, a suitable flow control is employed to allow a user to set the pressure/flow rate of gases drawn by pump 88. Gases collected by pump 88 are then transmitted to filter 90 through an outlet port 98 of console 52. In one embodiment, filter 90 is a charcoal filter that removes unused isoflurane and outputs primarily oxygen enriched air into the ambient room.

[0035] Figs. 3A and 3B illustrate front and back views, respectively, of console 52 in accordance with one embodiment of the present invention. As shown, a housing 101 supports many of the components described with respect to Fig. 2 as well as numerous other interface mechanisms. For example, switch 102 (Fig. 3A) permits control of oxygen on/off valve 66 of Fig. 2. Exhaust pump 88 is controlled using pump on/off switch 104, which is disposed beside flowmeter 91. On/off switches and flowmeters are also illustrated for each of the gas outlets. Referring to Fig. 3B, numerous conduits 105 are shown that interconnect many of the functional components of console 52 and the inlet and outlet ports 59, 69, 71, 81, and 97. In a specific embodiment 6,4 mm (1/4 inch)OD orbital welded stainless steel tubes are used as conduits 105 and are fixed to their respective ports and components using standard industrial gas fittings. Alternately, 9,5 mm (3/8 inch) OD 60 durometer viton rubber tubing may be used for conduits with significant bending.

[0036] Having briefly discussed the functional arrangement of anesthesia delivery system 50 in accordance with one embodiment of the present invention, several gas delivery components of the system will now be described in further detail.

III. Induction Chamber

[0037] FIGs. 4B-4E illustrate induction chamber 54 of FIG. 1 in accordance with one embodiment of the present invention. Induction chamber 54 allows a user to anesthetize one or more living specimens that fit within induction chamber 54 and is also commonly referred to as a' knockdown box'. Induction chamber 54 includes an interior cavity sized to receive living specimens and a gas inlet that allows a user to supply an anesthesia gas to the interior cavity, as described in greater detail below. While induction chamber 54 will now be described as an apparatus, those skilled in the area will recognize that the present invention encompasses a method of using the apparatus based on the functional components of the induction chamber.

[0038] FIG. 4B illustrates a top prospective view of the front of induction chamber 54. FIG. 4C illustrates a top prospective view of the back of induction chamber 54. FIG. 4D illustrates a top prospective view of the underside of door 224. FIG. 4E illustrates a side cross sectional view of induction chamber 54 taken through the lateral midpoint of side walls 212a and 222d.

[0039] Referring initially to FIG. 4B, induction chamber 54 includes four vertical side walls 222a-d fixed to a bottom 223. Side walls 222a-d include front wall 222a, side wall 222b, side wall 222c, and back wall 222d. Side walls 222, bottom 223 and top wall 227 then comprise a set of walls that define an interior cavity 228 (FIG. 4E) within

induction chamber 54. As shown in FIG. 4E, interior cavity 228 is sufficiently sized to receive multiple living specimens 234. A cavity volume of about 2 to about 8 liters is suitable for many induction chambers. In one embodiment, interior cavity 228 has a cavity volume of about 3 to about 4 liters. Interior cavity 228 may include a stage 243 or platform that the living specimens rest upon.

[0040] Side walls 222, bottom 223 and top wall 227 each comprise a transparent material that allows a user to view interior chamber 228. In a specific embodiment, lexan, a transparent plastic such as polycarbonate, or a transparent acrylic, are used for the walls of induction chamber 54. Using transparent walls for induction chamber 54 advantageously allows a user to view the interior of induction chamber 54. Walls for induction chamber 54 may very in thickness from about 1/8 inch thick to about 1 inch thick, for example.

[0041] Induction chamber 54 comprises a door or user access port that allows user access to interior cavity 228. As shown in FIG. 4B, induction chamber 54 comprises a door 227 that comprises the top wall of chamber 54 and spans the entire top surface area of induction chamber 54 as defined by side walls 222a-d. Door 227 is hingeably coupled to back wall 222d using a pin 244 (FIG. 4C) that passes through a channel 241 included in the hinges 225 (FIG. 4B) and a channel 242 included in door 227 (FIG. 4D). Hinges 225 are screwed to the outside of back wall 222d (FIG. 4C).

[0042] Door 227 is movable between a closed condition and various opened conditions. FIG. 4B illustrates the closed condition for door 227 and induction chamber 54. In the closed condition as shown, door 227 rests upon the top portion of walls 222 and seals interior cavity 228 from the environment exterior to induction chamber 54. In one opened condition, door 227 enables user access into interior chamber to insert or remove living specimens. In the opened conditions, door 227 enables gaseous communication, or gaseous flow, between interior cavity 228 and the environment exterior to induction chamber 54 through an opening. The size and profile of the opening will depend on the angle door 227 makes relative to its closed position as shown. Thus, when door 227 is initially opened from the position shown in FIG. 4B, a crack between the top portion of each side wall 222 in door 227 creates an opening that allows anesthesia gas and oxygen to flow between interior chamber 228 and the environment external to induction chamber 54.

[0043] Seal 245 is disposed in a recess that runs perimetrically about a top portion of each side wall 222 (FIG. 4C). As shown in FIG. 2C, seal 245 runs 360 degrees about the top opening of interior cavity 228. Seal 245 is a compressible material that prevents gaseous communication between interior cavity 228 and the environment exterior to induction chamber 54 when door 227 is in the closed condition. More specifically, when door 227 is in the closed position, the bottom surface of door 227 cooperates with seal 245 to prevent gaseous communication between interior cavity 228 and the environment external to induction chamber 54. In a specific embodiment, seal 245 comprises a rubber or silicone.

[0044] Clamp 247 is attached side wall 222a using screws 251. Clamp 247 allows a user to secure door 227 in the closed position. In addition, clamp 247 is vertically disposed such that securing clamp 247 provides a compressive force between the bottom surface of door 227 and seal 245.

[0045] Induction chamber receives low-pressure oxygen and anesthesia gas from console 52 and conduit 60. Induction chamber 54 thus includes at least one gas inlet capable of providing anesthesia gas to the interior cavity 228. As shown, induction chamber 54 includes a single gas inlet 230 that supplies both anesthesia gas and oxygen from the exterior of induction chamber 54 to the interior cavity 228. Gas inlet 230 is disposed in a lower portion of back wall 222d and includes a circular port or hole through back wall 222d that allows gaseous communication between the inside and outside of induction chamber 54. As shown, gas inlet 230 includes an exterior interface 223 (FIG. 4C) that receives conduit 60 (FIG. 1). Referring back to FIG. 1, console 52 provides a mixture of oxygen and anesthesia gas to induction chamber 54. This mixture is passed into induction chamber 54 through gas inlet 230. Console 52 includes controls that allow a user to turn on/off or vary the flowrate of oxygen and anesthesia gas provided to induction chamber 54. It is understood that induction chamber 54 may include multiple gas inlets. For example, one inlet may be dedicated to anesthesia gas was a second gas inlet is dedicated to oxygen supply.

[0046] The present invention employs an anesthesia gas to sedate living specimens. As the term is used herein, an anesthesia gas refers to any gas or agent that is used to induce any level of anesthetic state, unconsciousness, lack of awareness, or local or general insensibility to pain for a specimen interacting with induction chamber 54. The amount of anesthesia gas is typically determined by the control console 52 of FIG. 1. A vaporizer included in console 52 may be used to produce the anesthesia gas and add it to low-pressure oxygen. The output of the vaporizer typically comprises a controlled and variable gas mixture of life sustaining gases and anesthetizing gases. In a specific embodiment, isoflurane is added to low pressure oxygen by passing oxygen across a vaporizer that evaporates isoflurane. In this case, the low-pressure oxygen acts as a carrier for the isoflurane, which is added to the oxygen according to the physical characteristics of isoflurane liquid and its temperature. Although the present invention is primarily described with respect to using only a single anesthesia gas, isoflurane, it is understood that an anesthesia gas of the present invention may include multiple anesthesia gases, as one of skill in the art will appreciate.

[0047] Induction chamber 54 also comprises a gas outlet 250 capable of drawing anesthesia gas from interior cavity 228 when door 227 is in an opened condition. Gas outlet 250 is disposed in an upper portion of back wall

222d and includes a circular port or hole through back wall 222d that allows gaseous communication between the inside and outside of induction chamber 54. As shown, gas outlet 250 includes an exterior interface 252 (FIG. 4C) that receives conduit 79, which exhausts gases from induction chamber 54 to filter 81 (FIG. 1).

[0048] Gas outlet 250 actively draws and collects anesthesia gas from interior cavity 228, and actively draws and collects anesthesia gas from the environment external to induction chamber 54 when door 227 is in an opened condition. To do so, gas outlet 250 is in gaseous communication with a negative pressure supply such as vacuum pump 83 (FIG. 1). Vacuum pump 83 provides a negative pressure to gas outlet 250 that draws gases into outlet 250. In one embodiment, the negative pressure is negative relative to the pressure within interior chamber 228. In another embodiment, the native pressure is negative relative to the environment exterior to induction chamber 54. This creates a draft through any opening created by door 227 that causes air and anesthesia gas to be drawn from the external environment, to pass in through interior cavity 228, and into gas outlet 250. Thus, with suitable pressure from pump 83, anesthesia gases about to escape interior cavity 228 when door 227 opens may be drawn and collected by gas outlet 250 before they escape. In addition, anesthesia gases that have escaped interior cavity 228 may be drawn and collected by gas outlet 250 when door 227 is opened.

[0049] The flowrate of anesthesia gas and other gases through gas outlet 250 may vary. In one embodiment, gas outlet 250 draws gases from interior cavity 228 at a flowrate through gas outlet 250 greater than the volume of interior cavity 228 per minute. In a specific embodiment, gas outlet 250 draws gases from interior cavity 228 at a flowrate from about 0L/min to about 8 L/min.

[0050] Induction chamber 54 also comprises a gas outlet obstruction 260 that varies flow of anesthesia gas from interior cavity 228 through gas outlet 250 based on the position of door 227. Typically, gas outlet obstruction 260 varies flow of anesthesia gas from interior cavity 228 by obstructing gas outlet 250 in some manner. Thus, gas outlet obstruction 260 may plug, cap, cork, block, prevents, or otherwise impair gas flow through gas outlet 250.

[0051] As shown in FIG. 4E, gas outlet obstruction 260 comprises a bracket 262 having a portion 262a that rests flat against the bottom side of door 227 and is attached to door 227 using screws 264. Bracket 262 also has a portion 262b that is positioned proximate to gas outlet 250 when door 227 is in the closed position. Attached to portion 262b is screw 266, washer 268, compressible material 270 and spacer 272. Screw 266 fastens washer 268, compressible material 270 and spacer 272 to portion 262b. Compressible material 270 interfaces with gas outlet 250 to seal the outlet 250 when door 227 is in the closed position. Thus, portion 262b is proximate to gas outlet 250 such that compressible material 270 seals the outlet 250 when door 227 is in the closed position. Together, portion 262b and perimeter material of gas outlet 250 combine to compress the compressible material 270 when door 227 is in the closed condition. FIG. 4E illustrates gas outlet obstruction 260 when door 227 is in the closed position. Here, gas outlet obstruction 260 restricts flow of anesthesia gas from interior cavity 228 through gas outlet 250. Washer 268 allows screw 266 to tighten without compressing a localized portion of compressible material 270. Spacer 272 allows a user to change the thickness of compressible material 270 or vary the force applied between portion 262b and the perimeter material of gas outlet 250 on compressible material 270.

[0052] In a simplified embodiment, gas outlet obstruction 260 simply comprises a compressible material that spans the entire back side of portion 262b of bracket 262. Similar to the previous case, the this simplified embodiment seals gas outlet 250 when door 227 is in the closed condition. In addition, the gas outlet obstruction 260 allows gas to flow from interior cavity 228 through gas outlet 250 when door 227 is in an opened condition.

[0053] In one embodiment, gas outlet 250 is disposed in the top half of a side wall 222. In this case, anesthesia gas is introduced near the bottom of induction chamber 54 and collects in interior cavity 228 when door 227 is closed. When door 227 opens, gas outlet 250 draws anesthesia gas from the top portion of interior cavity 228. As long as the flowrate through gas outlet 250 is not excessive, this may result in a temporary two layer gaseous formation within interior cavity 228. The top layer comprises gases that move toward gas outlet 250. The bottom layer comprises anesthesia gas and oxygen supplied by gas inlet 223. An advantage of this design is that even with door 227 opened for short periods of time, living specimens is disposed near the bottom of induction chamber 54 may not be entirely devoid of anesthesia gas.

[0054] In one embodiment, passive gas outlet 232 is disposed in back wall 222c. Passive gas outlet 232 passively exhausts anesthesia gas from interior cavity 228 based on positive pressure in interior cavity 228 relative to conduit 77. Typically, this occurs when door 227 is in the closed condition and gas outlet 250 is obstructed. In this case, continual anesthesia gas and oxygen supply into interior cavity 228 builds pressure within the interior cavity and causes passive gas flow through gas outlet 232. Gas outlet 232 is disposed in a lower portion of back wall 222d and includes a circular port or hole through back wall 222d that allows gaseous communication between the inside and outside of induction chamber 54. As shown, passive gas outlet 232 includes an exterior interface 229 (FIG. 4C) that receives conduit 77, which exhausts gases from induction chamber 54 to filter 78 (FIG. 1).

[0055] FIG. 6 illustrates a process flow 200 for using induction chamber 54 of FIG. 1 in accordance with one embodiment of the present invention. Processes in accordance with the present invention may include up to several additional steps not described or illustrated herein in order not to obscure the present invention.

[0056] In operation, a user opens door 227 (FIG. 1) and places a living specimen within the interior of induction chamber 54. Closing door 227 seals the interior of induction chamber 54 from the ambient room via the interface of seal 245 and door 227. In addition, a gas outlet obstruction obstructs gas flow through gas outlet 250 when door 227 is in the closed condition (206). In one embodiment, the gas outlet obstruction is attached to the door 227 and fully seals gas outlet 250 when door 227 is in the closed condition and fully prevents gas from flowing through gas outlet 250.

[0057] After induction chamber 54 is sealed, oxygen and anesthesia gas are supplied to the interior via console 52 and gas inlet 230 (202). As pressure in the interior cavity builds, exhaust gases may be passively removed from induction chamber 54 via a passive exhaust included in induction chamber 54. Gas flows into the passive exhaust based on a positive pressure accumulating inside the chamber relative to the passive exhaust. After anesthesia delivery to the living specimen is complete, e.g. when the specimen has been anesthetized, door 227 may be opened to allow a lab technician to remove one or more specimens.

[0058] Opening door 227 ceases obstruction of gas outlet 250. This causes anesthesia gas to be drawn through gas outlet 250 from the interior chamber when door 227 is in the opened condition (204). In one embodiment, the gas outlet draws gases from the interior chamber at a flow rate greater than the volume of the interior chamber per minute. This is done with a negative pressure that is applied to the gas outlet, e.g., via a pump.

[0059] When used in the imaging system 10 of FIG. 1, process flow 200 may also include various additional actions related to imaging the specimen after it has been sedated. For example, a user may remove a living specimen from the interior cavity when the door is in the opened condition, place the living specimen in the imaging box, and image the living specimen, or a portion thereof, using the imaging system.

[0060] In early tests, induction chamber 54 produced improved results for its intended purpose when gas outlet 250 and obstruction 260 were used. In many cases, induction chamber 54 reduced the amount of anesthesia gas introduced into the ambient surroundings by a factor of about 4-5 relative to an induction chamber without an active gas outlet and gas outlet obstruction. In addition, opening door 227 did not result in frequent living specimen arousal - for both specimens being removed and resting temporarily while another specimen was being removed.

[0061] Fig. 4A illustrates induction chamber 54 in accordance with another embodiment of the present invention. Induction chamber 54 allows a user to anesthetize a living specimen that fits within induction chamber 54. As shown, induction chamber 54 includes four vertical walls 122a-d fixed to a bottom 123. Lid 124 is hingeably coupled to back wall 122c using hinges 125 that are fixed to the outer sides of wall 122c and lid 124. Walls 122, bottom 123 and lid 124 define an interior of induction chamber 54. Lid 124 is movable between an opened condition that defines an opening into chamber 54 and enables access to the interior, and a closed condition that prevents access to the interior. Seal 127 is disposed along the inner surfaces of walls 122 and mates with lid 124, when lid 124 is in the closed position, to provide a seal that prevents gaseous communication between the interior of induction chamber 54 and the environment exterior to induction chamber 54. In a specific embodiment, seal 127 comprises a rubber or silicone seal and walls 122, bottom 123, and lid 124 or all made from a transparent plastic such polycarbonate. Using transparent walls for induction chamber 54 advantageously allows a user to view the interior of induction chamber 54.

[0062] Inlet port 130 is disposed in back wall 122c and allows gaseous communication between the inside and outside of induction chamber 54. As shown, inlet port 130 receives conduit 60, which provides low-pressure oxygen and anesthesia gas from port 71 of console 52.

[0063] In one embodiment, outlet port 132 is disposed in back wall 122c and allows gaseous communication between the inside and outside of induction chamber 54. As shown, outlet port is coupled to conduit 77, which exhausts gases from induction chamber 54 to filter 78 (Fig. 2). As shown, anesthesia gases are exhausted from induction chamber 54 based on positive pressure build up in the interior and relief using conduit 77. In an active removal embodiment, conduit 77 may be associated with negative pressure via a vacuum pump, such as vacuum pump 88 of Fig. 2, to actively draw gases from induction chamber 54.

[0064] In another embodiment, induction chamber 54 employs a scavenging system to collect and exhaust anesthesia gases. After anesthetizing a living specimen within induction chamber 54, a user typically opens lid 124 to move the specimen into imaging box 12. At this point, gases from within the chamber 54 interior - including anesthesia gases - may escape from induction chamber 54. Often, the anesthesia gas is denser than air and spills over the side walls 122. To facilitate the capture of anesthesia gas that escapes from induction chamber 54, the scavenging system employed by induction chamber 54 includes holes 138 disposed in a skirt 140. The top of skirt 140 is attached to bottom 123. Holes 138 are peripherally disposed about walls 122 and associated with channels (not shown) within skirt 140 that collectively lead to an outlet port, or similar interface, that couples to a conduit and vacuum pump 88. Vacuum pump 88 provides a suitable negative pressure that draws gases into holes 138 and creates a downdraft about the outside of induction chamber 54. Thus, anesthesia gases that escape the induction chamber 54 interior may be collected by holes 138, pass through their associated channels, and flow to the vacuum pump for subsequent filtering. In one embodiment, skirt 140 comprises from about 10 to about 50 holes, each having a diameter of about .061 to about 2.54 mm. In a specific embodiment, skirt 140 com-

prises 17 holes, each having a diameter of about 1.86 mm.

[0065] Inlet port 134 is also disposed in back wall 122c and allows gaseous communication between the inside and outside of induction chamber 54. Inlet port 134 is coupled to conduit 65, which provides working pressure oxygen communication with purge valve 120. Referring back to Fig. 2, purge valve 120 allows a user to flood induction chamber 54 with working pressure oxygen from the regulated supply pressure oxygen source. Flow restricter 122 reduces the flow rate of oxygen provided by the external oxygen supply before entering induction chamber 54. Conduit 65 is coupled to oxygen induction chamber outlet 69 of console 52 and an oxygen inlet port 134 of induction chamber 54.

[0066] In operation, a user opens lid 124 and places a living specimen within the interior of induction chamber 54. After closing lid 124, and sealing the interior of induction chamber 54 from the ambient room, oxygen and anesthesia gas are supplied to the interior via console 52 and inlet port 130. During this time, exhaust gases may be actively removed from induction chamber 54 using a pump or one of the exhaust techniques described above. After anesthesia delivery to the living specimen is complete, e.g. when the specimen has been put to sleep, lid 124 is opened and the specimen removed. Scavenging exhaust 136 may collect and exhaust anesthesia gases that escapes induction chamber 54.

IV. Specimen Interface

[0067] The present invention also relates to a gas delivery device that is capable of delivering an anesthesia gas and oxygen to multiple living specimens. Fig. 5A illustrates a top perspective view of gas delivery device 56 of Fig. 1 in accordance with one embodiment of the present invention. Gas delivery device 56 is particularly useful for operation in imaging box 12 to maintain the anesthetic state of a specimen induced by induction chamber 54. Gas delivery device 56 comprises a front face 157 having many features suitable for gas interchange with multiple living specimens. In a specific embodiment, gas delivery device 56 is a solid structure machined from one or more pieces of black anodized aluminum.

[0068] Gas delivery device 56 comprises an inlet port 152 on one end for coupling to a conduit that delivers an anesthesia gas and oxygen, such as conduit 62 from outlet port 81 of console 52. Five specimen interfaces 156a-e are horizontally disposed along a front face 157 of gas delivery device 56. Inlet port 152 opens into a channel 154 (Fig. 5B) that substantially spans the length of device 56. Fig. 5B illustrates a top cutaway view of gas delivery device 56 showing channel 154 taken through the vertical midsection of each specimen interfaces 156. Channel 154 communicates anesthesia gases and oxygen from inlet port 152 to each specimen interface 156.

[0069] Channel 154 is larger in cross-sectional area than inlet port 152 includes a buffer volume sufficiently large to substantially reduce flow rate inconsistencies and fluctuations to and from the specimen interfaces 156. In this manner, a large volume of gas accumulated within channel 154 may act as a buffer to facilitate substantially constant flow of anesthesia gas and oxygen from interfaces 156 despite fluctuations in the delivery of anesthesia gas and oxygen. In a specific embodiment, channel 154 is circular in cross-section and has a diameter in the range of about 15 to about .75 inches and a length spanning device 56 save the outside walls. In a specific embodiment, channel 154 is circular in cross-section and has a diameter of about 313 inches.

[0070] In one embodiment, a disposable sleeve is inserted each specimen interface 156. The disposable sleeve has a smaller orifice at channel 154 and a larger orifice distal from channel 154 at front face 157. In a specific embodiment, the disposable sleeve includes a substantially frustoconical shape that increases in diameter as it extends away from channel 154. In operation, the head of a specimen is disposed in, or proximate to, the disposable sleeve. When the disposable sleeve is used with a sleeping mouse for example, the head of the mouse may rest on the disposable sleeve. Since the cross-sectional area that the anesthesia gas encounters increases as it travels through the disposable sleeve, the flow rate of the gas decreases as it approaches the specimen. As a result, the anesthesia gas may have a more laminar and less turbulent flow from each specimen interface 156.

[0071] Gas delivery device 56 also comprises a scavenger system capable of drawing and collecting waste anesthesia gases. Since the amount of anesthesia gas supplied usually exceeds the amount necessary for the specimen, scavenging according to the present invention decreases box 12 pollution - and pollution in the surrounding room after the door to box 12 is opened.

[0072] As shown, the scavenger exhaust comprises an array of holes 160 perimetrically located about each of the specimen interfaces 156. Holes 160 locally draw and collect anesthesia gas when a suitable negative pressure is applied thereto. An exhaust port 161 acts as an exterior outlet from gas delivery device 56 and allows external attachment to a conduit. A longitudinal channel (not shown) allows gaseous communication between exhaust port 161 and each of the holes 160. The longitudinal channel runs the face 157 length of gas delivery device 56 from exhaust port 161 to the opposite end. Internal channels (not shown) extend within gas delivery device 56 between each of the holes 160 and the longitudinal channel. In one embodiment, a conduit connected to exhaust port 161 actively draws gases through holes 160, through their associated internal channels, through the longitudinal channel, and through exhaust port 161 using a negative pressure, e.g. via a pump such as pump 88. Holes 160 are particularly useful for drawing in anesthesia gas output towards a specimen by the specimen interfaces 156. In one embodiment, oxygen and anesthe-

sia gases flow from each specimen interface 156 is supplied in a laminar and substantially non-turbulent manner. Holes 161 may then locally draw anesthesia gas in a minimally turbulent manner; thereby minimizing gas escape into imaging box 12.

[0073] In another embodiment, holes 162 are also disposed in the upper surface of gas delivery device 56 to capture anesthesia gas not locally captured by holes 161 and to capture anesthesia gas above gas delivery device 56. Internal channels (also not shown) extend from holes 162 downward towards the longitudinal channel and deliver collected gases to exhaust port 161.

[0074] When imaging is performed on multiple living specimens using gas delivery device 56, it may be desirable to prevent light emitted by one specimen from reaching an adjacent specimen. To this end, gas delivery interface also includes a vertical slot 164 disposed between each of the adjacent specimen interfaces 156. Each vertical slot 164 is capable of receiving and vertically holding a light barrier, such as a paper or otherwise suitable opaque barrier. In a specific embodiment, each vertical slot 164 is between 0.76 and 1.0 mm (.03 and .04 inches) thick and penetrates 6,4 mm (¼ inch) into face 157.

[0075] While this invention has been described in terms of several preferred embodiments, there are alterations, permutations, and equivalents which fall within the scope of this invention which have been omitted for brevity's sake. For example, although gas delivery device 56 is described as a solid structure machined from aluminum, it is understood that other designs may the structure of the gas delivery device and include tube and bellows systems that equally deliver anesthesia gas to multiple living specimens. It is therefore intended that the scope of the invention should be determined with reference to the appended claims.

Claims

1. A gas delivery system (50) capable of delivering an anesthesia gas to a plurality of gas outlets, the system comprising:

   an oxygen inlet (59) that receives oxygen from an oxygen source;
   a pressure regulator (68) having an inlet that receives oxygen from the oxygen inlet and having an outlet that provides oxygen at a lower pressure;
   an anesthesia gas source (70) having an inlet coupled to receive low pressure oxygen from the outlet of the pressure regulator and capable of adding anesthesia gas to the low pressure oxygen to produce a combination of anesthesia gas and low pressure oxygen;
   a first gas delivery outlet (81) coupled to a gas delivery device (56) having one or more living specimen interfaces and capable of providing the combination of anesthesia gas and oxygen to the one or more living specimen interfaces; and
   a second gas delivery outlet (71) coupled to an induction chamber (54) and capable of providing the combination of anesthesia gas and oxygen to the induction chamber.

2. The gas delivery system of claim 1 wherein the first gas delivery outlet comprises an outlet port and a first flow control (85) disposed between the anesthesia gas source and the outlet port.

3. The gas delivery system of claims 2 or 3 wherein the second gas delivery outlet comprises an outlet port and a second flow control (75) disposed between the anesthesia gas source and the outlet port.

4. The gas delivery system of claim 3 wherein the first (85) and second (75) flow control each allow independent control of gases to the first gas delivery outlet (81) and to the second gas delivery outlet (71).

5. The gas delivery system of claim 4 wherein the first flow control allows control of gases from about 0 L/min to about 5 L/min to the first gas delivery outlet.

6. The gas delivery system of claims 1-5 wherein the gas delivery device (56) further comprises an inlet (27) for receiving the combination of anesthesia gas and oxygen and at least one channel for communicating anesthesia gas and oxygen between the inlet and the one or more specimen interfaces.

7. The gas delivery system of claim 6 wherein the at least one channel comprises a buffer volume sufficiently large to substantially reduce flow rate fluctuations from the multiple specimen interfaces.

8. The gas delivery system of claim 6 wherein the gas delivery device (56) is configured to rest in an imaging box (12) included in an imaging system (10), the imaging box having a set of walls enclosing an interior cavity and a camera mount (16) configured to position a (20) camera relative the interior cavity.

Patentansprüche

1. Gasbeförderungssystem (50), welches zum Befördern eines Anästhesiegases zu mehreren Gasauslässen geeignet ist, wobei das System umfasst:

   einen Sauerstoffeinlass (59), welcher Sauerstoff von einer Sauerstoffquelle empfängt;
   einen Druckregler (68) mit einem Einlass, welcher Sauerstoff von dem Sauerstoffeinlass

empfängt, und mit einem Auslass, welcher Sauerstoff bei einem niedrigen Druck bereitstellt;
eine Anästhesiegasquelle (70) mit einem Einlass, welcher gekoppelt ist, um Sauerstoff mit niedrigem Druck von dem Auslass des Druckreglers zu empfangen, welche geeignet ist, Anästhesiegas zu dem Sauerstoff mit niedrigem Druck hinzuzufügen, um eine Kombination aus Anästhesiegas und Sauerstoff mit niedrigem Druck herzustellen;
einen ersten Gasbeförderungsauslass (81), welcher mit einer Gasbeförderungsvorrichtung (56) gekoppelt ist, die ein oder mehrere Schnittstellen zu lebenden Proben aufweist, und geeignet ist, die Kombination von Anästhesiegas und Sauerstoff zu den ein oder mehreren Schnittstellen zu lebenden Proben bereitzustellen; und
einen zweiten Gasbeförderungsauslass (71), welcher mit einer Induktionskammer (54) gekoppelt ist, und geeignet ist, die Kombination von Anästhesiegas und Sauerstoff für die Induktionskammer bereitzustellen.

2. Gasbeförderungssystem nach Anspruch 1, wobei der erste Gasbeförderungsauslass einen Auslassanschluss und eine erste Flusssteuerung (85), welche zwischen der Anästhesiegasquelle und dem Auslassanschluss angeordnet ist, umfasst.

3. Gasbeförderungssystem nach Anspruch 2, wobei der zweite Gasbeförderungsauslass einen Auslassanschluss und eine zweite Flussteuerung (75), welche zwischen der Anästhesiegasquelle und dem Auslassanschluss angeordnet ist, umfasst.

4. Gasbeförderungssystem nach Anspruch 3, wobei die erste (85) und zweite (75) Flusssteuerung jeweils eine unabhängige Steuerung des Gases zu dem ersten Gasbeförderungsauslass (81) und zu dem zweiten Gasbeförderungsauslass (71) ermöglicht.

5. Gasbeförderungssystem nach Anspruch 4, wobei die erste Flusssteuerung eine Steuerung von Gasen von näherungsweise 0 l/min bis näherungsweise 5 l/min zu dem ersten Gasbeförderungsauslass ermöglicht.

6. Gasbeförderungssystem nach Anspruch 1-5, wobei die Gasbeförderungsvorrichtung (56) ferner einen Einlass (27) zum Aufnehmen der Kombination von Anästhesiegas und Sauerstoff und mindestens einen Kanal zum Übertragen von Anästhesiegas und Sauerstoff zwischen dem Einlass und der einen oder mehreren Schnittstellen zu Proben umfasst.

7. Gasbeförderungssystem nach Anspruch 6, wobei der mindestens eine Kanal ein Puffervolumen umfasst, welches ausreichend groß ist, um Strömungsgeschwindigkeitsschwankungen von den mehreren Schnittstellen zu Proben wesentlich zu reduzieren.

8. Gasbeförderungssystem nach Anspruch 6, wobei die Gasbeförderungsvorrichtung (56) ausgestaltet ist, in einem bildgebenden Kasten (12), welcher in einem bildgebenden System (10) enthalten ist, zu liegen, wobei der bildgebende Kasten einen Satz von Wänden, welche einen inneren Hohlraum umschließen, und eine Kamerabefestigung (16), welche ausgestaltet ist, eine Kamera (20) relativ zu dem inneren Hohlraum zu positionieren, aufweist.

Revendications

1. Système d'administration de gaz (50) capable d'administrer un gaz anesthésiant à une pluralité de sorties de gaz, le système comprenant :

une entrée d'oxygène (59) qui reçoit l'oxygène d'une source d'oxygène ;
un régulateur de pression (68) ayant une entrée qui reçoit l'oxygène de l'entrée d'oxygène et ayant une sortie qui fournit l'oxygène à une pression plus basse ;
une source de gaz anesthésiant (70) ayant une entrée couplée pour recevoir l'oxygène à basse pression provenant de la sortie du régulateur de pression et capable d'ajouter du gaz anesthésiant à l'oxygène à basse pression afin de produire une combinaison de gaz anesthésiant et d'oxygène à basse pression ;
une première sortie d'administration de gaz (81) couplée à un dispositif d'administration de gaz (56) ayant une ou plusieurs interfaces de spécimen vivant et capable de fournir la combinaison de gaz anesthésiant et d'oxygène à une ou plusieurs interfaces de spécimen vivant ; et
une seconde sortie d'administration de gaz (71) couplée à une chambre d'induction (54) et capable de fournir la combinaison de gaz anesthésiant et d'oxygène à la chambre d'induction.

2. Système d'administration de gaz selon la revendication 1, dans lequel la première sortie d'administration de gaz comprend un orifice de sortie et une première commande d'écoulement (85) disposée entre la source de gaz anesthésiant et l'orifice de sortie.

3. Système d'administration de gaz selon les revendications 2 ou 3, dans lequel la seconde sortie d'administration de gaz comprend un orifice de sortie et une seconde commande d'écoulement (75) disposée entre la source de gaz anesthésiant et l'orifice de sortie.

4. Système d'administration de gaz selon la revendication 3, dans lequel les première (85) et seconde (75) commandes d'écoulement permettent chacune la commande indépendante des gaz vers la première sortie d'administration de gaz (81) et vers la seconde sortie d'administration de gaz (71).

5. Système d'administration de gaz selon la revendication 4, dans lequel la première commande d'écoulement permet la commande des gaz d'environ O L/min jusqu'à environ 5 L/min jusqu'à la première sortie d'administration de gaz.

6. Système d'administration de gaz selon les revendications 1 à 5, dans lequel le dispositif d'administration de gaz (56) comprend en outre une entrée (27) pour recevoir la combinaison de gaz anesthésiant et d'oxygène et au moins un canal pour faire communiquer le gaz anesthésiant et l'oxygène entre l'entrée et les une ou plusieurs interfaces de spécimen.

7. Système d'administration de gaz selon la revendication 6, dans lequel le au moins un canal comprend un volume tampon suffisamment important pour réduire sensiblement les fluctuations de débit provenant des multiples interfaces de spécimen.

8. Système d'administration de gaz selon la revendication 6, dans lequel le dispositif d'administration de gaz (56) est configuré pour reposer dans une boîte d'imagerie (12) comprise dans un système d'imagerie (10), la boîte d'imagerie ayant un ensemble de parois enfermant une cavité intérieure et un pied de caméra (16) configuré pour positionner une caméra (20) par rapport à la cavité intérieure.

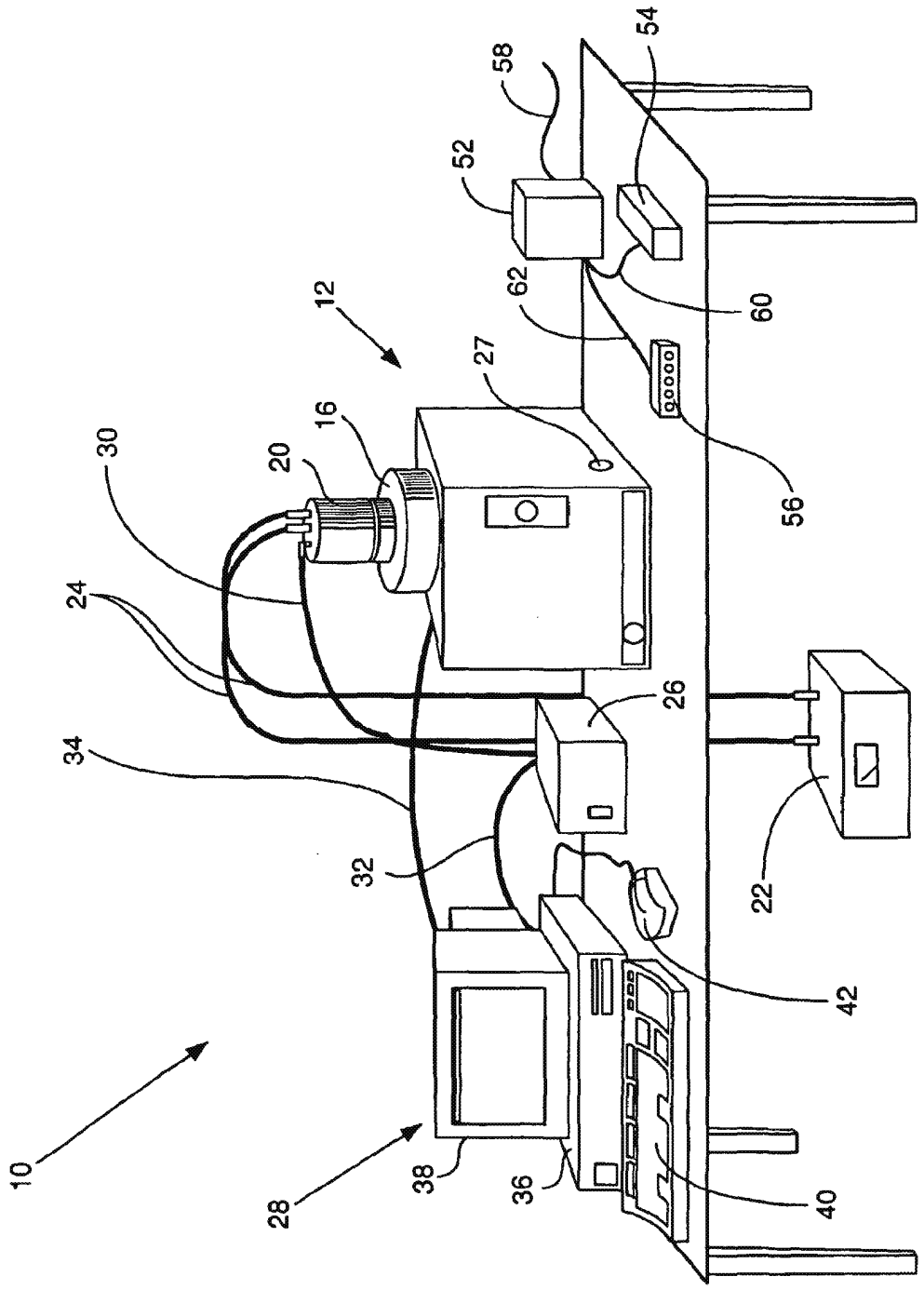


FIG. 1

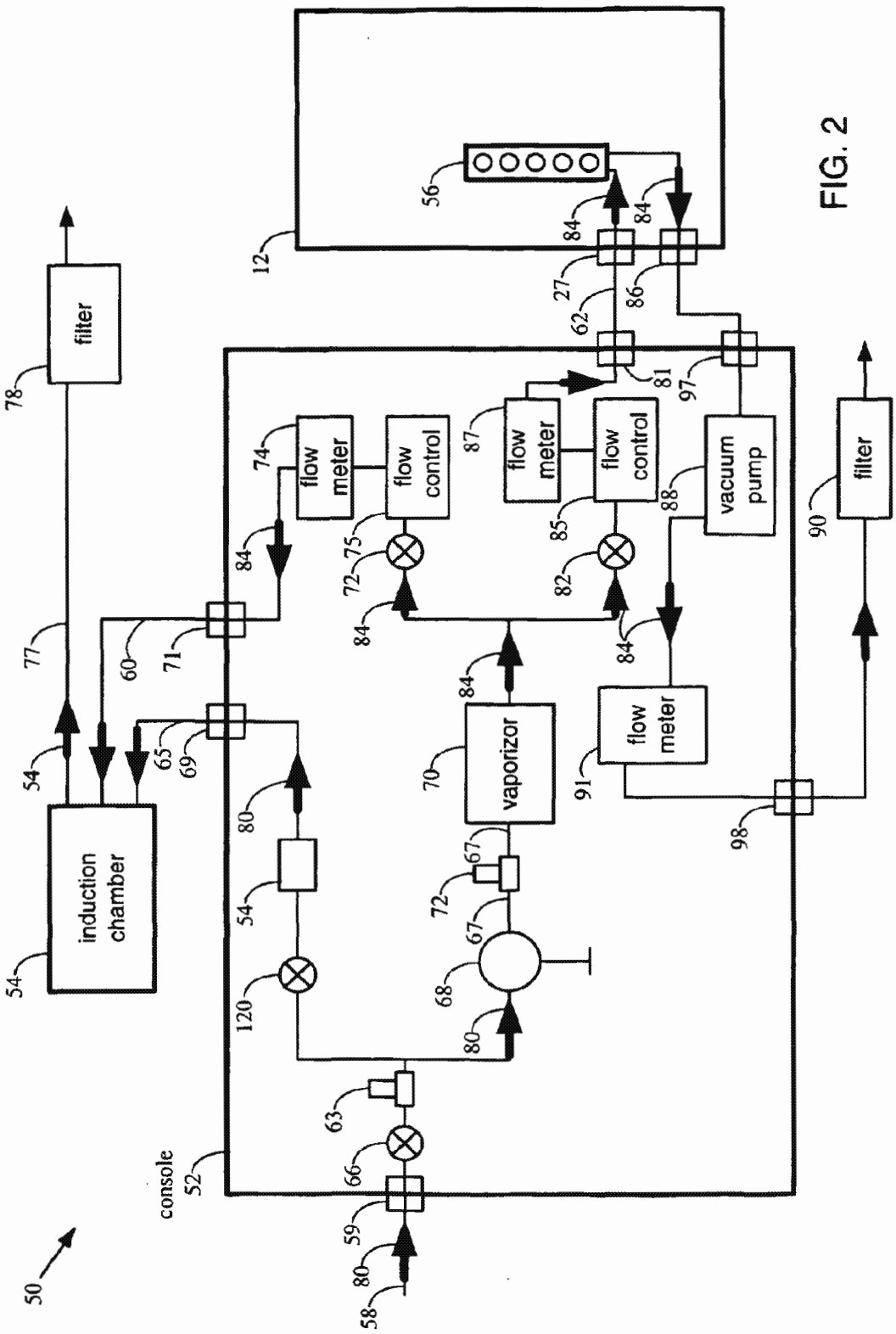


FIG. 2

SPECTRAL_0000250

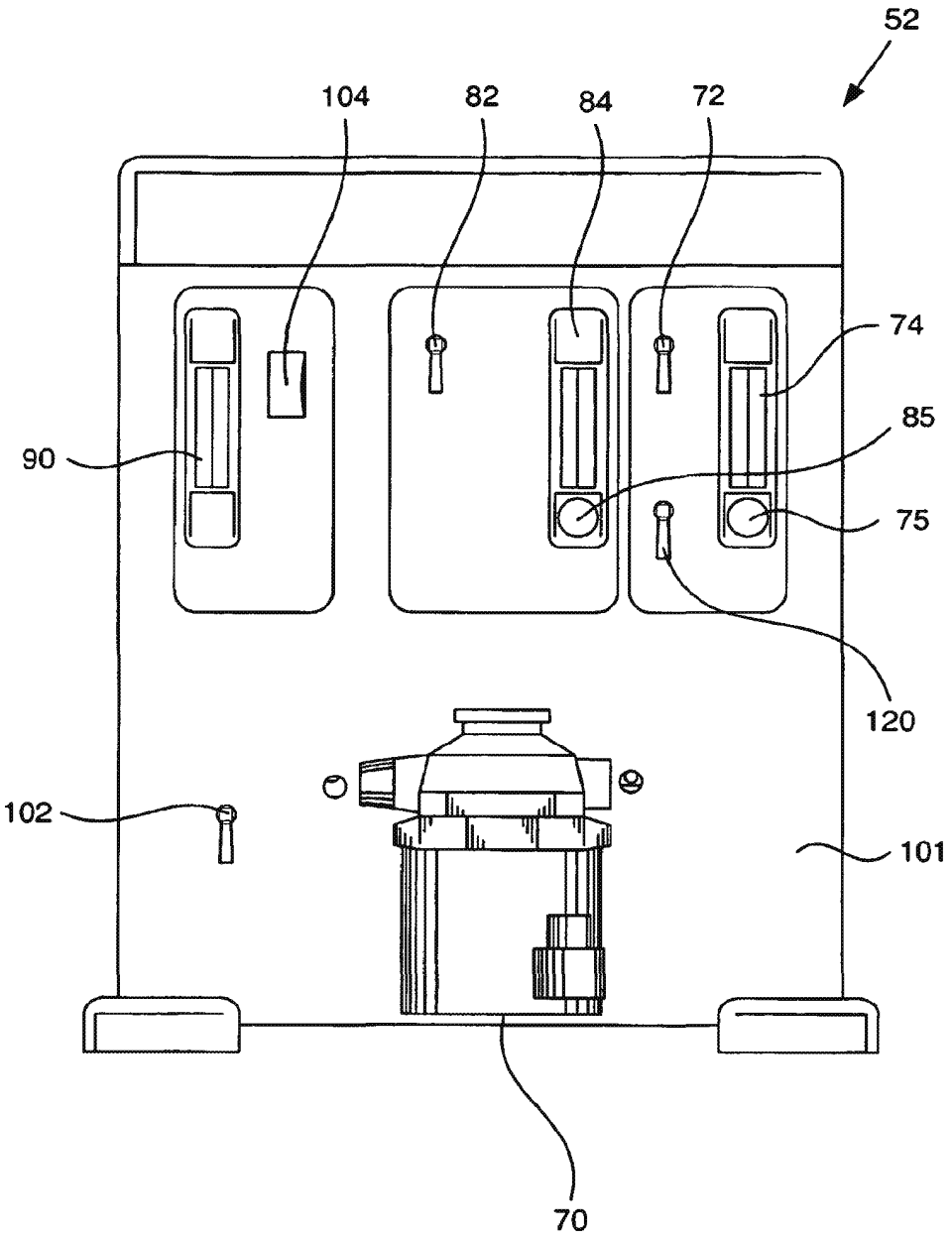


FIG. 3A

SPECTRAL_0000251

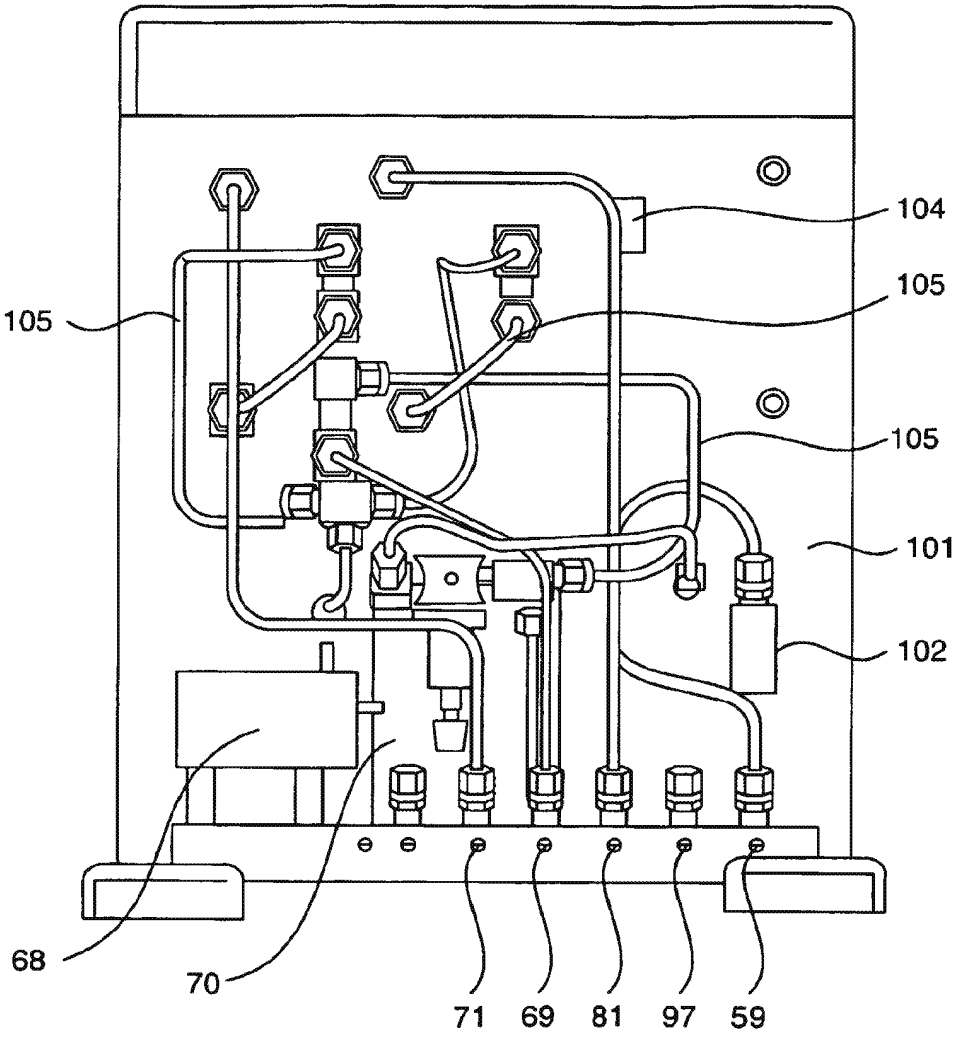


FIG. 3B

SPECTRAL_0000252

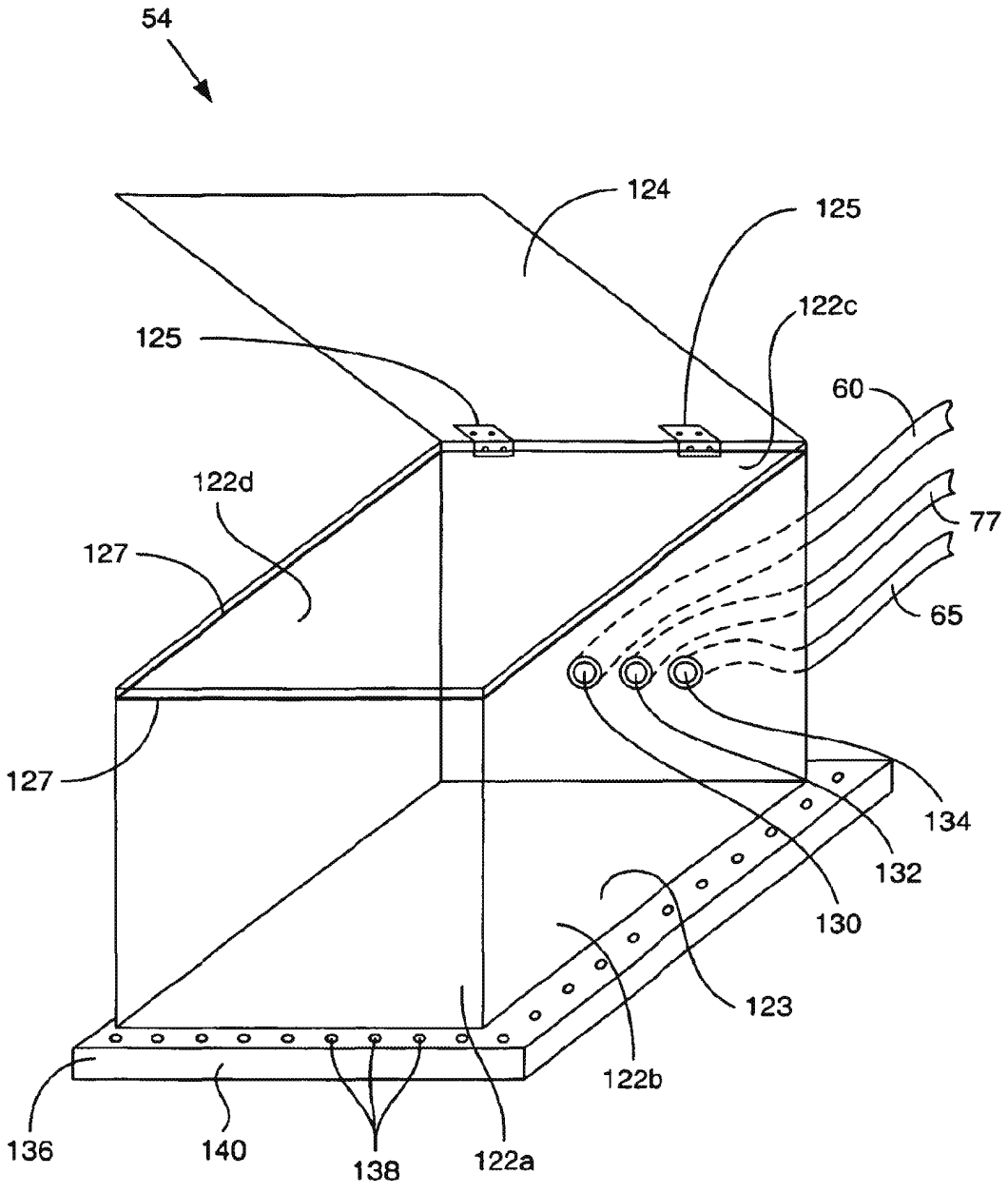

54
124
125
125
122c
60
122d
77
127
65
127
134
132
130
123
122b
136
140
138
122a

FIG. 4A

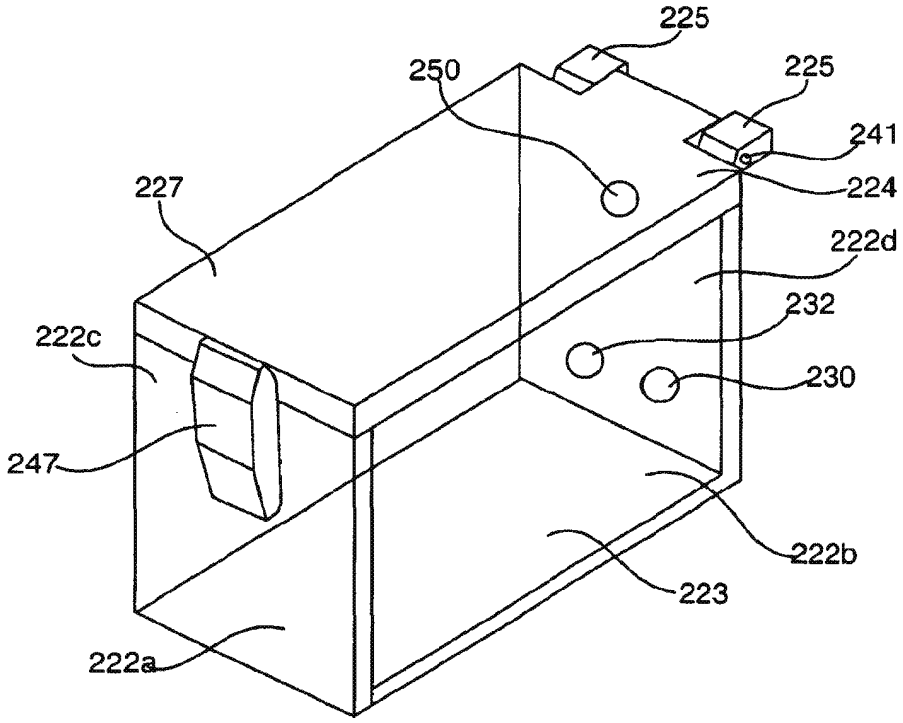


FIG. 4B

SPECTRAL_0000254

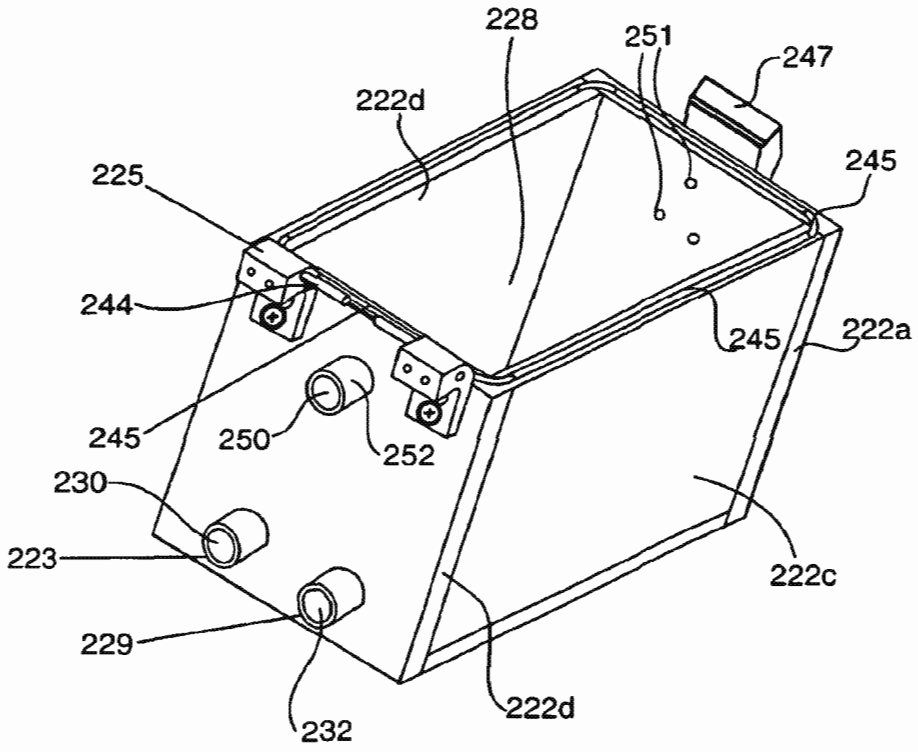

228
251
247
222d
225
245
244
245
222a
245
250
252
230
223
222c
229
222d
232

FIG. 4C

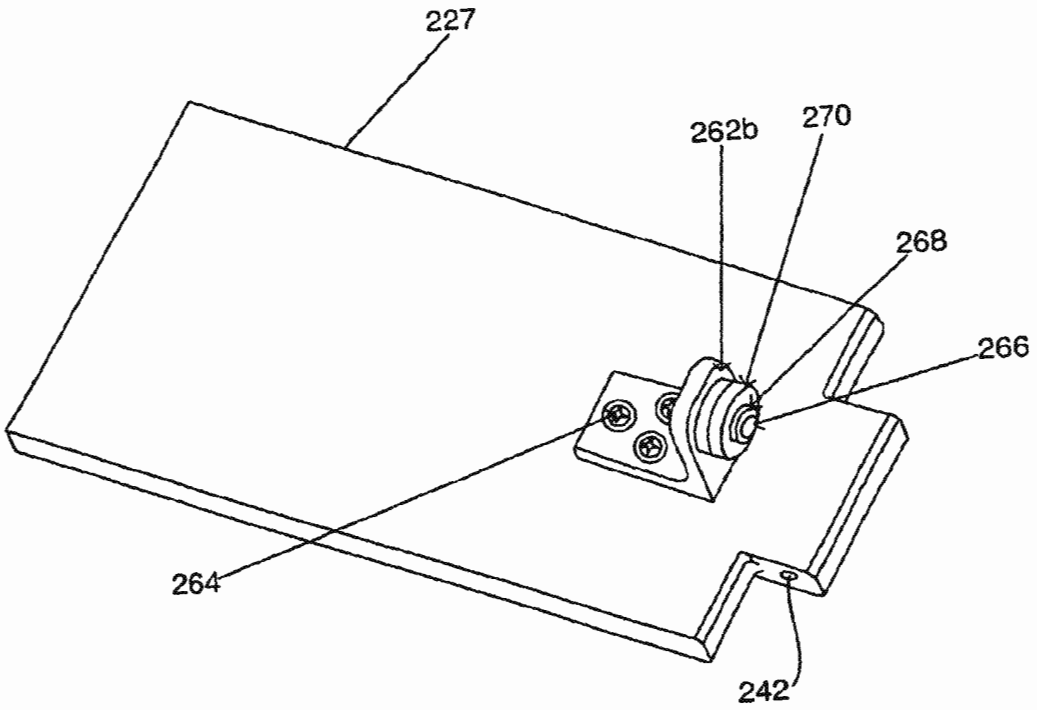


FIG. 4D

SPECTRAL_000025

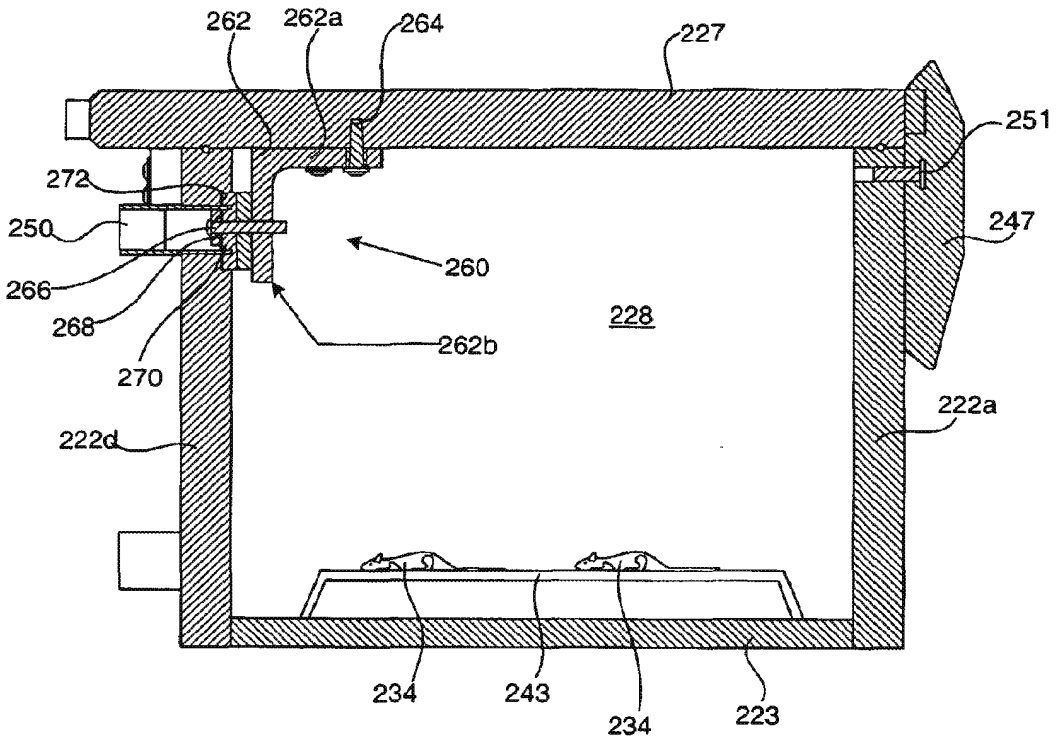

262
262a
264
227
251
272
250
247
260
266
228
268
262b
270
222a
222d
234
243
234
223

FIG. 4E

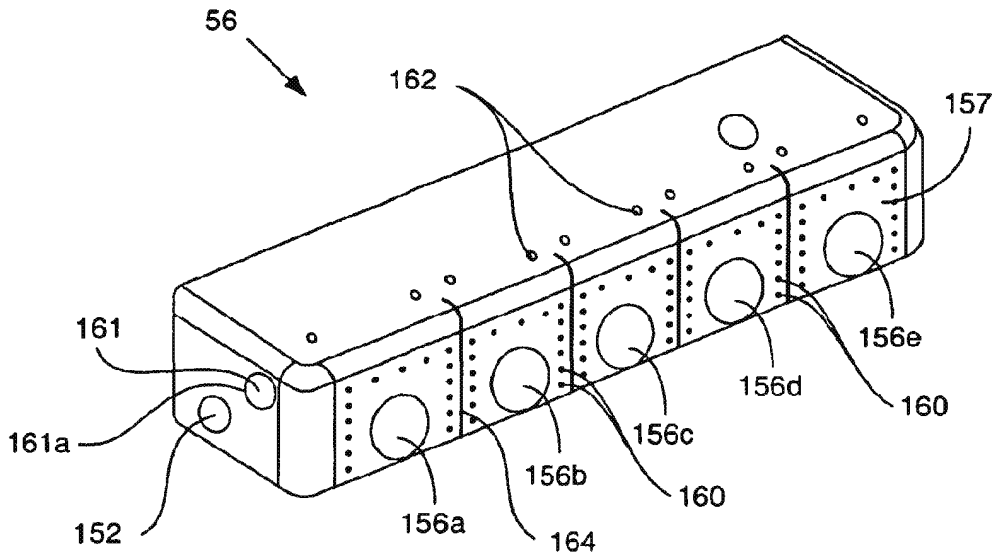

56
162
157
161
156e
156d
161a
160
156c
156b
160
152
156a
164

FIG. 5A

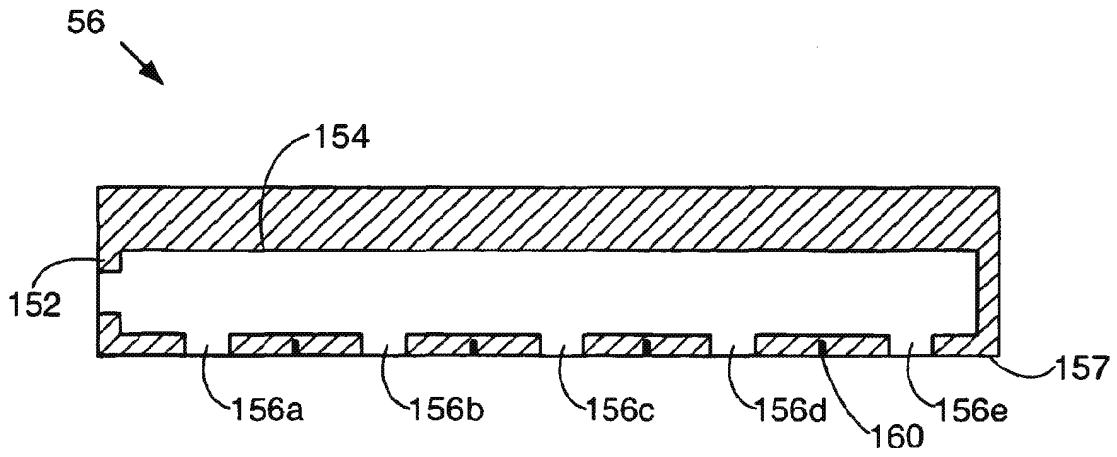

56
154
152
157
156a
156b
156c
156d
160
156e

FIG. 5B

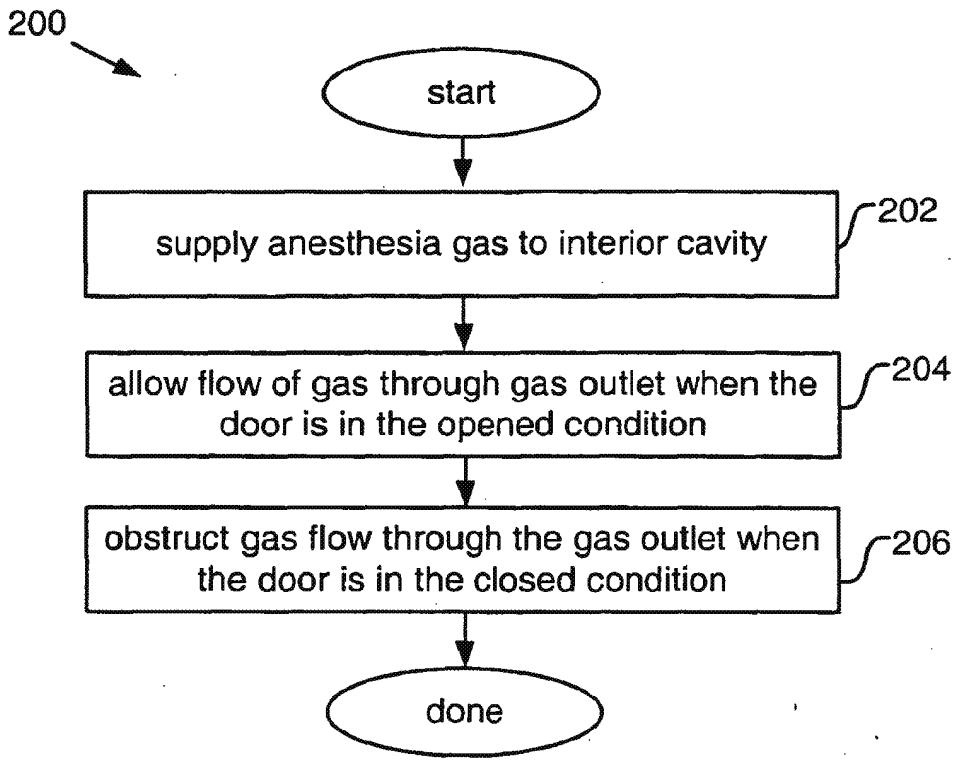

200
start
supply anesthesia gas to interior cavity
202
allow flow of gas through gas outlet when the door is in the opened condition
204
obstruct gas flow through the gas outlet when the door is in the closed condition
206
done

FIG. 6

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 478 916 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
09.07.2008 Bulletin 2008/28

(21) Application number: **03713577.9**

(22) Date of filing: **21.02.2003**

(51) Int Cl.:
G01N 21/64 *(2006.01)* ***G02B 6/38*** *(2006.01)*

(86) International application number:
PCT/US2003/005199

(87) International publication number:
WO 2003/073079 (04.09.2003 Gazette 2003/36)

(54) **FLUORESCENCE ILLUMINATION ASSEMBLY FOR AN IMAGING APPARATUS**

FLUORESZENZ-BELEUCHTUNGSSYSTEM FÜR EIN BILDGEBENDES GERÄT

ENSEMBLE D'ECLAIRAGE FLUORESCENT POUR APPAREIL D'IMAGERIE

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PT SE SI SK TR

(30) Priority: **22.02.2002 US 359663 P**
03.07.2002 US 189886

(43) Date of publication of application:
24.11.2004 Bulletin 2004/48

(73) Proprietor: **Xenogen Corporation**
Alameda, CA 94501 (US)

(72) Inventors:
- **NILSON, David**
  Walnut Creek, CA 94596 (US)
- **RICE, Brad**
  Danville, CA 94506 (US)
- **TROY, Tamara**
  San Francisco, CA 94131 (US)

(74) Representative: **Alton, Andrew**
Urquhart-Dykes & Lord LLP
Tower North Central
Merrion Way
Leeds LS2 8PA (GB)

(56) References cited:
WO-A-00/49938
WO-A-01/61324
WO-A-01/63247
WO-A-99/08233
US-A- 5 039 868
US-A- 5 542 012
US-A- 5 680 492
US-A- 5 898 802
US-A- 5 986 271
US-A- 6 097 025
US-A1- 2001 028 510

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Printed by Jouve, 75001 PARIS (FR)

EP 1 478 916 B1

Description

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation-in-part of a U.S. Patent Application entitled "FLUORESCENCE ILLUMINATION ASSEMBLY FOR AN IMAGING APPARATUS" by Nilson et al., filed July 3, 2002, U.S. Application Ser. No. 10/081,040, which in turn claims priority under 35 U.S.C. 119(e) from U.S. Provisional Patent Application No. 60/359,663, entitled same and filed February 22, 2002.

TECHNICAL FIELD

[0002] The present invention relates generally to imaging systems, and more particularly, relates to macroscopic fluorescent illumination sources and their associated components to illuminate imaging systems.

BACKGROUND

[0003] One specialized type of imaging involves the capture of low intensity fluorescence. Briefly, fluorescence is a molecular phenomenon in which a substance absorbs light of a particular wavelength and emits light of a longer wavelength. The absorption of light is referred to as the "excitation", and the emission of longer wave lights as the "emission". Both organic and inorganic substances can exhibit fluorescent properties.

[0004] Fluorescence imaging is performed by illuminating a sample to excite fluorescence molecules in the sample, and then capturing an image of the sample as it fluoresces using a camera. Such imaging applications present particular challenges to the design of a box or chamber in which the sample is contained during imaging. This is especially true in macroscopic applications where the field-of-view is about 10 cm - 30 cm in diameter, as compared to microscopic applications where the field-of-view is less than about 1 cm.

[0005] Typically, intensified or cooled charge-coupled device (CCD) cameras are used to detect the fluorescence of low intensity light radiating from the sample. These cameras are generally complex, may require specialized cooling, and are typically fixed to a single location on the top of a specimen chamber. A user places a sample at a predetermined position in the specimen chamber within the field of view for the overhead camera.

[0006] Due to this static design, one particular challenge to imaging apparatus design is the diverse lighting needs required during image capture. Fluorescent image capture, of course, involves the sample being illuminated with an in-box illumination source, while the minute amounts of fluoresced light from the "excited" sample are detected using a light detector, e.g., a CCD camera.

[0007] One problem associated with the capture of overhead images in macroscopic applications is that the relatively large CCD camera is typically centrally located directly over the sample platform which supports the sample. A single illumination source is thus often positioned in the light box at a location off-set from the camera lens, and angularly directed at the sample platform. Thus, for relatively non-planar samples supported atop the platform, substantially uniform illumination is difficult to achieve. Such is also the case when multiple illumination sources are applied which often causes detrimental shadowing, and thus, non-uniform lighting.

[0008] Another problem associated with fluorescent imaging in macroscopic applications is that the current imaging apparatus generally employ dichroic mirrors to perform partial filtering functions. Briefly, dichroic mirrors are typically used in fluorescence microscopes to provide an additional amount of separation for the excitation and emission wavelengths. The dichroic mirror is usually mounted at about a 45 degree angle to excitation and emission light. The excitation light is reflected by the dichroic mirror onto the specimen, while the emission light passes through the dichroic mirror, the emission filter, the lens, and is incident on the CCD camera. Dichroic mirrors are commonly used on microscopes because the beam size is very small and so the mirrors are quite compact (usually 2.54 cm (1 inch) or less in diameter).

[0009] For a macroscopic application, as mentioned , the required field-of-view is much larger (i.e., 10 cm - 30 cm) than that for a microscopic application (less than about 1 cm). This of course necessitates the use of a much larger lens which in turn renders the use of a dichroic mirror impractical. Due to the size and orientation of such a mirror in the imaging compartment of the imaging box, the footprint of the imaging box is unfeasibly large. In view of the foregoing, an improved illumination assembly for a light box that enables the substantially uniform lighting for fluorescent image capture of the sample would be highly desirable.

[0010] US-2001/0028510 provides a digital imaging system comprising a lens sub-assembly and an imaging sub-assembly disposed behind the lens sub-assembly. The lens sub-assembly comprises a lens, including a front lens element and having an optical axis. The lens sub-assembly further comprises a source of light disposed within the lens. The source of light is disposed behind the front lens element and constructed so as to direct light towards the front lens element and out of the lens.

[0011] WO-01/63247 provides an imaging box comprising a body including an interior cavity, and having a front wall defining an opening into the cavity, and a door having a rear wall. The door is moveable between an opened condition providing access to the cavity through a cavity opening, and a closed condition, positioning the rear wall of the door adjacent the body front wall to prevent access to the cavity. In the closed position the rear wall of the door and the body front wall define a capture space extending perimetrically about the cavity opening. A compressible material is disposed within the capture space. Light entering the capture space from the exterior

of the body is intercepted by the compressible material.

[0012] WO-99/08233 provides an apparatus comprising a grid generator providing a matrix of nominal target locations including a probe template of predefined, two dimensional size and shape at each of a plurality of fixed, predetermined grid points on the specimen. The apparatus includes a probe template locator which is jointly responsive to pixel intensity and the spacial distribution of pixel intensity in an image of the specimen at a plurality of locations in the vicinity of a nominal target location, to determine the most probable location of the probe template corresponding to a specific target.

[0013] The present invention provides a macroscopic fluorescence imaging assembly including an imaging apparatus having an enclosure wall defining a view port into a light-tight imaging compartment thereof, and a specimen platform positioned in the imaging compartment having a support surface facing toward the view port. An illumination assembly includes an illumination device including a bundle of fiber optic strands having proximal ends and illumination output distal ends. Proximal ends of the strands are in optical communication with a light source and corresponding illumination output distal ends are positioned proximate to and peripherally encircling the view port. The illumination output ends are disposed directly into the imaging compartment to attain substantially uniform illumination of the support surface of the specimen platform by light passing directly from the illumination output distal ends to the support surface.

[0014] The illumination assembly can include a frame substantially peripherally encircling said view port, and adapted to support the output end of said illumination device.

[0015] Preferably, the frame of the illumination assembly is curvilinear-shaped.

[0016] The illumination output distal ends thereof can terminate at the frame to emit a conical directional beam of light onto said specimen platform, said output ends of said fiber optic strands being sufficiently spaced peripherally about the view port such that the plurality of directional beams collectively illuminate the specimen platform in the substantially uniform manner.

[0017] The light assembly can include an opaque material sleeve extending substantially around the bundle of fiber optic strands to substantially prevent external light leakage. The material sleeve can be comprised of a shrink-wrap material.

[0018] The illumination assembly can further include a filter assembly having an excitation interference filter positioned between the light source and the proximal ends of the fiber optic strands of the bundle to filter the light before passing there through.

[0019] Preferably, the illumination output distal ends of said fiber optic strands are positioned in the range of about 120.0 mm to about 380.0 mm from said specimen platform. More preferably they are spaced at 190mm,

[0020] Said fiber optic strand bundle can include a first bundle portion and a second bundle portion, said first bundle portion including said proximal end of said fiber optic strand bundle and a transmission end positioned proximate an upstream side of the interference filter to transmit substantially all of the light there through, and said second bundle portion including said distal end of said fiber optic strand bundle and a receiving end positioned proximate a downstream side of the interference filter to receive substantially all of the transmitted light passing there through.

[0021] The imaging assembly can further include a filter housing containing the interference filter therein in a substantially light-tight recess, and a collimating lens disposed in said housing between the transmission end of the first bundle portion and said upstream side of the interference filter to collimate substantially all of the light passing there through.

[0022] The imaging assembly can further include a collecting lens disposed in said housing between the receiving end of the second bundle portion and said downstream side of the interference filter to collect substantially all of the collimated light passing there through.

[0023] Said filter assembly can further include a collecting lens disposed in said housing between the proximal ends of the fiber optic strands of the bundle and a downstream side of the interference filter to collect substantially all of the collimated light passing through filter.

[0024] Said filter assembly can include a downstream connector portion housing the collecting lens and adapted to removably couple to the proximal ends of the fiber optic strands of the bundle portion.

[0025] Said downstream connector portion can be adapted to pass through an aperture in a wall of the imaging apparatus and into the imaging compartment thereof, said bundle of fiber optic strands having an optical connector to removably mount to the downstream connector portion in a manner placing the proximal ends of the fibre optic strands in optical communication with said collecting lens.

[0026] The illumination assembly can further include a light baffle disposed in said upstream connector portion upstream from said collimating lens, said light baffle configured to intercept light rays transmitted from the distal transmission end of the transmission device which are sufficiently skewed from a direction substantially parallel to the optical axis of the distal transmission end.

[0027] Said light baffle can include an opaque plate member disposed substantially adjacent an upstream abutting surface of said collimating lens, said light baffle having an aperture extending there through having a transverse cross-sectional area smaller than that of the collimating lens abutting surface.

[0028] Said light baffle can include a plurality of opaque plate members nested substantially adjacent one another, and disposed substantially adjacent an upstream abutting surface of said collimating lens, each said plate member having a respective aperture extending there through, and having a respective transverse cross-sectional area smaller than that of the collimating lens abut-

ting surface. A longitudinal axis of said aperture can be substantially co-axially aligned with a longitudinal axis of said collimating lens.

[0029] The ratio of the transverse cross-sectional area of each aperture to that of the collimating lens abutting surface can be in the range of about 0.64:1 to about 0.8:1. Each aperture can taper outwardly in a direction toward the collimating lens abutting surface such that an entrance diameter of the respective aperture is less than an exit diameter thereof.

[0030] The respective entrance diameter and exit diameter of each aperture of the respective plate member can be successively larger than that of an adjacent upstream plate member.

[0031] The respective entrance diameter of each aperture of the respective plate member can be successively smaller than the respective exit diameter of the aperture of the adjacent upstream plate member.

[0032] Each outward taper can be in the range of about 30° to about 60° from the longitudinal axis.

[0033] Each plate member can have a thickness in the range of about 1.0 mm to about 4.0 mm.

[0034] Each end plate can be made from black anodised aluminium.

[0035] Said plate member can include a plurality of annular ribs extending inwardly into said aperture,

[0036] A downstream side of each annular rib can taper outwardly to intersect the upstream side of each adjacent downstream annular rib. The diameter of each downstream annular rib can be successively larger than that of the adjacent upstream annular rib.

[0037] The fibre optic strand bundle can include a transmission portion having the proximal ends of the fiber optic strands on one end and transmission ends on an opposite end thereof, and a receiving portion having the illumination output distal ends of the fiber optic strands on one end and receiving ends on an opposite end thereof, and said system can further include a connector body mounted to the imaging apparatus, and defining a passage extending therethrough for optical communication from outside the enclosure to the imaging compartment thereof, said body having a proximal portion adapted to couple to the transmission portion of the bundle such that the transmission end thereof terminates in said passage, and said body having a distal portion adapted to couple to the receiving portion of the bundle such that the receiving end thereof terminates in said passage in opposed relationship to the bundle transmission end; and a diffuser device disposed in said passage between the bundle transmission end and the bundle receiving end, and adapted to diffuse the light transmitted from the transmission end for receipt in the receiving end.

[0038] The diffuser can be composed of frosted glass and can be plate-like having substantially flat, opposed surfaces, and can be disposed across the transverse cross-sectional dimension of the connector passage.

[0039] Preferably, connector body can be adapted to position the first bundle transmission end and the second bundle receiving end a distance in the range of about 8.0 mm to about 15.0 mm from the respective opposed surface of the diffuser device.

[0040] The proximal portion of the connector body can be adapted to removably mount to the first bundle transmission end. The distal portion of the connector body can be adapted to removably mount to the second bundle receiving end.

[0041] The light dispersion assembly can include a plurality of bracket devices peripherally spaced about the view port of the interior wall. The distal ends of the fiber optic strands can be separated into a plurality of distal end groups. Each group cooperates with a corresponding bracket device such that the plurality of directional beams extending generally peripherally around the view port collectively illuminate the specimen platform in the substantially uniform manner.

[0042] Each of said bracket devices can include a diffuser positioned in the path of the directional beams from the respective distal end group to diffuse the light toward the specimen platform.

[0043] The light dispersion assembly can include a diffuser positioned in the path of the directional beams to diffuse the light toward the specimen platform. The diffuser can include a reflector device having a substantially planar reflective surface oriented at an angle about 45° relative the direction substantially parallel to the optical axis of the distal ends of the fiber optic strands. This orientation reflects the directional beams of light emitted therefrom toward the specimen platform.

[0044] Said reflector device can be elongated, and the distal ends of said fiber optic bundle can be aligned in a linear array extending substantially along the elongated reflective surface. Preferably, the distal ends of said fiber optic strands can be positioned about 8 mm from the reflective surface of the reflector device. Preferably, the reflective surface of each said reflector is positioned in the range of about 120 mm to about 460 mm from said specimen platform.

[0045] The substantially parallel optical axes of the distal ends of the fiber optic bundle can be oriented in said cavity substantially perpendicular to a plane containing said view port of the support surface; and said reflector device can include a substantially planar reflective surface oriented at an angle about 45° relative the direction substantially parallel to the optical axes of the distal ends of the fiber optic strands to reflect the directional beams of light emitted there from through said view port.

[0046] Said reflective surface can include a diffuser material to diffuse the light toward said view port in a substantially uniform manner.

[0047] Said diffuser material can include a roughened aluminum plate to diffuse the light generally toward the view port. The plate can be black anodized aluminium. The plate can have a thickness in the range of 1mm to 4mm. Said diffuser material can include SPECRALON®

[0048] The distal ends of said fiber optic strands can be positioned in the range of about 6 mm to about 11 mm

from the reflective surface of the reflector device.

[0049] In a preferred embodiment the bundle of fibre optic strands is composed of substantially pure fused silica. Preferably the substantially pure fused silica can be one of plastic clad fused silica and silica clad fused silica.

[0050] The dispersion assembly can include at least four bracket devices.

BRIEF DESCRIPTION OF THE DRAWING

[0051] The assembly of the present invention has other objects and features of advantage which will be more readily apparent from the following description of the best mode of carrying out the invention and the appended claims, when taken in conjunction with the accompanying drawing, in which:

FIGURE 1 is a top perspective view of an imaging apparatus, with the door removed, incorporating an illumination assembly constructed in accordance with the present invention.

FIGURE 2 is a bottom perspective view of the imaging apparatus of FIGURE 1, and illustrating a light ring component of the illumination assembly.

FIGURE 3 is a front elevation view of the illumination assembly of FIGURE 1 incorporated in the imaging apparatus.

FIGURE 4 is a side perspective view of a light source and a filter wheel assembly of the illumination assembly of FIGURE 1 optically coupled to the imaging apparatus.

FIGURE 5 is an enlarged, bottom plan view of the light ring component of the of the illumination assembly of FIGURE 1.

FIGURE 6 is an enlarged, side elevation view, in cross-section, of the filter wheel assembly of the illumination assembly of FIGURE 1.

FIGURE 7 is an enlarged, exploded, side elevation view, in cross-section, of the plate members of a baffle device of the filter wheel assembly of FIGURE 6.

FIGURE 8 is an enlarged, fragmentary side elevation view, in cross-section, of an optical connection assembly of the illumination assembly of FIGURE 1.

FIGURE 9 is an enlarged, side elevation view, in cross-section, of the optical connection assembly of FIGURE 8.

FIGURE 10 is an enlarged, bottom plan view of the light ring component of the of the illumination assembly of FIGURE 1, and illustrating a second light filter thereof in phantom lines.

FIGURE 11 is a side elevation view, in cross-section, of the light-tight seal mechanism and second filter wheel of in FIGURE 10.

FIGURE 12 is an enlarged, bottom perspective view of the imaging apparatus of FIGURE 1, and illustrating an alternative embodiment illumination assembly.

FIGURE 13 is an enlarged, bottom perspective view of the alternative embodiment illumination assembly of FIGURE 12 mounted to the upper interior wall of the imaging apparatus.

FIGURE 14 is a bottom plan view of the alternative embodiment illumination assembly of FIGURE 12.

FIGURE 15 is an enlarged, bottom perspective view of a light dispersion assembly of the illumination assembly of FIGURE 12.

FIGURE 16 is an enlarged, bottom perspective view of the of the light dispersion assembly of FIGURE 12.

FIGURE 17 is an enlarged, bottom perspective view of the alternative embodiment illumination assembly of FIGURE 12 illustrating the protective cover device shown in phantom lines.

FIGURE 18 is a rear perspective view of a light source and a filter wheel assembly of the illumination assembly of FIGURE 12 optically coupled to the imaging apparatus.

FIGURE 19 is an enlarged, side elevation view, in cross-section, of the filter wheel assembly of the illumination assembly mounted to the imaging apparatus of FIGURE 18.

FIGURE 20 is a top perspective view of an alternative embodiment bottom illumination assembly providing bottom illumination of the specimen constructed according to the present invention.

FIGURE 21 is an enlarged, top perspective view of the bottom illumination assembly of FIGURE 20 without the specimen thereatop.

FIGURE 22 is an enlarged, top perspective view of the bottom illumination assembly of FIGURE 20 with the cover plate removed.

FIGURE 23 is an enlarged, top perspective view of the bottom illumination assembly of FIGURE 20 with a slide device positioned thereatop.

FIGURE 24 is an exploded top perspective view of the bottom illumination assembly of FIGURE 20.

BEST MODE OF CARRYING OUT THE INVENTION

[0052] While the present invention will be described with reference to a few specific embodiments, the description is illustrative of the invention and is not to be construed as limiting the invention. Various modifications to the present invention can be made to the preferred embodiments by those skilled in the art without departing from the true spirit and scope of the invention as defined by the appended claims. It will be noted here that for a better understanding, like components are designated by like reference numerals throughout the various figures.

[0053] Referring now to FIGURES 1-5, a fluorescence imaging assembly, generally designated 20, is provided which includes a light-tight sample box or imaging apparatus 21 having an enclosure wall or upper housing 22 defining a view port 23 (FIGURE 5) into a light-tight imaging compartment 25 thereof. A specimen platform 26 is positioned in the imaging compartment 25 which includes a support surface 27 facing toward the view port 23. The imaging assembly 20 further includes an illumination assembly, generally designated 28, having an illumination device 30 disposed in the imaging compartment 25, and positioned proximate to and substantially peripherally encircling the view port 23 such that said specimen platform 26 is illuminated in a substantially uniform manner.

[0054] Accordingly, by illuminating the specimen platform 26 from an illumination device peripherally extending around or continuously surrounding the camera view port 23, a specimen (not shown) positioned on the platform 26 will be substantially uniformly illuminated, symmetrically about the optical axis. Such uniform illumination is not attainable in the off-set lighting techniques currently applied without repositioning the lighting or the specimen.

[0055] Moreover, as will be apparent below, the improved filtering performance, the careful selection of low auto fluorescent materials for the filters and lighting components, as well as the formation of light tight seals essentially from the light source to the imaging compartment of the imaging apparatus, collectively enable sufficient filtered fluorescent lighting without the need for additional filtering through dichroic mirrors. Thus, in this macroscopic fluorescent imaging application, these relatively large dichroic filters can be eliminated. Consequently, the overall footprint of the imaging enclosure is substantially reduced.

[0056] Briefly, FIGURES 1-4 illustrate an imaging apparatus 21 suitable for capturing photographic, fluorescent or luminescence images in accordance with one embodiment of the present invention. The imaging apparatus 21 includes an upper housing 22 defining the view port in which a lens system of a high sensitivity camera 31 is mounted. This camera is preferably an intensified or cooled integrating Charge-Coupled Device (CCD) camera 31 which is positioned on top of the imaging apparatus 21 and positioned above the upper housing 22. The CCD camera 31 is capable of capturing fluorescent, luminescent and photographic (i.e., reflection based images) images of the sample within the imaging apparatus 21. The CCD camera 31 may be cooled by a suitable source such as a refrigeration device that cycles a cryogenic fluid through the CCD camera via conduits. A suitable refrigeration device is the "CRYOTIGER" compressor, which can be obtained from IGC-APD Cryogenics Inc., Allentown, PA. Other methods, such as liquid nitrogen, may be used to cool the CCD camera 31.

[0057] An image processing unit optionally interfaces between camera 31 and a computer through cables. The computer, which may be of any suitable type, typically comprises a main unit that contains hardware including a processor, memory components such as random-access memory (RAM) and read-only memory (ROM), and disk drive components (e.g., hard drive, CD, floppy drive, etc.). The computer also includes a display and input devices such as a keyboard and mouse. The computer is in communication with various components in the imaging apparatus 21 via cable. To provide communication and control for these components, the computer includes suitable processing hardware and software configured to provide output for controlling any of the devices in the imaging apparatus 21. The processing hardware and software may include an I/O card, control logic for controlling any of the components of the imaging assembly 20, and a suitable graphical user interface for the imaging assembly 20. The computer may also include suitable processing hardware and software for the camera 31 such as additional imaging hardware, software, and image processing logic for processing information obtained by the camera 31. Components controlled by the computer may include the camera 31, the motors responsible for camera 31 focus, the motors responsible for position control of a platform supporting the sample, the camera lens, filter wheels, f-stop, etc. The logic in computer may take the form of software, hardware or a combination thereof. The computer also communicates with a display for presenting imaging information to the user. By way of example, the display may be a monitor, which presents an image measurement graphical user interface (GUI) that allows the user to view imaging results and also acts as an interface to control the imaging assembly 20.

[0058] Referring now to FIGURES 2, 4 and 5, the illumination assembly 28 includes a frame 32 supporting the illumination device 30 which is mounted to the upper housing through a nut plate 33. The frame 32 is preferably a rigid, ring-shaped structure having an interior diameter slightly larger than that of the view port 23 (FIGURE 5) so as to peripherally surround it without obstructing the view from the lens system. Although the illustrated illumination device and the supporting frame 32 are circular, other geometric forms may be applied as long as the

illumination device extends generally around the view port 23.

[0059] In one specific embodiment, the illumination device is provided by a fiber optic lighting system having a plurality or bundle 35 of fiber optic strands extending into the imaging compartment 25. The proximal ends 36 of the strands of the bundle 35 are positioned in optical communication with a light source 37 to transmit collected light to the distal ends 38 of the fiber optic strands. To optimize the system for use fluorescent image capture in accordance with the present invention, the material composition of the fiber optic strands are selected to have low auto-fluorescence properties. All materials (glasses, etc.), it will be understood, will fluoresce at some level. Ordinary optical glasses (E.g., float glass, BK7) contain impurities that can fluoresce. Although the autofluorescence of glass is fairly low, the extremely sensitive cameras utilized in the present invention will easily detect the autofluorescence of these materials. The glass (or other material) autofluorescence passes through the emission filter and creates noise in the fluorescence signal, so every effort is made to minimize autofluorescence. One material particularly suitable for the fiber optic strands and filters is high purity fused silica, such as plastic clad fused silica or silica clad fused silica, which has very low autofluorescence.

[0060] As shown in FIGURE 5, a bottom face 40 of the frame 32 defines an annular slit 41 upon which the distal ends 38 of the strands terminate at a position generally perpendicular to the face 40. Accordingly, the plurality of distal ends 38 of the fiber optic strands each independently emit a conical directional beam of light (illustrated by broken lines 39) onto the specimen platform.

[0061] Preferably, a collective cone of light (illustrated by broken lines 39) is emitted having with a full angle of about 55°, wherein each strand emits light in the range of about 50° to about 60°. By positioning the strand distal ends 38 at least about 140 mm to about 380 mm from the specimen platform, the collective ring of conical light beams emitted from strand distal ends 38 sufficiently overlap (FIGURE 3) to produce relatively uniform illumination of the specimen. That is, the illumination intensity does not vary by more than about ± 25% over the entire field of view, which is between about 10 cm to about 25 cm in these macroscopic applications. Moreover, these diverging beams, as will be discussed below, are substantially directed onto the specimen platform to illuminate only the specimen, and to reduce detrimental florescence of the other box components in the imaging compartment.

[0062] The strand distal ends are positioned substantially continuously around the annular slit 41, and are about 3-6 strands wide. It will be appreciated, however, that complete continuity of the strand distal ends is not required to provide uniform lighting in accordance with the present invention. One example of such ring light guides is model 70001148001 by Dolan Jenner Industries of Lawrence, MA. These fixtures are typically utilized in non-light-tight microscopic applications. Accordingly, these applications are non-fluorescent in nature.

[0063] To protect the exterior fiber optic bundle portions 50 and 51 of the fiber optic bundle 35 and to reduce the introduction of exterior light or noise into the fiber optic strands, a segmented, flexible metal jacket (not shown) is placed around these portions which is further surrounded by a flexible PVC sleeve 42. This sleeve is opaque (black), and blocks all external ambient light that might enter the fiber bundle.

[0064] While this protective sleeve combination is sufficient to substantially reduce the introduction of exterior noise into the fibers (i.e., for use in the exterior bundle portions 50 and 51 outside the of imaging box), it may be phosphorescent and is therefore unsuitable for use inside the box since, in these low-intensity imaging applications, even an amount of light emanating from within the protective sleeve will detrimentally influence the fluorescent imaging of the specimen.

[0065] Accordingly, internal to the imaging box, the sleeve material 43 surrounding the bundle portion 53 of the fiber optic bundle 35 is replaced with a non-phosphorescent material to substantially eliminate the possibility of spurious light sources from the fiber optic bundle 35 within the imaging box. One particular flexible material which has low phosphorescence is a polyolefin heat shrink tubing material.

[0066] The proximal end 36 of the fiber optic bundle 35 is coupled to a fiber optic light source 37 which optically couples the proximal end faces of the fiber optic strands (not shown) with a direct light of the light source. Preferably, the light source includes a housing 45 which provides a connector 46 to position the proximal end faces of the fiber optic strands substantially adjacent the light source so that the light can be transmitted through the fiber optic strands of the bundle 35. One example of such a Fiber Optic Illuminator is model PL 900, by Dolan Jenner Industries of Lawrence, MA.

[0067] In one embodiment, the direct light is provided by a bulb contained in the housing 45, and positioned at the proximal end faces of the fiber optic strands. A preferred light comprises a tungsten halogen lamp, which emits a wide spectrum of bright white light suitable to fluoresce objects. Other applicable light sources include xenon lamps, mercury lamps and lasers.

[0068] Typically, the usable fluorescence spectrum is in the range of 400 nm to about 900 nm. Thus, depending upon the desired fluorescence spectra, the composition of the sample material and the fluorescent material, the remaining light emitted by the light source must be filtered out. Optical filters are applied, accordingly, to filter out non-fluorescence spectra as well as unwanted fluorescence spectra. Depending upon the application, there have been selected optical filters or filter wheels disposed in the imaging compartment of an imaging apparatus 21 just after the off-set light source. Such an arrangement, however, would not be practical in the lighting technique of the present invention since the diameter of the ring-

shaped frame 32 is significantly larger. Moreover, proportionate to the size of the imaging compartment, a filter wheel could not be deployed.

[0069] In accordance with the present invention, a filter wheel assembly, generally designated 47, is positioned "in-line" in the fiber optic bundle 35 (FIGURES 1, 4 and 6). Preferably, the filter wheel assembly 47, which includes a plurality of optical filters, is positioned in close proximity to the transmission box. This enables the filter wheel assembly and the transmission box to be supported on a common support frame 48, and to be packaged together as a single unit.

[0070] Briefly, as best illustrated in FIGURES 2, 4 and 5, the fiber optic bundle 35 includes a first bundle portion 50, extending between the light source 37 and the filter wheel assembly 47, and a second bundle portion 51, extending between the filter wheel assembly 47 and an optical connector assembly 52 mounted to the imaging apparatus 21. Finally, the fiber optic bundle includes a third bundle portion 53 extending from the optical connector assembly 52 (as will be described in greater detail below) on the inside of the imaging compartment 25 to the frame 32. This third bundle portion 53, as above-mentioned, includes the heat shrink material sleeve 43 which has low phosphorescence.

[0071] The optical filters are typically interference-type filters which include bandpass filters, longpass filters and shortpass filters. These filters are preferably provided as a filter set contained on a filter wheel 55 of the filter wheel assembly 47 which is placed in-line with the fiber optic bundle 35. Thus, the filter wheel 55, rotatably mounted in a recess 56 of the housing 57, can be selectively rotated to position the selected filter in the path of the fiber optic strands.

[0072] Each interference filter is selected to allow the passage of select spectra of light. Another useful parameter, in accordance with the present invention, is selecting a filter with sharp cut-offs or edges so that the gap between the excitation and emission filter bands can be minimized. This is beneficial in that the Stokes shift for many common fluorescent dyes and proteins is relatively small. The Stokes shift is the separation in wavelength between the excitation and emission peak. Usually, a filter gap of about 20 nm is chosen. These filters also are characterized by very high rejection outside the passband or "out-of-band" blocking properties, with a typical rejection of $>10^6$. Furthermore, the filters are also preferably constructed from low auto-fluorescent materials. Accordingly, the application of these higher quality excitation and emission filters allows the use of a single excitation-emission filter pair without an associated dichroic filter.

[0073] Examples of such filters include the Alpha Technology filters from Omega Optical, Inc. of Brattleboro, Vermont. For a general discussion on fluorescence and filtering, see the Handbook of Optical Filters for Fluorescence Microscopy; by Jay Richman of the Chroma Technology Corp, June 2000. The housing 57 of the filter wheel assembly 47 is substantially light-tight so that detrimental exterior light is not introduced as the light is transmitted through the filters. The housing, as viewed in FIGURE 6, further includes an input port 58 and an output port 60 upon which the selected filter optically aligns therewith for the filtering of the light. Accordingly, a first connector 61 is included which is adapted to optically align an optical output end 62 of the first bundle portion 50 within the input port 58 of the housing for transmission of the light through the filter 63. Similarly, the filter wheel assembly 47 includes a second connector 65 which is adapted to optically align an optical input end 66 of the second bundle portion 51 within the output port 60 of the housing for reception of the filtered light from the filter 63.

[0074] To facilitate transmission of the light through the filter, a collimating lens 67 is positioned in the input port 58 between the optical output end 62 of the first bundle portion 50 and the filter 63. In order for the excitation filter to function properly, the light rays must be fairly well collimated (parallel to the optical axis) through the filter. Therefore, as the light passes through the collimating lens, it is collimated in a direction substantially perpendicular to the planar face of the filter which minimizes detrimental reflection there from. Further, by selecting the first bundle portion 50 of the fiber optic bundle 35, extending between the light source 37 and the filter wheel assembly 47, to be about 0,64 cm (¼ inch) in diameter, most of the exiting light rays have a maximum cone angle in the range of about 30° to about 40°. Consequently, after passing through the collimating lens 67, the angle of incidence is reduced to a maximum ray angle of less than or equal to about 12° The output of the excitation filter/lens assembly couples into the 1,27 cm (½ inch) diameter fiber optic bundle portion 51 in order to mate up with the ring light, which also as a 1,27 cm (1/2) inch bundle size.

[0075] A focusing lens 68 is further disposed downstream from the filter 63 to focus and direct the collimated and filtered light, exiting the filter 63, into the optical input end 66 of second bundle portion 51 for transmission through the fiber optic strands thereof. FIGURE 6 best illustrates that the focusing lens 68 is positioned in the output port 60 between the filter 63 and the optical input end 66 of the second bundle portion 51 of the fiber optic bundle 35. Typical of these filter wheel assemblies, by way of example, is model FA-448, by Acton Research of Acton, Massachusetts. It will be appreciated, however, that light-tight filter cassettes and filter bars may be employed as well.

[0076] While the collective optical arrangement of a conventional filter wheel assembly is applicable for most optical applications, this set-up is not suitable for fluorescent imaging. This is due to the fact that trace amounts of unfiltered light often leak around the periphery of the filter which detrimentally affect the fluorescent imaging of the sample in the imaging compartment. Although the housing 57 of these conventional filter wheel assemblies

is considered light-tight, when the angle of transmission of some rays of light exiting the optical output end 62 of the first bundle portion 50 are sufficiently skewed from a direction parallel to the optical axis of the output end, unfiltered light can pass along the outer edges of the collimating lens 67, and thus, past the filter 63 and into the focusing lens.

[0077] The skewing of the light rays exiting the collimating lens depends on the distance of the bundle distal end to the collimating lens, as well as the diameter of the bundle and collimating lens, the f-number of the lens and the numerical aperture of the bundle. By way of example, when the diameter of the bundle 62 is in the range of about 6.3 mm to 6.4 mm, the diameter of the collimating lens 67 is in the range of about 16.5 mm to about 17 mm, the f-number is one and the distance between the optical output end 62 and the collimating lens 67 is in the range of about 19 mm to about 20 mm, a substantially skewed light ray exiting the collimating lens would be one in the range of greater than about 14 degrees from the direction substantially parallel to the longitudinal axis of the perpendicular to the optical output end 62.

[0078] In accordance with another aspect of the present invention as is best illustrated in FIGURE 6, a light baffle device, generally designated 70, is deployed between the optical output end 62 and the collimating lens 67 to intercept light these skewed light rays. Accordingly, the baffle device 70 will substantially prevent skewed rays from reflecting off of interior walls and entering the collimating lens 67 and thus leak around the filter 63.

[0079] The light baffle device 70, in one embodiment, includes an opaque plate member 75 disposed substantially adjacent an upstream abutting surface 71 of the collimating lens. Centrally disposed in the plate member is an aperture 72 extending there through, and having a transverse cross-sectional area smaller than that of the collimating lens abutting surface 71. Preferably, the ratio of the transverse cross-sectional area of the aperture 72 to that of the abutting surface 71 of the collimating lens 67 is in the range of about 0.64:1 1 to about 0.8:1.

[0080] Thus, the skewed light rays impinging upon the upstream surface 73 of the plate member 75 are intercepted, while the remaining portion of the light transmitted from the first bundle portion 50 pass through aperture 72. Further, to reduce reflection of these impinging light rays, the plate member 75 is either coated with a material which absorbs light, such as black anodize, or is composed of opaque materials having absorption properties, such as black Delrin.

[0081] In the preferred form, the aperture 72 of the baffle device 70 is substantially central to the abutting surface 71 of the collimating lens 67. Thus, a longitudinal axis of the aperture 72 is substantially co-axially aligned with a longitudinal axis of the collimating lens 67.

[0082] In still another configuration, a plurality of plate members 75_A-75_D are nested together in a side-by-side manner in abutment with the collimating lens abutting surface. Briefly, while four plate members 75_A-75_D are shown and described, it will be appreciated that more or less plate members may be applied.

[0083] FIGURES 6 and 7 further illustrates that each plate member 75_A-75_D defines a respective central aperture 72_A-72_D which is co-axially aligned with the longitudinal axis of the abutting surface 71 of the collimating lens. A threaded ring 76 or the like is deployed in the input port 58 and matably engaged with the first connector 61 to affix the plate member 75_A-75_D against the abutting surface 71 of the collimating lens 67. Further, each aperture 72_A-72_D has a respective transverse cross-sectional area smaller than that of the collimating lens abutting surface 71. However, each adjacent downstream plate member 75_B-75_D defines a respective aperture 72_B-72_D having a diameter incrementally larger than its adjacent upstream plate member 75_A-75_C. Preferably, the area of each successive downstream aperture 72_B-72_D is about 10% to about 25% larger.

[0084] Each aperture 72_A-72_D further tapers outwardly in a direction toward the collimating lens 67 such that a respective entrance diameter (D_{en}) of the respective aperture 72_A-72_D is smaller than the corresponding exit diameter (D_{ex}) thereof. Preferably, the outward taper of each aperture is in the range of about 30° to about 60° from the longitudinal axis, and the thickness of each plate member is in the range of about 0.5 mm to about 2.0 mm.

[0085] However, in accordance with the present invention, while the overall transverse cross-sectional dimension of each successive downstream aperture 72_B-72_D is progressively larger, the entrance Diameter (D_{en}) of the corresponding downstream aperture 72_B-72_D of the plate member 75_B-75_D is smaller than the exit Diameter (D_{ex}) of the adjacent aperture 72_A-72_C of the adjacent upstream plate member 75_A-75_C. Accordingly, if this nested configuration of the plate members were provided as an integral single unit, a plurality of annular ribs and adjacent troughs would be defined with each annular rib being successively larger in diameter as the plate member is positioned closer to the collimating lens 67.

[0086] This is beneficial in that should a sufficiently skewed light ray pass through the upstream aperture 72, it may be intercepted in the annular trough between two adjacent ridges.

[0087] In another aspect of the present invention, a light-tight fiber optic connection assembly, generally designated 52, is included for optically connecting the distal transmission end 77 of second bundle portion 51 to the proximal receiving end 78 of third bundle portion 53. This optical connector assembly 52, as best viewed in FIGURES 2, 4, 8 and 9, includes a connector body 80 mounted to a side wall 85 of the imaging apparatus 21 for optical communication into the light-tight imaging compartment 25.

[0088] The connector body 80 includes a proximal portion 81 and an opposite distal portion 82, and defines an elongated passage 83 extending from the proximal portion 81 to the distal portion 82. The proximal portion 81

of the connector body is adapted to removably couple to a distal optical connector 86 mounted to the second bundle portion 51, while the distal portion 82 of the connector body is adapted to removably couple to a proximal optical connector 87 mounted to the third bundle portion 53. Each of the distal optical connector 86 and the proximal optical connector are conventional female optical connectors which are threaded to the corresponding threaded male end of the connector body.

[0089] When the optical connectors 86, 87 are properly mounted to the connector body 80, the transmission ends 77 of the fiber optic strands of the second bundle portion 51 terminate in the passage 83 in opposed relationship to the receiving ends 78 of the fiber optic strands of the third bundle portion 53. A diffuser device 88 is further disposed in the passage 83 in the gap region 90 between the second bundle transmission end 77 and the third bundle receiving end 78. This diffuser device 88 is adapted to diffuse the light transmitted from the transmission end of the fiber optic strands to facilitate receipt in the receiving end.

[0090] As the collective light is diffused while passing through diffuser device 88, it is substantially uniformly distributed about the proximal receiving ends 78 of the third bundle portion 53. Consequently, the light ring mounted peripherally about the view port 23 uniformly illuminates the specimen platform 26. Without this diffuser, the collimated and filtered light transmitted across the gap may develop "hot-spots" on the proximal receiving end 78 of the third bundle portion 53 which causes distribution non-uniformity from the light ring.

[0091] The diffuser may be any substantially transparent device capable of uniform diffusion of the light passing there through. Preferably, the diffuser device 88 is composed of a rigid material, such as plastic or glass which is seated across the transverse cross-sectional dimension of the connector body passage 83. To provide uniform light diffusion, at least one surface of the diffuser device 88 upon which the light must pass through is frosted. Typical of these diffusers, by way of example, is model L45-652, by Edmund Scientific of Barrington, NJ.

[0092] As illustrated in FIGURE 9, the diffuser device 88 is preferably plate-like having substantially flat, opposed surfaces, of which at least one is frosted. Preferably, an interior wall 91 of the connector body, defining the passage 83, is substantially cylindrical-shaped. An insert sleeve 89 having an outer diameter substantially similar to but smaller than that of the passage 83 is slideably inserted between the transmission ends 77 and the receiving ends 78 of the strands in the gap region 90. The insert sleeve 89 includes an annular shoulder portion 92 upon which the outer circumferential edge of diffuser device 88 seats. When the insert sleeve 89 is slideably disposed in the gap region 90 of the passage 83, the opposed surfaces of the diffuser device 88 extend across the entire transverse cross-sectional dimension of the passage 83 to assure the light transmitted from the distal end of the second bundle portion 51 of the fiber optic strand bundle passes through the diffuser device.

[0093] By way of example, for a fiber optic bundle with a diameter of about 12.6 mm to about 12.7 mm, the connector body passage 83 may have a diameter in the range of about 12.7 mm to about 12.8 mm. Upon mating of the optical connectors to the connector body, the gap between the opposed ends of the second bundle portion 51 and the third bundle portion 53 may be in the range of about 8.0 mm to about 15.0 mm. The diffuser device 88 accordingly, may have a thickness in the range of about 1.0 mm to about 2.2 mm.

[0094] Referring now to FIGURES 10 and 11, an emission filter wheel 96 is housed in the imaging chamber 25 to filter out the excitation light rays, leaving substantially only the fluorescent rays emanating from the sample for capture by the CCD camera 31. Depending upon which excitation filter is necessary in the excitation filter wheel 57 to excite the desired fluorescent spectra in the imaging compartment 25, a corresponding emission filter 95 is selected to absorb the excitation rays and allow passage of the fluorescent emission into the lens 97 of the camera 31.

[0095] In one specific embodiment, emission filter wheel 96 is rotatably mounted to the upper housing 22 of the imaging apparatus 21, between the frame 32 of the light ring 30 and lens 97 of the camera 31 (FIGURE 11). As best viewed in FIGURE 10, the filter wheel includes a plurality of filters each of which can be selectively rotated about optical filter wheel axis 98, and into optical alignment with the aperture of the light ring 30.

[0096] Due to size constraints and limitations, a crescent-shaped gap 100 may be formed between the outer peripheral edge 101 of the frame 99 of the filter wheel 96 and the view port 23 of the upper housing 22 (FIGURE 10). While this gap 100 is not large, it is sufficient to permit the passage of some unfiltered light rays along the path of arrow 102 in FIGURE 11. Accordingly, to prevent this light leakage, a light-tight seal device 105 is positioned between the filter wheel frame 99 and the upper wall 103 of the upper housing.

[0097] This seal device 105 is sufficiently dense and/or opaque to prevent the passage of light there through yet sufficiently flexible to allow relative rotation of the second filter wheel without exerting undue stress thereon. Preferably, the seal device 105 is provided by a pair of brush devices positioned in the gap. One such brush material is a plastic brush, by Amesbury Group, Inc. of Statesville, North Carolina.

[0098] Referring now to FIGURES 12-14, 18 and 19, another specific embodiment of the macroscopic fluorescence illumination assembly 28 is illustrated. In this configuration, the illumination assembly 28 includes a fluorescent light source 37, and a light dispersion assembly 110 positioned proximate the view port 23 of the interior wall 103. The illumination assembly 28 further includes a bundle 111 of fiber optic strands composed of substantially pure fused silica. The proximal ends 112 thereof in optical communication with the light source 37 and distal

ends 113 thereof terminate proximate the view port 23. The distal ends 113 each emit a conical directional beam of light originating from the light source 37 and cooperating with the light dispersion assembly 110 such that the plurality of directional beams 115 (shown in phantom lines) collectively illuminate the specimen platform 26 in a substantially uniform manner.

[0099] As mentioned above, all materials (glasses, etc.) fluoresce at some level. Ordinary optical glass materials applied for fiber optic strands contain impurities that can fluoresce. It has been observed that high purity or substantially pure fused silica exhibits very low autofluorescence. This is of course beneficial to reduce undesirable autofluorescence of the fiber optic strand material which passes through the emission filter and may be mistaken for "sample" fluorescence. In particular, the fiber optic strands and filters are composed of as plastic clad fused silica or silica clad fused silica.

[0100] High purity or substantially pure fused silica is less flexible than glass or other conventional materials applied for fiber optic strands. Thus, the permissible bending radius or radius of curvature of a bundle of fiber optic strands (i.e., the minimum suggested usable bending radius of the fiber optic bundle without fracture of the strands) composed of such fused silica is substantially greater than that for conventional fiber optic strand materials. A fused silica core/clad diameter must be significantly reduced to obtain the same radius of curvature. Thus, about twice as many fused silica fibers must be used to transmit the same amount of light, and the cost becomes a significant factor.

[0101] In this specific embodiment, since the bundle of fiber optic strands originates from an interior side wall 85 of the imaging apparatus 21, as shown in FIGURE 12, similar to the light-ring embodiment above, the optical axes of the bundle are contained in a generally horizontal plane which is generally parallel with that of the specimen platform 26. Thus, the optical axis of the distal ends of the strands needs to be redirected and repositioned in a direction toward the specimen platform of illumination thereof. However, due to the relatively large radius of curvature of the substantially pure fused silica strands, the overall vertical footprint of the imaging apparatus and costs are significantly increased.

[0102] To address this problem, the dispersion assembly 110 is configured to cooperate with the distal ends 113 of the fiber optic strands to redirect the directional beams 115 (shown in phantom lines) collectively toward the specimen platform 26 for illumination thereof in a substantially uniform manner. Accordingly, the optical axes of the distal ends 113 of the fiber optic strands may be retained generally parallel to the specimen platform 26, while the directional beams are directed (E.g., through reflective surfaces 116) downwardly toward the specimen platform 26. The overall height of the imaging apparatus 21, thus, is significantly reduced since the distal ends of the substantially pure fused silica fibers themselves need not be curved toward the platform 26, and the overall cost is significantly reduced.

[0103] Referring now to FIGURES 15 and 16, the light dispersion assembly 110 includes a bracket device 117 adapted to mount and secure the distal ends 113 of the fiber optic strands to the upper interior wall 103 of the imaging apparatus 21. These bracket devices 117 are preferably substantially rigid, and are composed of black anodized aluminum to reduce auto fluorescence.

[0104] In one specific embodiment, to redirect the directional beams emitted from each distal end 113 of the strands, the dispersion assembly 110 includes a reflective surface 116 angled to reflect the directional beams toward the specimen platform 26. This permits the entire fiber optic bundle 111 to be maintained in generally the same plane which is essentially parallel to the specimen platform 26.

[0105] To reflect the directional beams about 90° from the optical axis of the distal ends of the strands and toward the specimen platform, the relatively planar reflective surface 116 should be oriented about 45° relative the direction of the optical axis. It will be appreciated that depending upon the particular position of the bracket device 117 and the exact orientation of the optical axis from the relative the desired position along the specimen platform to be illuminated, the angle of the reflective surface can be altered accordingly.

[0106] In one application, illumination "hot spots" can be reduced by diffusing the directional beams as they reflect off of the reflective surface 116. This improves the light distribution across the specimen platform so that the illumination is substantially uniform. One diffuser technique is to provide a diffusing surface 114 which cooperates with the reflective surface 116 to uniformly diffuse the directional beams emitted from the strand distal ends 113. For example, the reflective surface 116 may be provided by an aluminum plate with a roughened surface or by SPECRALON®, which diffuses the reflected light as it impinges the surface thereof.

[0107] Another diffuser technique is to provide slightly convex reflective surface configured to provide substantially uniform illumination across the specimen platform 26. Other conventional diffuser techniques or a combination thereof may be employed without departing from the true nature and scope of the present invention. Moreover, the desired diffusion pattern after reflection off of the reflective surface is dependent in-part on the particular shape and radius of the convex reflective surface, the distance of the surface from the reflective surface, and the distance from the reflective surface to the specimen platform.

[0108] Each bracket device 117 includes a mounting section 118 adapted to removably secure the distal ends 113 of the fiber optic bundle in a manner directing their emitted directional beams of light against the reflective surface 116. As best viewed in FIGURES 15 and 16, the mounting section defines a passage 120 which is formed and dimensioned for sliding receipt of the fiber optic bundle 111 there through. An adjustable fastener 121 affixes

the bundle to the bracket device to enable the distal ends 113 of the fiber optic strands to be displaced closer to or further away from the reflective surface 116 during calibration. In one example, the fastener may be simply be a set screw or the like.

[0109] FIGURE 16 best illustrates that the distal ends 113 of the strands are positioned relatively close to the reflective surface 116 of the mirror, without actual contact therewith. By positioning the distal ends relatively close to the reflective surface, the diffusion of the reflected directional beams are better controlled to be substantially within the specimen platform 26 boundaries. However, it should be appreciated that the distal ends should not be too close or in contact with the reflective surface so as to minimize reflection back through the strand distal ends 113.

[0110] For example, the distal ends of the fiber optic strands, in one specific configuration, are positioned in the range of about 6 mm to about 11 mm from the reflective surface, and more preferably about 8 mm. In this example, the reflective surface 116 of the reflector 122 is positioned in the range of about 120 mm to about 460 mm from the specimen platform. Thus, the collective vertical footprint of this configuration is significantly reduced by applying this dispersion assembly.

[0111] Preferably, the dispersion assembly includes a plurality of bracket devices 117 peripherally spaced about the view port 23 of the interior wall 103. Such peripheral spacing even more uniformly distributes the reflected directional beams about the view port 23, and further reduces shadowing. Four (4) to eight (8) bracket devices 117 have been found sufficient to assure illumination uniformity, but more or less may be applied as well.

[0112] Referring now to FIGURES 12 and 17, the illumination assembly 28 may include a protective cover device 123 (shown in phantom lines in FIGURE 17). This cover provides protection for the lighting components as well as reduce the residual auto fluorescence of the lighting components. The protective cover device 123 includes corresponding apertures 124 (FIGURE 12) each aligned with the respective reflective surface 116, and extending through the bottom wall of the cover device to permit passage of the reflected directional beams. Further, a larger aperture 125 corresponding to and aligned with the view port 23 is provided to enable passage of the light to the camera.

[0113] As shown in FIGURES 18 and 19, the filter assembly 47 may include a mounting bracket (not shown) which affixes the housing 57 to one wall of the imaging apparatus. The side wall 85 of the housing 57 includes a passage 126 sized for sliding receipt of the downstream connector portion 127 of the filter housing 57 there through. A threaded nut 128 may also be provided which is fastened from inside the imaging chamber 25 to affix the filter assembly 47 to the imaging apparatus. The proximal connector end 130 of the fiber optic bundle 111 can then be removably attached to the downstream connector portion.

[0114] This arrangement is particularly suitable for this application since the length of the fused silica clad silica fiber optic bundle can be minimized. In effect, the path length of the filtered light passing through the fused clad silica fiber optic bundle is reduced significantly to minimize any auto fluorescence thereof, even though such material already has low auto fluorescence properties. Moreover, high purity or substantially pure fused clad silica is very expensive, and minimizing the length of the bundle ultimately reduces costs.

[0115] Referring now to FIGURES 20-24, in still another aspect of the present invention, an alternative embodiment macroscopic fluorescence illumination assembly, generally designated 140, is provided for use with the imaging apparatus 21. In this configuration, a bottom illumination configuration is provided that significantly reduces background fluorescent or autofluorescent signals emitted from the endogenous animal tissue itself. Briefly, this is one of the most significant limitations to fluorescent imaging contrast. Biological tissues have an inherent fluorescent signal that is strongly excited by excitation light that is in the blue/green region of the spectrum. These autofluorescent signals limit the sensitivity of detection by reducing the signal to background of the desired fluorescent labeling reagent.

[0116] There are several techniques that can reduce this background fluorescent signal, one of which includes moving the excitation light source wavelength towards the infrared region of the spectrum. In this region, the tissue autofluorescence is reduced, but not totally eliminated. The drawback to this technique is that it is not always feasible to use an infrared dye which would be necessary. Another approach to minimize the tissue autofluorescence signal from the sample is to alter the illumination configuration. The illumination design in the above-mentioned configuration is that of a front illumination profile, sometimes referred to as top illumination, where the excitation light source in the fluorescent kit and the detection equipment (i.e., camera 31) are on the same side of the specimen. Since the excitation light intensity is highest on the side of the specimen facing the camera 31, the tissue autofluorescent signal emitted is also highest on this side. Thus, the ratio of the signal to background noise of the desired fluorescent labeling reagent is significantly reduced, limiting the sensitivity of detection by the camera.

[0117] In accordance with the bottom illumination assembly design of the present invention, the excitation light source is located on the opposite side of the specimen as the detection system. Generally referred to as transillumination or bottom illumination, this illumination configuration reduces the autofluorescent signal from the sample by trapping the autofluorescent light on the opposite or bottom side of the animal relative to the camera.

[0118] Referring to FIGURES 20, 21 and 23, the bottom side illumination assembly 140 is shown including a specimen support surface 141 sized and dimensioned for receipt in the imaging compartment 25 atop the spec-

imen platform 26 of the imaging apparatus 21 (e.g., as shown in FIGURE 1). The support surface 141 is substantially opaque and defines a window portion 142 that enables the passage of light there through which is oriented to face toward the view port 23 thereof. The window portion is selectively sized and dimensioned such that when the specimen is supported atop the support surface 141, it can be positioned and seated fully over the window portion in a manner forming a light-tight seal substantially there between. The illumination assembly 140 further includes an excitation light source 37, and a bundle of fiber optic strands 143 having proximal ends thereof in optical communication with the light source 37. The distal ends of the strands terminate proximate the window portion of the support surface. The distal ends each emit a respective beam of light originating from the light source 37 which are then collectively directed toward the window portion 142 and into a bottom side of the specimen 145.

[0119] Accordingly, since biological tissue is a turbid medium, the excitation light entering the specimen becomes multiply scattered and diffused where it propagates throughout the entire specimen, thereby exciting the fluorophore. The fluorophore emission is also scattering by tissue, and eventually, some of the scattered excitation light exits the topsides of the specimen 145, and is captured through the view port 23 and into the camera 31.

[0120] In one specific configuration, the bottom illumination assembly 140 includes a specimen illumination platform, generally designated 146, having a support structure 147 and a cover plate 150 removably mounted atop the support structure. The support structure 147 is preferably rectangular shaped having four upstanding side walls 151 surrounding an interior cavity 152 thereof. The support structure 147 is preferably substantially rigid, and may be fabricated from one or more pieces of black anodized aluminum, however, to reduce auto fluorescence. Other rigid materials may be applied, as noted above. The interior surface of the upstanding walls 151 are preferably coated with a material which absorbs light, such as black anodize, or is composed of opaque materials having absorption properties, such as black Delrin. It will further be appreciated, of course, that the support structure can be other shapes as well.

[0121] Mounted atop the upper edges of the upstanding walls 151 is the cover plate 150 that incorporates the support surface 141 to support the specimen 145. The cover plate 150 is also preferably composed of a rigid material such as black anodized aluminum to reduce autofluorescence. The peripheral footprint of the cover plate is rectangular, and is sized to match that of the support structure 147 when mounted thereto. A plurality of set screws 156 (eight as shown) cooperate with aligned screw holes 157', 157 (FIGURE 24) in the respective cover plate 150 and support structure 147 to removably mount the two together. A gasket or the like may be provided between the interface to assure a light-tight seal there between.

[0122] Extending through the cover plate from the support surface 141 to a bottom side is an aperture 153 that enables the excitation light to pass from the interior cavity 152, and into the specimen. Thus, in some configurations, the aperture 153 functions as the window portion 142 of the support surface 141. This aperture is preferably rectangular shaped, but can be any size and/or shape to better coordinate with the shape of the specimen supported over the aperture. When the aperture 153 functions as the window portion, the specimen must be large enough to form a light-tight seal all around the edge of the aperture 153 when it is properly seated atop the support surface 141. Thus, essentially, the peripheral footprint of the aperture 153 must be sufficiently smaller than that of the properly oriented specimen 145 to form such a seal. It will be understood that without the formation of this light-tight seal between of the specimen with the edge defining the aperture, unscattered excitation light would leak into the imaging compartment 25 of the imaging apparatus 21 and be detected by the sensitive camera 31.

[0123] In one specific example, the opening (i.e., window portion 142) upon which light may pass through from the interior cavity 152 of the specimen illumination platform 146, and into the specimen can be sized and dimensioned to assure the formation of such a light-tight seal. This may be performed by providing a removable slide device 155 (FIGURES 23 and 24) or the like that is sized for aligned positioning into a receiving slot 158 formed in the support surface 141 of the cover plate 150. This receiving slot 158 is generally rectangular shaped, and is of a height profile such that when the slide device 155 is received in the receiving slot 158, the top surface of the slide device 155 is substantially flush with the top surface of the cover plate 150, thus becoming the support surface 141.

[0124] The slide device 155 is preferably formed of a transparent material, such as a glass slide, sized and dimensioned for receipt in the receiving slot 158 (FIGURES 21 and 24). By painting the top or bottom side of the glass with an opaque paint material, such as Krylon black paint, the transparent window portion 142 that permits the passage of the excitation light into the specimen can be formed from a void in the painted surface. Using a plurality or set of slide devices 155, a variety of different size and shape window portions 142 can be provided. Accordingly, the size of the window portion 142 can be altered by merely changing the slide device 155. As mentioned, should the specimen have a footprint large enough, the slide device 155 can be removed altogether and the specimen be placed directly atop and over the aperture 153.

[0125] To assure cleanliness of the slide device 155, a disposable lining (not shown) may be included which is positioned atop the support surface 141 of the slide device. This disposable lining, which may be composed of paper, is preferably dimensioned to seat into and along the peripheral lining edge 160 surrounding the receiving

slot 158 of the cover plate 150. This lining edge performs the function of aligning and seating the disposable lining, similar to that between the receiving slot 158 and the slide device 155. The lining includes an opening larger than the window portion 142 of the slide device 155 so as not to obstruct the passage of excitation light into the specimen.

[0126] Similar to the top illumination configuration above-mentioned, the bottom illumination assembly 140 includes a bundle 143 of fiber optic strands composed of materials selected to have low autofluorescence properties. Again, one material particularly suitable for the fiber optic strands and filters is high purity fused silica, such as plastic clad fused silica or silica clad fused silica, which has very low autofluorescence. Further, as indicated above, all exterior fiber optic bundle portions of the fiber optic bundle 35 include a segmented, flexible metal jacket (not shown) placed around these portions together with an exterior flexible PVC sleeve 42 to reduce the introduction of exterior light or noise into the fiber optic strands. This sleeve is opaque (black), and blocks all external ambient light that might enter the fiber bundle.

[0127] The fiber optic bundle portions internal to the imaging box are surrounded by a non-phosphorescent material to substantially eliminate the possibility of spurious light sources from the fiber optic bundle within the imaging box. One particular flexible material which has low phosphorescence is a polyolefin heat shrink tubing material. Again, all optical filters (e.g., interference-type filters including bandpass filters, longpass filters and shortpass filters), filter wheel assemblies, illumination sources, fiber optic connections external to and into the imaging apparatus 21 are similar to that discussed above. Further, while the composition of the fiber optic strands for the internal fiber optic bundle may be composed of fused silica, as mentioned above, they are preferably composed of conventional fiber optic glass. Relatively costly fused silica has lower autofluorescence properties, but this is not as critical for bottom illumination where the autofluorescence is trapped under the specimen where it is not detected or seen by the camera.

[0128] In this specific embodiment, the bundle of fiber optic strands for the bottom illumination assembly 140 originates from an interior side wall 85 of the imaging apparatus 21 similar to the top illumination assemblies such as the light-ring embodiment above. In the bottom illumination assembly 140, however, the optical connector into the imaging apparatus 21 may be positioned lower to the bottom specimen platform 26 so that the bundle of fiber optic strands can extend into the interior cavity 152 of the support structure 147 without being significantly bent.

[0129] FIGURES 22 and 24 best illustrates that the distal ends 161 of the fiber optic bundle 143 terminate in the interior cavity 152 of the specimen illumination platform 146. A bundle slot 162 is provided in one of the upstanding walls 151 of the support structure 147 for receipt of the fiber optic bundle portion 143 there through. In one configuration, the distal ends 161 of the fiber strands of the fiber optic bundle 143 are oriented to direct the conical beams of light emitted there from directly through the window portion 142 and into the specimen seated thereatop.

[0130] Similar to the dispersion assembly 110 of the top illumination assembly above, a reflector device 163 is included in the interior cavity 152 of the support structure 147 that is configured to cooperate with the distal ends 161 of the fiber optic strands to redirect the directional beams collectively through the window portion 142 of the slide device 155. Accordingly, the optical axes of the distal ends 161 of the fiber optic strands may be retained generally parallel to the horizontal plane of the fiber optic bundle portion extending through the bundle slot 162 and into the interior cavity 152 of the support structure 147, while the directional beams emitted from the strand distal ends are reflected (E.g., through reflector device 163) upwardly through the window portion 142 and into the specimen 145. The overall height of the bottom illumination assembly 140 can, thus, be significantly reduced since the distal ends of the fibers themselves need not be curved upward toward the window portion.

[0131] The reflector device 163 includes a reflective surface 165 oriented at an angle about 45° relative the direction substantially parallel to the optical axes of the distal ends of the fiber optic strands to reflect the directional beams of light about 90° from that emitted from the distal ends. To orient the reflector device 163 properly relative the distal ends 161 of the fiber optic bundle, mounting supports 166 are provided in the interior cavity 152 of the support structure upon which a backside of the reflector device seats. The reflector device 163 is then either permanently seated against the mounting supports through an adhesive, or removably mounted to permit interchangeability of the reflector device.

[0132] The reflective surface 165 is preferably mirror-like for non-diffuse reflection of the excitation light fully into the bottom side of the specimen. In this configuration, the turbid medium of the biological tissue is relied upon to multiply scatter and uniformly propagate the excitation light though the entire specimen. In other configurations, the reflective surface 165 may include a diffuse material that diffuses the light reflected through the window portion 142. As mentioned above, these diffuser materials may include a roughed aluminum surface or SPECRALON®.

[0133] The reflective surface 165 is preferably substantially planar. It is conceivable, however, that the reflective surface could be partially concave to reflectively focus the reflected excitation light toward a smaller area or through discretely shaped window portions.. This would be particularly valuable should the size and shape of the window portion be smaller than the size and shape of the layout distribution of the distal ends of the fiber optic bundle. In the configuration shown in FIGURES 22 and 24, however, the reflective surface 165 is preferably rectangular-shaped. Both the reflective surface 165 and

the layout distribution of the distal ends of the fiber optic bundle, as will be described, are sized and dimensioned to match one another. Accordingly, both the reflective surface and the layout distribution of the distal ends of the fiber optic bundle 143 are elongated and rectangular-shaped (i.e., conforming to the rectangular-shaped aperture 153 and window portion 142).

[0134] As shown, the distal ends of said fiber optic bundle are thus aligned in a linear array extending substantially along the elongated reflective surface 165. Preferably, the distal ends 161 in the fiber optic cable are rearranged from a round bundle (about 0.7 cm in diameter) to a line or an elongated rectangle array of fibers that is approximately 6 cm long and 0.2 cm wide. One example of a round to linear fiber cable is the distribution unit 167 that commercially available from Dolan Jenner Industries of Lawrence, MA, model no. BL405. In this example, the interior cavity 152 of the support structure 147 is sized to slideably receive the rectangular housing of the distribution unit 167 in a manner automatically aligning and spacing the distal ends 161 along and relative to the reflector device 163.

[0135] Although only a few embodiments of the present inventions have been described in detail, it should be understood that the present inventions may be embodied in many other specific forms without departing from the spirit or scope of the inventions.

Claims

1. A macroscopic fluorescence imaging assembly (20) comprising:

   an imaging apparatus (21) having an enclosure wall (22) defining a view port (23) into a light-tight imaging compartment (25) thereof;
   a specimen platform (26) positioned in said imaging compartment (25) having a support surface (27) facing toward said view port (23);
   an illumination assembly (28) having an illumination device (30) including a bundle (35) of fiber optic strands having proximal ends (36) thereof in optical communication with a light source (37), and corresponding illumination output distal ends (38) positioned proximate to and peripherally encircling said view port (23); and

   characterised in that the illumination output distal ends (38) are disposed directly into the imaging compartment (25) to attain substantially uniform illumination of the support surface (27) of the specimen platform (26) by light passing directly from the illumination output distal ends (38) to the support surface (27).

2. The imaging assembly (20) as defined by claim 1, wherein
   said illumination assembly (28) includes a frame (32) substantially peripherally encircling said view port (23), and adapted to support the output end of said illumination device (30).

3. The imaging assembly (20) as defined by claim 2, wherein
   said illumination output distal ends (38) thereof terminating at the frame (32) to emit a conical directional beam of light onto said specimen platform (26), said output ends (32) of said fiber optic strands (35) being sufficiently spaced peripherally about the view port (23) such that the plurality of directional beams collectively illuminate the specimen platform (26) in the substantially uniform manner.

4. The imaging assembly (20) as defined by claim 1, wherein
   the illumination output distal ends (38) of said fiber optic strands (35) are positioned substantially continuously about the periphery of said view port (23).

5. The imaging assembly (20) as defined by claim 1, wherein
   the illumination output distal ends (38) of said fiber optic strands (35) are positioned in the range of about 120.0 mm to about 380.0 mm from said specimen platform (26).

6. The imaging assembly (20) as defined by claim 5, wherein
   the illumination output distal ends (38) of said fiber optic strands (35) are positioned about 190 mm from said specimen platform (26).

7. The imaging assembly (20) as defined by claim 1, further including:

   an opaque material sleeve (42) extending substantially around the bundle of fiber optic strands (35) to substantially prevent external light leakage.

8. The imaging assembly (20) as defined by claim 1, further including:

   an excitation interference filter positioned in the path of the fiber optic strands (35) to filter the light passing therethrough.

9. The imaging assembly (20) as defined in claim 8, wherein
   said fiber optic strand bundle (35) includes a first bundle portion (50) and a second bundle portion (51), said first bundle portion (50) including said proximal end (36) of said fiber optic strand bundle (35) and a transmission end positioned proximate an upstream side of the interference filter to transmit substantially

all of the light therethrough, and said second bundle portion (51) including said illumination output distal ends (38) of said fiber optic strand bundle (35) and corresponding receiving ends positioned proximate a downstream side of the interference filter to receive substantially all of the transmitted light passing therethrough.

10. The imaging assembly (20) as defined in claim 9, further including:

a filter housing (57) containing the interference filter therein in a substantially light-tight recess (56), and
a collimating lens (67) disposed in said housing (57) between the transmission end of the first bundle portion (50) and said upstream side of the interference filter to collimate substantially all of the light passing therethrough.

11. The imaging assembly (20) as defined in claim 10, further including:

a collimating lens (67) disposed in said housing (57) between the receiving end of the second bundle portion (51) and said downstream side of the interference filter to collect substantially all of the collimated light passing therethrough.

12. The imaging assembly (20) as defined in claim 11, wherein
said housing (57) includes an upstream connector housing (61) the collimating lens (67) and adapted to removably couple to the transmission end of the first bundle portion (50).

13. The imaging assembly (20) as defined in claim 12, wherein
said housing (57) includes a downstream connector housing (65) the collecting lens (67) and adapted to removably couple to the receiving end of the second bundle portion (51).

14. The imaging assembly (20) as defined in claim 14, further including:

a light baffle (70) disposed in said connector (61) between the transmission end of the first bundle portion (50) and said collimating lens (67), said light baffle (70) configured to intercept light rays transmitted from the distal end (38) of the first bundle portion (50) which are sufficiently skewed from a direction substantially parallel to the optical axis of the distal end (38).

15. The imaging assembly (20) as defined in claim 14, wherein
said light baffle (70) includes an opaque plate member (75) disposed substantially adjacent an upstream abutting surface (71) of said collimating lens (67), said light baffle (70) having an aperture (72) extending therethrough having a transverse cross-sectional area smaller than that of the collimating lens abutting surface (71).

16. The imaging assembly (20) as defined in claim 14, wherein
said light baffle (70) includes a plurality of opaque plate members (75) nested substantially adjacent one another, and disposed substantially adjacent an upstream abutting surface (71) of said collimating lens (67), each said plate member (75) having a respective aperture (72) extending therethrough, and having a respective transverse cross-sectional area smaller than that of the collimating lens abutting surface (71).

17. The imaging assembly (20) as defined in claim 15 or 16, wherein
a longitudinal axis of said aperture (72) is substantially co-axially aligned with a longitudinal axis of said collimating lens (67).

18. The imaging assembly (20) as defined in claim 15 or 16, wherein
the ratio of the transverse cross-sectional area of the aperture (72) to that of the collimating lens abutting surface (71) is in the range of about 0.64:1 to about 0.8:1.

19. The imaging assembly (20) as defined in claim 15, wherein
the aperture (72) tapers outwardly in a direction toward the collimating lens abutting surface (71).

20. The imaging assembly (20) as defined in claim 16, wherein
each aperture (72) tapers outwardly in a direction toward the collimating lens abutting surface (71) such that an entrance diameter (Den) of the respective aperture (72) is less than an exit diameter (Dex) thereof.

21. The imaging assembly (20) as defined in claim 20, wherein
the respective entrance diameter (Den) and exit diameter (Dex) of each aperture (72) of the respective plate member (75) is successively larger than that of an adjacent upstream plate member (75).

22. The imaging assembly (20) as defined in claim 21, wherein
the respective entrance diameter (Den) of each aperture of the respective plate member (75) is successively smaller than the respective exit diameter (Dex) of the aperture (72) of the adjacent upstream

plate member (75).

23. The imaging assembly (20) as defined in claim 19 or 22, wherein
the outward taper is in the range of about 30° to about 60° from the longitudinal axis.

24. The imaging assembly (20) as defined in claim 16, wherein
each plate member (75) has a thickness in the range of about 1.0 mm to about 4 mm.

25. The imaging assembly (20) as defined in claim 15, wherein
said plate member (75) includes a plurality of annular ribs extending inwardly into said aperture (72).

26. The imaging assembly (20) as defined in claim 25, wherein
a downstream side of each annular rib tapers outwardly to intersect the upstream side of each adjacent downstream annular rib.

27. The imaging assembly (20) as defined in claim 26, wherein
the diameter of each downstream annular rib is successively larger than that of the adjacent upstream annular rib.

28. The imaging assembly (20) as defined in claim 1, wherein
said fiber optic strand bundle (35) includes a transmission portion having the proximal ends (36) of the fiber optic strands (35) on one end and transmission ends (77) on an opposite end thereof, and a receiving portion having the illumination output distal ends (38) of the fiber optic strands (35) on one end and receiving ends (78) on an opposite end thereof, said system further including:

a connector body (80) mounted to the imaging apparatus (21), and defining a passage (83) extending therethrough for optical communication from outside the enclosure to the imaging compartment (25) thereof, said body (80) having a proximal portion (81) adapted to couple to the transmission portion of the bundle (35) such that the transmission end (77) thereof terminates in said passage, and said body (80) having a distal portion (82) adapted to couple to the receiving portion of the bundle (35) such that the receiving end (78) thereof terminates in said passage (83) in opposed relationship to the bundle transmission end; and
a diffuser device (88) disposed in said passage (83) between the bundle transmission end (77) and the bundle receiving end (78), and adapted to diffuse the light transmitted from the transmission end (77) for receipt in the receiving end (78).

29. The imaging assembly (20) as defined in claim 28, wherein
said diffuser (88) is composed of frosted glass.

30. The imaging assembly (20) as defined in claim 29, wherein
said diffuser (88) is plate-like having substantially flat, opposed surfaces, and is disposed across the transverse cross-sectional dimension of the connector passage (83).

31. The imaging assembly (20) as defined in claim 30, wherein
said connector body (80) is adapted to position the bundle transmission end (77) and the bundle receiving end (78) a distance in the range of about 8.0 mm to about 15.0 mm from the respective opposed surface of the diffuser device (88).

32. The imaging assembly (20) as defined in claim 28, wherein
said proximal portion (81) of the connector body (80) is adapted to removably mount to the bundle transmission end (77).

33. The imaging assembly (20) as defined in claim 28, wherein
said distal portion (82) of the connector body (80) is adapted to removably mount to the bundle receiving end (78).

34. The imaging assembly (20) as defined in claim 1, further including:

a light dispersion assembly (110) positioned in the optical path of the conical-shaped directional beams emitted from the illumination output distal ends (38) for dispersion thereof to collectively illuminate the specimen platform (26) in said substantially uniform manner.

35. The imaging assembly (20) as defined in claim 34, wherein
said dispersion assembly (110) includes a plurality of bracket devices (117) peripherally spaced about said view port (23) of the interior wall,
wherein the illumination output distal ends (38) of said fiber optic strands (35) are separated into a plurality of distal end groups (38), each of which cooperates with a corresponding bracket device (117) such that the plurality of directional beams extending generally peripherally around the view port (23) collectively illuminate the specimen platform (26) in the substantially uniform manner.

36. The illumination assembly (20) as defined by claim

35, wherein
each said bracket device (117) includes a diffuser (88) positioned in the optical path of the directional beams from the respective distal end group (38) to diffuse the light toward the specimen platform (26).

37. The illumination assembly (20) as defined by claim 36, wherein
each said diffuser (86) includes a reflector device.

38. The illumination assembly (20) as defined by claim 37, wherein
each said reflector device (163) includes a substantially planar reflective surface oriented at an angle about 45° relative the direction substantially parallel to the optical axis of the respective distal end group (38) of the fiber optic strands (35) to reflect the respective directional beams of light emitted therefrom toward the specimen platform (26).

39. The illumination assembly (20) as defined by claim 38, wherein
each said reflective surface (163) includes a roughened aluminum plate to diffuse the light generally toward the specimen platform (26) for collective illumination thereof in the substantially uniform manner.

40. The illumination assembly (20) as defined by claim 38, wherein
each illumination output distal ends (38) of the respective distal end group of said fiber optic strands (35) are positioned in the range of about 6 mm to about 11 mm from the reflective surface of the respective reflector device (163).

41. The illumination assembly (20) as defined by claim 1, wherein
said bundle of fiber optic strands (35) is composed of substantially pure fused silica.

42. The illumination assembly (20) as defined by claim 40, wherein
the reflective surface of each said reflector (163) is positioned in the range of about 120 mm to about 460 mm from said specimen platform (26).

43. The illumination assembly (20) as defined by claim 41, wherein
said substantially pure fused silica is one of plastic clad fused silica and silica clad fused silica.

44. The illumination assembly (20) as defined by claim 35, wherein
said dispersion assembly (110) includes at least four bracket devices.

Patentansprüche

1. Makroskopische Fluoreszenzbildgebungsanordnung (20), umfassend:

eine Bildgebungsvorrichtung (21) mit einer Gehäusewand (22), die eine Aufnahmeöffnung (23) in einen lichtdichten Bildgebungsraum (25) davon vorgibt;
eine Probenstandfläche (26), die in dem Bildgebungsraum (25) angeordnet ist, der eine Haltefläche (27) aufweist, die der Aufnahmeöffnung (23) gegenüberliegt;
eine Beleuchtungsanordnung (28) mit einer Beleuchtungsvorrichtung (30), die ein Bündel (35) Lichtwellenleiterstränge beinhaltet, deren proximale Enden (36) in optischer Verbindung mit einer Lichtquelle (37) stehen und die jeweiligen distalen Beleuchtungsausgangsenden (38) benachbart zu und die Aufnahmeöffnung (23) peripher umgebend angeordnet sind; und

dadurch gekennzeichnet, dass
die distalen Beleuchtungsausgangsenden (38) unmittelbar in den Bildgebungsraum (25) hinein angeordnet sind, um eine im Wesentlichen gleichmäßige Beleuchtung der Haltefläche (27) der Probenstandfläche (26) durch Licht zu erreichen, das direkt von den distalen Beleuchtungsausgangsenden (38) auf die Haltefläche (27) fällt.

2. Bildgebungsanordnung (20) gemäß Anspruch 1, worin
die Beleuchtungsanordnung (28) einen Rahmen (32) beinhaltet, der die Aufnahmeöffnung (23) im Wesentlichen peripher umgibt und ausgelegt ist, das Ausgangsende der Beleuchtungsvorrichtung (30) zu halten.

3. Bildgebungsanordnung (20) gemäß Anspruch 2, worin
die distalen Beleuchtungsausgangsenden (38) davon an dem Rahmen (32) enden, um einen kegelförmigen, gerichteten Lichtstrahl auf die Probenstandfläche (26) abzustrahlen, wobei die Ausgangsenden (32) der Lichtwellenleiterstränge (35) derart ausreichend peripher um die Aufnahmeöffnung (23) beabstandet sind, dass die mehreren gerichteten Strahlen gemeinsam die Probenstandfläche (26) in der im Wesentlichen gleichmäßigen Weise beleuchten.

4. Bildgebungsanordnung (20) gemäß Anspruch 1, worin
die distalen Beleuchtungsausgangsenden (38) der Lichtwellenleiterstränge (35) im Wesentlichen fortlaufend um den Umfang der Aufnahmeöffnung (23) angeordnet sind.

5. Bildgebungsanordnung (20) gemäß Anspruch 1, worin
die distalen Beleuchtungsausgangsenden (38) der Lichtwellenleiterstränge (35) im Bereich von etwa 120,0 mm bis etwa 380,0 mm von der Probenstandfläche (26) angeordnet sind.

6. Bildgebungsanordnung (20) gemäß Anspruch 5, worin
die distalen Beleuchtungsausgangsenden (38) der Lichtwellenleiterstränge (35) etwa 190 mm von der Probenstandfläche (26) angeordnet sind.

7. Bildgebungsanordnung (20) gemäß Anspruch 1, ferner beinhaltend:

einen opaken Materialmantel (42), der sich im Wesentlichen um das Bündel der Lichtwellenleiterstränge (35) erstreckt, um im Wesentlichen einen äußeren Lichtverlust zu verhindern.

8. Bildgebungsanordnung (20) gemäß Anspruch 1, ferner beinhaltend:

ein Anregungsinterferenzfilter, das in dem Weg der Lichtwellenleiterstränge (35) angeordnet ist, um das hindurch laufende Licht zu filtern.

9. Bildgebungsanordnung (20) gemäß Anspruch 8, worin
das Lichtwellenleiterstrangbündel (35) einen ersten Bündelteil (50) und einen zweiten Bündelteil (51) beinhaltet, wobei der erste Bündelteil (50) das proximale Ende (36) des Lichtwellenleiterstrangbündels (35) und ein Übertragungsende beinhaltet, das benachbart zu einer strahlaufwärts liegenden Seite des Interferenzfilters angeordnet ist, um im Wesentlichen das gesamte Licht hindurch zu übertragen, und der zweite Bündelteil (51) die distalen Beleuchtungsausgansgsenden (38) des Lichtwellenleiterstrangbündels (35) und entsprechende Empfangsenden beinhaltet, die benachbart zu einer strahlabwärts liegenden Seite des Interferenzfilters angeordnet sind, um im Wesentlichen das gesamte übertragene, hindurch laufende Licht zu empfangen.

10. Bildgebungsanordnung (20) gemäß Anspruch 9, ferner beinhaltend:

ein Filtergehäuse (57), das darin das Interferenzfilter in einer im Wesentlichen lichtdichten Aussparung (56) beinhaltet, und
eine Kollimatorlinse (67), die in dem Gehäuse (57) zwischen dem Übertragungsende des ersten Bündelteils (50) und der strahlaufwärts liegenden Seite des Interferenzfilters angeordnet ist, um im Wesentlichen das gesamte hindurch laufende Licht zu kollimieren.

11. Bildgebungsanordnung (20) gemäß Anspruch 10, ferner beinhaltend:

eine Kollimatorlinse (67), die in dem Gehäuse (57) zwischen dem Empfangsende des zweiten Bündelteils (51) und der strahlabwärts liegenden Seite des Interferenzfilters angeordnet ist, um im Wesentlichen das gesamte hindurch laufende Kollimationslicht zu sammeln.

12. Bildgebungsanordnung (20) gemäß Anspruch 11, worin
das Gehäuse (57) einen strahlaufwärts liegenden Verbinder (61) beinhaltet, der die Kollimatorlinse (67) aufnimmt und ausgelegt ist, das Übertragungsende des ersten Bündelteils (50) abnehmbar anzukoppeln.

13. Bildgebungsanordnung (20) gemäß Anspruch 12, worin
das Gehäuse (57) einen strahlabwärts liegenden Verbinder (65) beinhaltet, der die Sammellinse (67) aufnimmt und ausgelegt ist, das Empfangsende des zweiten Bündelteils (51) abnehmbar anzukoppeln.

14. Bildgebungsanordnung (20) gemäß Anspruch 14, ferner beinhaltend:

eine Lichtblende (70), die in dem Verbinder (61) zwischen dem Übertragungsende des ersten Bündelteils (50) und der Kollimatorlinse (67) angeordnet ist, wobei die Lichtblende (70) ausgestaltet ist, die von dem distalen Ende (38) des ersten Bündelteils (50) ausgesendeten Lichtstrahlen abzufangen, die ausreichend schräg zu einer im Wesentlichen zur optischen Achse des distalen Endes (38) parallelen Richtung verlaufen.

15. Bildgebungsanordnung (20) gemäß Anspruch 14, worin
die Lichtblende (70) ein opakes Scheibenelement (75) beinhaltet, das im Wesentlichen benachbart zu einer strahlaufwärts liegenden Stirnfläche (71) der Kollimatorlinse (67) angeordnet ist, wobei die Lichtblende (70) eine sich hindurch erstreckende Apertur (72) mit einer Transversalquerschnittsfläche aufweist, die kleiner ist als die der Kollimatorlinsenstirnfläche (71).

16. Bildgebungsanordnung (20) gemäß Anspruch 14, worin
die Lichtblende (70) mehrere opake Scheibenelemente (75) beinhaltet, die im Wesentlichen benachbart zueinander verschachtelt sind und im Wesentlichen angrenzend an eine strahlaufwärts liegende Stirnfläche (71) der Kollimatorlinse (67) angeordnet sind, wobei jedes Scheibenelement (75) eine ent-

sprechende sich hindurch erstreckende Apertur (72) mit einer jeweiligen Transversalquerschnittsfläche aufweist, die kleiner ist als die der Kollimatorlinsenstirnfläche (71).

17. Bildgebungsanordnung (20) gemäß Anspruch 15 oder 16, worin
eine Längsachse der Apertur (72) im Wesentlichen koaxial zu einer Längsachse der Kullimatorlinse (67) ausgerichtet ist.

18. Bildgebungsanordnung (20) gemäß Anspruch 15 oder 16, worin
das Verhältnis der Transversalquerschnittstläche der Apertur (72) zur Kollimatorlinsenstirnfläche (71) im Bereich von etwa 0,64:1 bis etwa 0,8:1 liegt.

19. Bildgebungsanordnung (20) gemäß Anspruch 15, worin
die Apertur (72) nach außen in Richtung der Kollimatorlinsenstirnfläche (71) konisch verläuft.

20. Bildgebungsanordnung (20) gemäß Anspruch 16, worin
jede Apertur (72) nach außen in Richtung der Kollimatorlinsenstirnfläche (71) derart konisch verläuft, dass ein Eingangsdurchmesser (Den) der entsprechenden Apertur (72) kleiner ist als ein Ausgangsdurchmesser (Dex) davon.

21. Bildgebungsanordnung (20) gemäß Anspruch 20, worin
der jeweilige Eingangsdurchmesser (Den) und Ausgangsdurchmesser (Dex) jeder Apertur (72) des entsprechenden Scheibenelements (75) stufenweise größer ist als der eines benachbarten, strahlaufwärts liegenden Scheibenelements (75).

22. Bildgebungsanordnung (20) gemäß Anspruch 21, worin
der jeweilige Eingangsdurchmesser (Den) jeder Apertur des entsprechenden Scheibenelements (75) stufenweise kleiner ist als der jeweilige Ausgangsdurchmesser (Dex) der Apertur (72) des benachbarten strahlaufwärts liegenden Scheibenelements (75).

23. Bildgebungsanordnung (20) gemäß Anspruch 19 oder 22, worin
der nach außen gerichtete Konus im Bereich von etwa 30° bis etwa 60° zur Längsachse liegt.

24. Bildgebungsanordnung (20) gemäß Anspruch 16, worin
jedes Scheibenelement (75) eine Dicke im Bereich von etwa 1,0 mm bis etwa 4,0 mm aufweist.

25. Bildgebungsanordnung (20) gemäß Anspruch 15, worin
das Scheibenelement (75) mehrere ringförmige Rippen beinhaltet, die sich nach innen in die Apertur (72) hinein erstrecken.

26. Bildgebungsanordnung (20) gemäß Anspruch 25, worin
eine strahlabwärts liegende Seite jeder ringförmigen Rippe nach außen konisch verläuft. um die strahlaufwärts liegende Seite jeder benachbarten strahlabwärts liegenden ringförmigen Rippe zu schneiden.

27. Bildgebungsanordnung (20) gemäß Anspruch 26, worin
der Durchmesser jeder strahlabwärts liegenden ringförmigen Rippe stufenweise größer ist als jener der benachbarten strahlaufwärts liegenden ringförmigen Rippe.

28. Bildgebungsanordnung (20) gemäß Anspruch 1, worin
das Lichtwellenleiterstrangbündet (35) einen Übertragungsteil mit proximalen Enden (36) der Lichtwellenleiterstränge (35) an einem Ende und Übertragungsenden (77) an einem abgewandten Ende davon und einen Empfangsteil mit den distalen Beleuchtungsausgangsenden (38) der Lichtwellenleiterstränge (35) an einem Ende und Empfangsenden (78) an einem abgewandten Ende davon beinhaltet, wobei das System ferner beinhaltet:

einen Verbinderkörper (80), der an der Bildgebungsvorrichtung (21) angebracht ist und einen sich hindurch erstreckenden Durchgang (83) zur optischen Verbindung von außerhalb des Gehäuses in den Bildgebungsraum (25) davon vorgibt, wobei der Körper (80) einen proximalen Teil (81) aufweist, der ausgelegt ist, sich mit dem Übertragungsteil des Bündels (35) derart zu verbinden, dass das Übertragungsende (77) davon in dem Durchgang endet, und der Körper (80) einen distalen Teil (82) aufweist, der ausgelegt ist, sich mit dem Empfangsteil des Bündels (35) derart zu verbinden, dass das Empfangsende (78) davon in dem Durchgang (83) in eine gegenüberliegenden Beziehung zum Übertragungsendbündel endet; und
eine Diffusorvorrichtung (88), die in dem Durchgang (83) zwischen dem Übertragungsendbündel (77) und dem Empfangsendbündel (78) angeordnet und ausgelegt ist, das aus dem Übertragungsende (77) für den Empfang in dem Empfangsende (78) ausgesandte Licht zu streuen.

29. Bildgebungsanordnung (20) gemäß Anspruch 28, worin

der Diffusor (88) aus Mattglas besteht.

30. Bildgebungsanordnung (20) gemäß Anspruch 29, worin
der Diffusor (88) scheibenförmig ist und im Wesentlichen flache abgewandte Oberflächen aufweist und quer über der Transversalquerschnittsabmessung des Verbindungsdurchgangs (83) angeordnet ist.

31. Bildgebungsanordnung (20) gemäß Anspruch 30, worin
der Verbinderkörper (80) ausgelegt ist, das Übertragungsendbündel (77) und das Empfangsendbündel (78) in einem Abstand im Bereich von etwa 8,0 mm bis etwa 15.0 mm von der entsprechenden gegenüberliegenden Oberfläche der Diffusorvorrichtung (88) anzuordnen.

32. Bildgebungsanordnung (20) gemäß Anspruch 28, worin
der proximale Teil (81) des Verbinderkörpers (80) ausgelegt ist, abnehmbar an dem Übertragungsendbündel (77) angebracht zu werden.

33. Bildgebungsanordnung (20) gemäß Anspruch 28, worin
der distale Teil (82) des Verbinderkörpers (80) ausgelegt ist, abnehmbar an dem Empfangsendbündel (78) angebracht zu werden.

34. Bildgebungsanordnung (20) gemäß Anspruch 1, ferner beinhaltend
eine Lichtstreuanordnung (110), die im optischen Weg der kegelförmigen gerichteten Strahlen, die von den distalen Beleuchtungsausgangsenden (38) abgestrahlt werden, zur Streuung davon angeordnet ist, um die Probenstandtläche (26) gemeinsam in der im Wesentlichen gleichmäßigen Weise zu beleuchten.

35. Bildgebungsanordnung (20) gemäß Anspruch 34, worin
die Streuanordnung (110) mehrere Winkelvorrichtungen (117) beinhaltet, die peripher um die Aufnahmeöffnung (23) der Innenwand beabstandet sind, worin die distalen Beleuchtungsausgangsenden (38) der Lichtwellenleiterstränge (35) in mehrere distale Endgruppen (38) unterteilt sind, von denen jede mit einer entsprechenden Winkelvorrichtung (117) derart zusammenwirkt, dass die mehreren gerichteten Strahlen, die sich allgemein peripher um die Aufnahmeöffnung (23) erstrecken, gemeinsam die Probenstandfläche (26) in der im Wesentlichen gleichmäßigen Weise beleuchten.

36. Bildgebungsanordnung (20) gemäß Anspruch 35, worin
jede Winkelvorrichtung (117) einen Diffusor (88) beinhaltet, der in dem optischen Weg der gerichteten Strahlen von der jeweiligen distalen Endgruppe (38) angeordnet ist, um das Licht in Richtung der Probenstandfläche (26) zu streuen.

37. Bildgehungsanordnung (20) gemäß Anspruch 36, worin
jeder Diffusor (86) eine Reflektorvorrichtung beinhaltet.

38. Bildgebungsanordnung (20) gemäß Anspruch 37, worin
jede Reflektorvorrichtung (163) eine im Wesentlichen ebene reflektierende Oberfläche beinhaltet, die in einem Winkel von etwa 45° relativ zur im Wesentlichen zur optischen Achse der jeweiligen distalen Endgruppe (38) der Lichtwellenleiterstränge (35) parallelen Richtung ausgerichtet ist, um die entsprechenden gerichteten davon abgestrahlten Lichtstrahlen in Richtung der Probenstandfläche (26) zu reflektieren.

39. Bildgebungsanordnung (20) gemäß Anspruch 38, worin
jede reflektierende Oberfläche (163) eine aufgeraute Aluminiumplatte beinhaltet, um das Licht allgemein in Richtung der Probenstandfläche (26) zur gemeinsamen Beleuchtung davon in der im Wesentlichen gleichmäßigen Weise zu streuen.

40. Bildgebungsanordnung (20) gemäß Anspruch 38, worin
jedes distale Beleuchtungsausgangsende (38) der entsprechenden distalen Endgruppe der Lichtwellenleiterstränge (35) im Bereich von etwa 6 mm bis etwa 11 mm von der reflektierenden Oberfläche der jeweiligen Reflektorvorrichtung (163) angeordnet ist.

41. Bildgebungsanordnung (20) gemäß Anspruch 1, worin
das Bündel der Liclitwellenleiterstränge (35) im Wesentlichen aus reinem Quarzglas gebildet ist.

42. Bildgebungsanordnung (20) gemäß Anspruch 40, worin
die reflektierende Oberfläche jedes Reflektors (163) im Bereich von etwa 120 mm bis etwa 460 mm von der Probenstandfläche (26) angeordnet ist.

43. Bildgebungsanordnung (20) gemäß Anspruch 41, worin
das im Wesentlichen reine Quarzglas Polymermantel-Quarzglas oder Quarzmantel-Quarzglas ist.

44. Bildgebungsanordnung (20) gemäß Anspruch 35, worin
die Streuanordnung (110) wenigstens vier Winkel-

vorrichtungen beinhaltet.

Revendications

1. Ensemble d'imagerie à fluorescence macroscopique (20) comprenant :

   un appareil d'imagerie (21) ayant une paroi d'enceinte (22) définissant un hublot (23) dans son compartiment d'imagerie étanche à la lumière (25) ;
   une plate-forme d'échantillon (26) positionnée dans ledit compartiment d'imagerie (25) ayant une surface de support (27) orientée vers ledit hublot (23) ;
   un ensemble d'éclairage (28) ayant un dispositif d'éclairage (30) comprenant un faisceau (35) de brins de fibre optique ayant leurs extrémités proximales (36) en communication optique avec une source de lumière (37), et des extrémités distales de sortie d'éclairage (38) correspondantes positionnées à proximité dudit hublot (23) et encerclant de manière périphérique ledit hublot (23) ; et

   caractérisé en ce que les extrémités distales de sortie d'éclairage (38) sont disposées directement dans le compartiment d'imagerie (25) pour obtenir un éclairage sensiblement uniforme de la surface de support (27) de la plate-forme d'échantillon (26) en faisant passer directement la lumière des extrémités distales de sortie d'éclairage (38) sur la surface de support (27).

2. Ensemble d'imagerie (20) selon la revendication 1, dans lequel :

   ledit ensemble d'éclairage (28) comprend un châssis (32) encerclant de manière sensiblement périphérique ledit hublot (23), et adapté pour supporter l'extrémité de sortie dudit dispositif d'éclairage (30).

3. Ensemble d'imagerie (20) selon la revendication 2, dans lequel :

   lesdites extrémités distales de sortie d'éclairage (38) de celui-ci se terminant au niveau du châssis (32) pour émettre un faisceau de lumière directionnel conique sur ladite plate-forme d'échantillon (26), lesdites extrémités de sortie (32) desdits brins de fibre optique (35) étant suffisamment espacées de manière périphérique autour du hublot (23) de sorte que la pluralité de faisceaux directionnels éclairent collectivement la plate-forme d'échantillon (26) d'une manière sensiblement uniforme.

4. Ensemble d'imagerie (20) selon la revendication 1, dans lequel :

   les extrémités distales de sortie d'éclairage (38) desdits brins de fibre optique (35) sont positionnées de manière sensiblement continue autour de la périphérie dudit hublot (23).

5. Ensemble d'imagerie (20) selon la revendication 1, dans lequel :

   les extrémités distales de sortie d'éclairage (38) desdits brins de fibre optique (35) sont positionnées d'environ 120,0 mm à environ 380,0 mm de ladite plate-forme d'échantillon (26).

6. Ensemble d'imagerie (20) selon la revendication 5, dans lequel :

   les extrémités distales de sortie d'éclairage (38) desdits brins de fibre optique (35) sont positionnées à environ 180 mm de ladite plate-forme d'échantillon (26).

7. Ensemble d'imagerie (20) selon la revendication 1, comprenant en outre :

   un manchon (42) en matériau opaque s'étendant sensiblement autour du faisceau de brins de fibre optique (35) pour empêcher sensiblement la fuite de lumière externe.

8. Ensemble d'imagerie (20) selon la revendication 1, comprenant en outre :

   un filtre d'interférence d'excitation positionné dans la trajectoire des brins de fibre optique (35) pour filtrer la lumière passant à travers celui-ci.

9. Ensemble d'imagerie (20) selon la revendication 8, dans lequel :

   ledit faisceau de brins de fibre optique (35) comprend une première partie de faisceau (50) et une seconde partie de faisceau (51), ladite première partie de faisceau (50) comprenant ladite extrémité proximale (36) dudit faisceau de brins de fibre optique (35) et une extrémité de transmission positionnée à proximité d'un côté amont du filtre d'interférence pour transmettre sensiblement toute la lumière à travers celui-ci, et ladite seconde partie de faisceau (51) comprenant lesdites extrémités distales de sortie d'éclairage (38) dudit faisceau de brins de fibre optique (35) et des extrémités de réception correspondantes positionnées à proximité d'un côté aval du filtre d'interférence pour recevoir sensiblement toute la lumière transmise passant à travers celui-ci.

10. Ensemble d'imagerie (20) selon la revendication 9, comprenant en outre :

un boîtier de filtre (57) contenant le filtre d'interférence à l'intérieur de celui-ci dans un évidement (56) sensiblement étanche à la lumière, et une électrode de collimation (67) disposée dans ledit boîtier (57) entre l'extrémité de transmission de la première partie de faisceau (50) et ledit côté amont du filtre d'interférence pour collimater sensiblement toute la lumière passant à travers celui-ci.

11. Ensemble d'imagerie (20) selon la revendication 10, comprenant en outre :

une électrode de collimation (67) disposée dans ledit boîtier (57) entre l'extrémité de réception de la seconde partie de faisceau (51) et ledit côté aval du filtre d'interférence pour collecter sensiblement toute la lumière collimatée passant à travers celui-ci.

12. Ensemble d'imagerie (20) selon la revendication 11, dans lequel :

ledit boîtier (57) comprend un connecteur amont (61) logeant l'électrode de collimation (67) et adapté pour se coupler de manière amovible à l'extrémité de transmission de la première partie de faisceau (50).

13. Ensemble d'imagerie (20) selon la revendication 12, dans lequel :

ledit boîtier (57) comprend un connecteur aval (65) logeant l'électrode de collimation (67) et adapté pour se coupler de manière amovible à l'extrémité de réception de la seconde partie de faisceau (51).

14. Ensemble d'imagerie (20) selon la revendication 13, comprenant en outre :

un déflecteur de lumière (70) disposé dans ledit connecteur (61) entre l'extrémité de transmission de la première partie de faisceau (50) et ladite électrode de collimation (67), ledit déflecteur de lumière (70) étant configuré pour intercepter les rayons lumineux transmis à partir de l'extrémité distale (38) de la première partie de faisceau (50) qui sont suffisamment déviés d'une direction sensiblement parallèle à l'axe optique de l'extrémité distale (38).

15. Ensemble d'imagerie (20) selon la revendication 14, dans lequel :

ledit déflecteur de lumière (70) comprend un élément de plaque opaque (75) disposé sensiblement de manière adjacente à une surface de butée amont (71) de ladite électrode de collimation (67), ledit déflecteur de lumière (70) ayant une ouverture (72) s'étendant à travers celui-ci ayant une surface transversale inférieure à celle de la surface de butée (71) de l'électrode de collimation.

16. Ensemble d'imagerie (20) selon la revendication 14, dans lequel :

ledit déflecteur de lumière (70) comprend une pluralité d'éléments de plaque opaque (75) emboîtés de manière sensiblement adjacente les uns par rapport aux autres et disposés de manière sensiblement adjacente à une surface de butée amont (71) de ladite électrode de collimation (67), chacun desdits éléments de plaque (75) ayant une ouverture (72) respective s'étendant à travers celui-ci et ayant une surface transversale respective inférieure à celle de la surface de butée (71) de l'électrode de collimation.

17. Ensemble d'imagerie (20) selon la revendication 15 ou 16, dans lequel :

un axe longitudinal de ladite ouverture (72) est aligné de manière sensiblement coaxiale avec un axe longitudinal de ladite électrode de collimation (67).

18. Ensemble d'imagerie (20) selon la revendication 15 ou 16, dans lequel :

le rapport de la surface transversale de l'ouverture (72) sur celle de la surface de butée (71) de l'électrode de collimation est de l'ordre d'environ 0,64:1 à environ 0,8:1.

19. Ensemble d'imagerie (20) selon la revendication 15, dans lequel :

l'ouverture (72) se rétrécit progressivement vers l'extérieur dans une direction allant vers la surface de butée (71) de l'électrode de collimation.

20. Ensemble d'imagerie (20) selon la revendication 16, dans lequel :

chaque ouverture (72) se rétrécit progressivement vers l'extérieur dans une direction allant vers la surface de butée (71) de l'électrode de collimation de sorte qu'un diamètre d'entrée (Den) de l'ouverture (72) respective est inférieur à son diamètre de sortie (Dex),

21. Ensemble d'imagerie (20) selon la revendication 20, dans lequel :

le diamètre d'entrée (Den) et le diamètre de sortie (Dex) respectif de chaque ouverture (72) de l'élément de plaque (75) respectif sont successivement plus grands que ceux d'un élément de plaque (75) adjacent amont.

22. Ensemble d'imagerie (20) selon la revendication 21, dans lequel :

le diamètre d'entrée (Den) respectif de chaque ouverture de l'élément de plaque (75) respectif est successivement plus petit que le diamètre de sortie (Dex) respectif de l'ouverture (72) de l'élément de plaque amont (75) adjacent.

23. Ensemble d'imagerie (20) selon la revendication 19 ou 22, dans lequel :

la conicité vers l'extérieur est de l'ordre d'environ 30° à environ 60° par rapport à l'axe longitudinal.

24. Ensemble d'imagerie (20) selon la revendication 16, dans lequel :

chaque élément de plaque (75) a une épaisseur de l'ordre d'environ 1,0 mm à environ 4 mm.

25. Ensemble d'imagerie (20) selon la revendication 15, dans lequel :

ledit élément de plaque (75) comprend une pluralité de nervures annulaires s'étendant vers l'intérieur dans ladite ouverture (72).

26. Ensemble d'imagerie (20) selon la revendication 25, dans lequel :

un côté aval de chaque nervure annulaire rétrécit progressivement vers l'extérieur pour couper le côté amont de chaque nervure annulaire aval adjacente.

27. Ensemble d'imagerie (20) selon la revendication 26, dans lequel :

le diamètre de chaque nervure annulaire aval est successivement plus grand que celui de la nervure annulaire amont adjacente.

28. Ensemble d'imagerie (20) selon la revendication 1, dans lequel :

ledit faisceau de brins de fibre optique (35) comprend une partie de transmission ayant les extrémités proximales (36) des brins de fibre optique (35) sur une extrémité et des extrémités de transmission (77) sur son extrémité opposée, et une partie de réception ayant les extrémités distales de sortie d'éclairage (38) des brins de fibre optique (35) sur une extrémité et des extrémités de réception (78) sur son extrémité opposée, ledit système comprenant en outre :

un corps de connecteur (80) monté sur l'appareil d'imagerie (21), et définissant un passage (83) s'étendant à travers celui-ci pour la communication optique de l'extérieur de l'enceinte jusqu'à son compartiment d'imagerie (25), ledit corps (80) ayant une partie proximale (81) adaptée pour se coupler à la partie de transmission du faisceau (35) de sorte que son extrémité de transmission (77) se termine dans ledit passage, et ledit corps (80) ayant une partie distale (82) adaptée pour se coupler à la partie de réception du faisceau (35) de sorte que son extrémité de réception (78) se termine dans ledit passage (83) en relation opposée à l'extrémité de transmission de faisceau ; et un dispositif diffuseur (88) disposé dans ledit passage (83) entre l'extrémité de transmission de faisceau (77) et l'extrémité de réception de faisceau (78), et adapté pour diffuser la lumière transmise par l'extrémité de transmission (77) pour la réception dans l'extrémité de réception (78).

29. Ensemble d'imagerie (20) selon la revendication 28, dans lequel :

ledit diffuseur (88) est composé de verre glacé.

30. Ensemble d'imagerie (20) selon la revendication 29, dans lequel :

ledit diffuseur (88) est en forme de plaque ayant des surfaces opposées sensiblement plates, et est disposé sur la dimension transversale du passage de connecteur (83).

31. Ensemble d'imagerie (20) selon la revendication 30, dans lequel :

ledit corps de connecteur (80) est adapté pour positionner l'extrémité de transmission de faisceau (77) et l'extrémité de réception de faisceau (78) sur une distance de l'ordre d'environ 8,0 mm à environ 15,0 mm de la surface opposée respective du dispositif diffuseur (88).

32. Ensemble d'imagerie (20) selon la revendication 28, dans lequel :

ladite partie proximale (81) du corps de connecteur (80) est adaptée pour se monter de manière amovible sur l'extrémité de transmission de faisceau (77).

33. Ensemble d'imagerie (20) selon la revendication 28, dans lequel :

ladite partie distale (82) du corps de connecteur (80) est adaptée pour se monter de manière amovible sur l'extrémité de réception de faisceau (78).

34. Ensemble d'imagerie (20) selon la revendication 1, comprenant en outre :

un ensemble de dispersion de lumière (110) disposé dans la trajectoire optique des faisceaux directionnels de forme conique émis par les extrémités distales de sortie d'éclairage (38) pour leur dispersion afin d'éclairer de manière collective la plate-forme d'échantillon (26) d'une manière sensiblement uniforme.

35. Ensemble d'imagerie (20) selon la revendication 34, dans lequel :

ledit ensemble de dispersion (110) comprend une pluralité de dispositifs formant console (117) espacés de manière périphérique autour dudit hublot (23) de la paroi intérieure,

dans lequel les extrémités distales de sortie d'éclairage (38) desdits brins de fibre optique (35) sont séparées en une pluralité de groupes d'extrémités distales (38), dont chacun coopère avec un dispositif formant console (117) correspondant de sorte que la pluralité de faisceaux directionnels s'étendant généralement de manière périphérique autour du hublot (23) éclairent collectivement la plate-forme d'échantillon (26) d'une manière sensiblement uniforme.

36. Ensemble d'éclairage (20) selon la revendication 35, dans lequel :

chacun desdits dispositifs formant console (117) comprend un diffuseur (88) positionné dans la trajectoire optique des faisceaux directionnels du groupe d'extrémités distales (38) respectif pour diffuser la lumière vers la plate-forme d'échantillon (26).

37. Ensemble d'éclairage (20) selon la revendication 36, dans lequel :

chacun desdits diffuseurs (86) comprend un dispositif de réflexion.

38. Ensemble d'éclairage (20) selon la revendication 37, dans lequel :

chacun desdits dispositifs de réflexion (163) comprend une surface réfléchissante sensiblement plane orientée selon un angle d'environ 45° par rapport à la direction sensiblement perpendiculaire à l'axe optique du groupe d'extrémités distales (38) respectif des brins de fibre optique (35) pour réfléchir les faisceaux directionnels respectifs de lumière émise à partir de ceux-ci vers la plate-forme d'échantillon (26).

39. Ensemble d'éclairage (20) selon la revendication 38, dans lequel :

chacune desdites surfaces réfléchissantes (163) comprend une plaque d'aluminium rugueuse pour diffuser la lumière généralement vers la plate-forme d'échantillon (26) pour son éclairage collectif d'une manière sensiblement uniforme.

40. Ensemble d'éclairage (20) selon la revendication 38, dans lequel :

chacune des extrémités distales de sortie d'éclairage (38) du groupe d'extrémités distales respectif desdits brins de fibre optique (35) est positionnée d'environ 6 mm à environ 11 mm de la surface réfléchissante du dispositif de réflexion (163) respectif.

41. Ensemble d'éclairage (20) selon la revendication 1, dans lequel :

ledit faisceau de brins de fibre optique (35) est composé de verre de silice sensiblement pur.

42. Ensemble d'éclairage (20) selon la revendication 40, dans lequel :

la surface réfléchissante de chacun desdits réflecteurs (163) est positionnée d'environ 120 mm à environ 460 mm de ladite plate-forme d'échantillon (26).

43. Ensemble d'éclairage (20) selon la revendication 41, dans lequel :

ledit verre de silice sensiblement pur est l'un parmi le verre de silice à gaine plastique et le verre de silice à gaine en silice.

44. Ensemble d'éclairage (20) selon la revendication 35, dans lequel :

ledit ensemble de dispersion (110) comprend

au moins quatre dispositifs formant console.

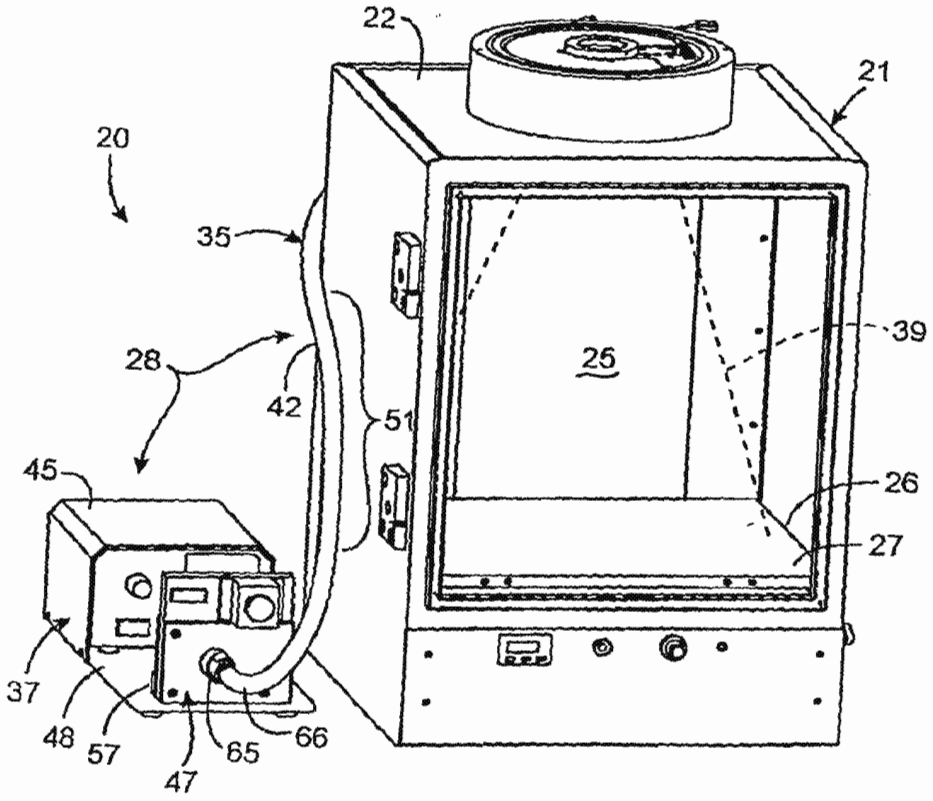


FIG. 1

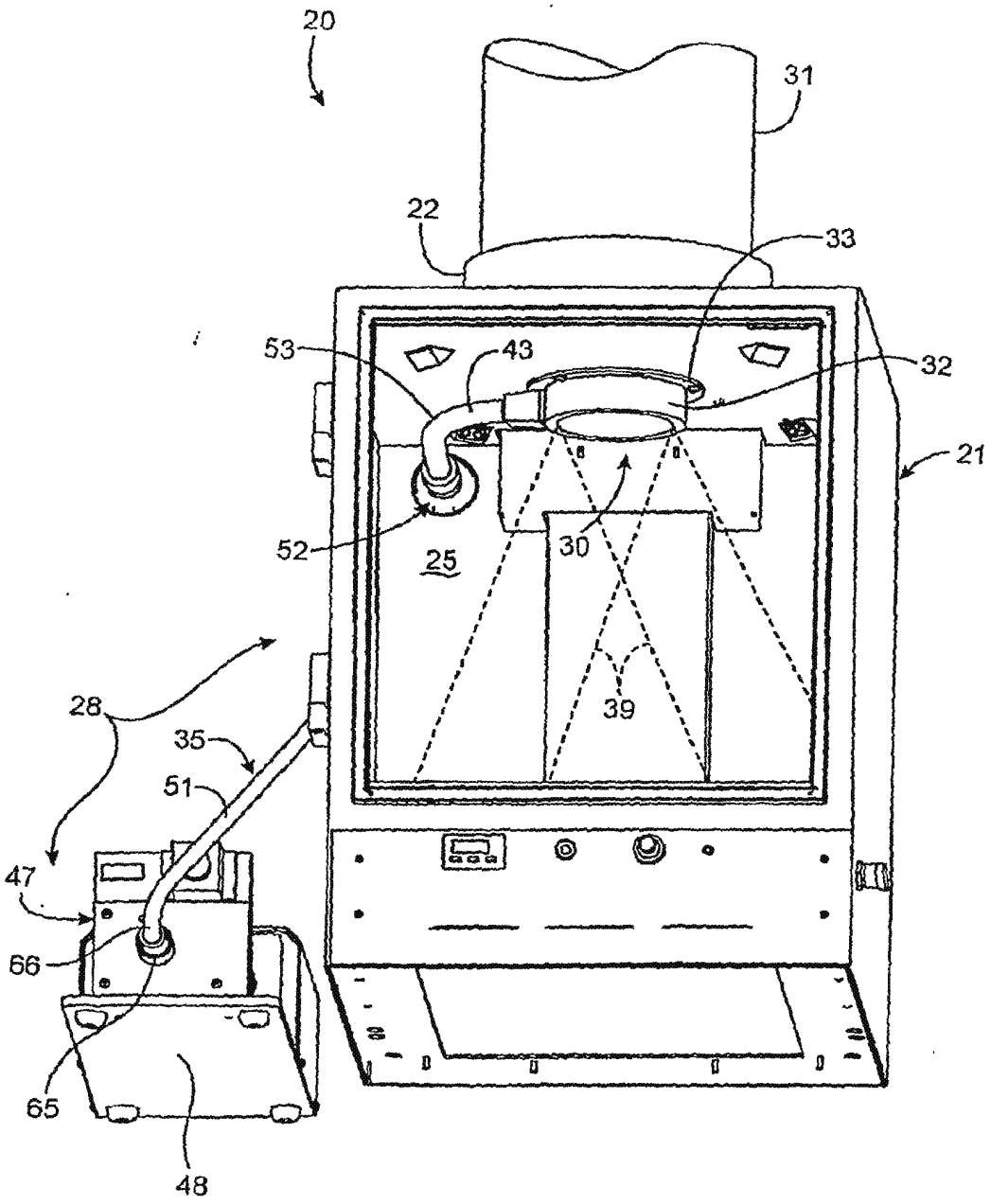


FIG. 2

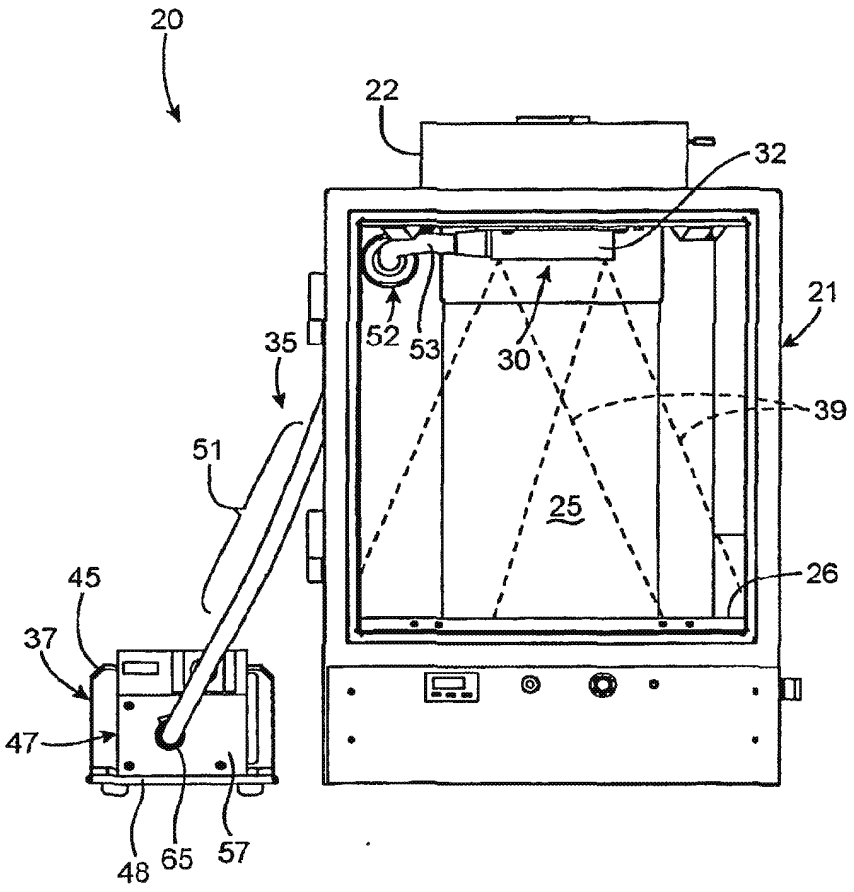


FIG. 3

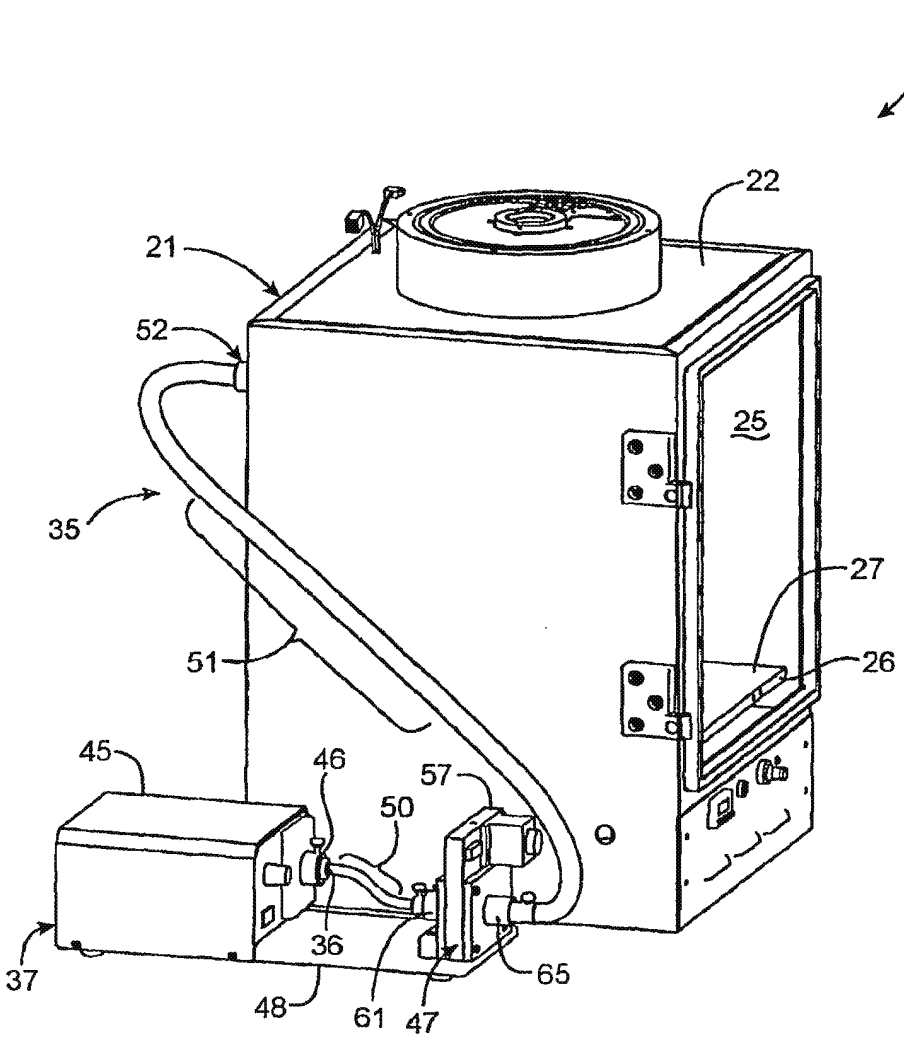


FIG. 4

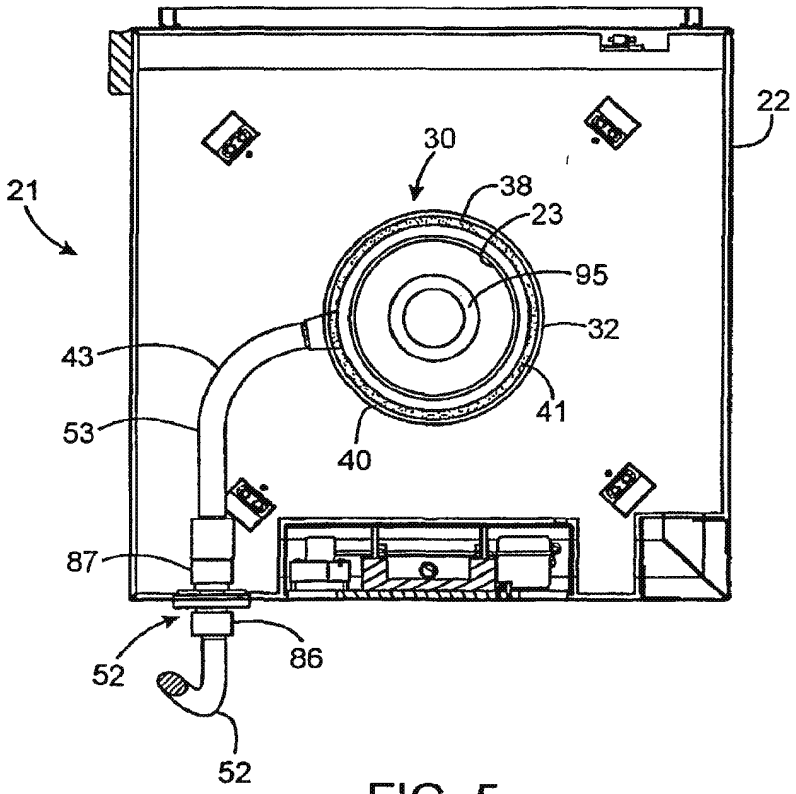


FIG. 5

SPECTRAL_0000290

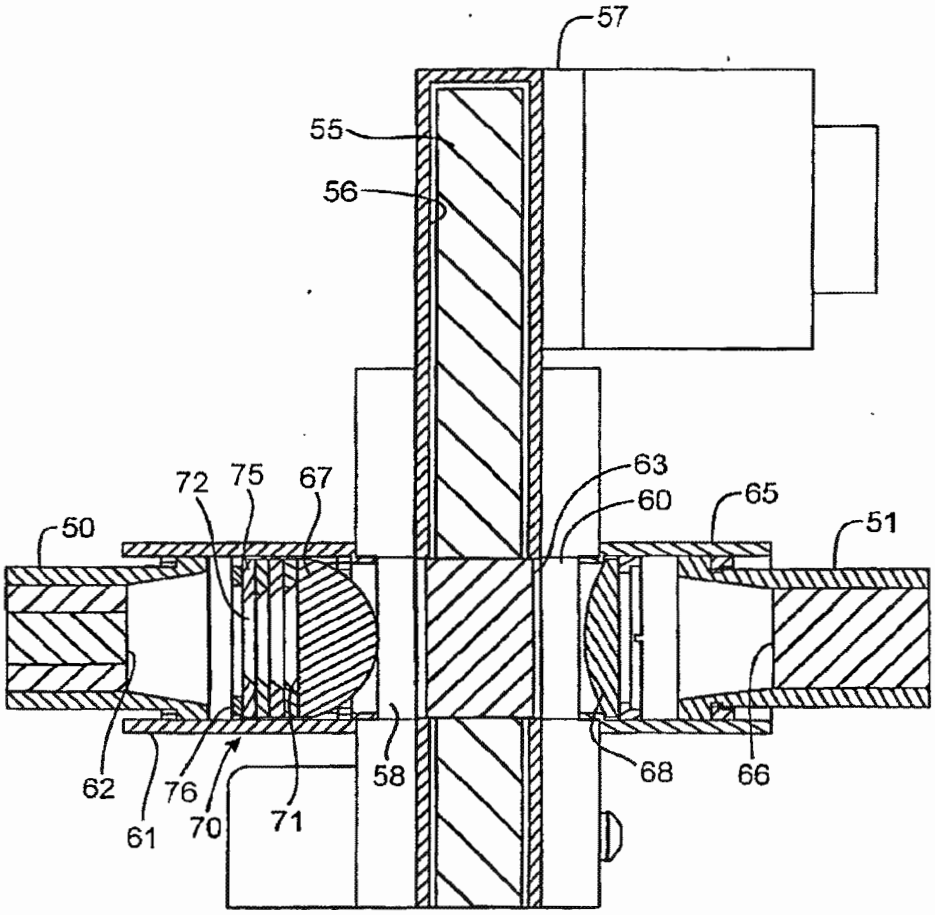


FIG. 6

SPECTRAL 0000291

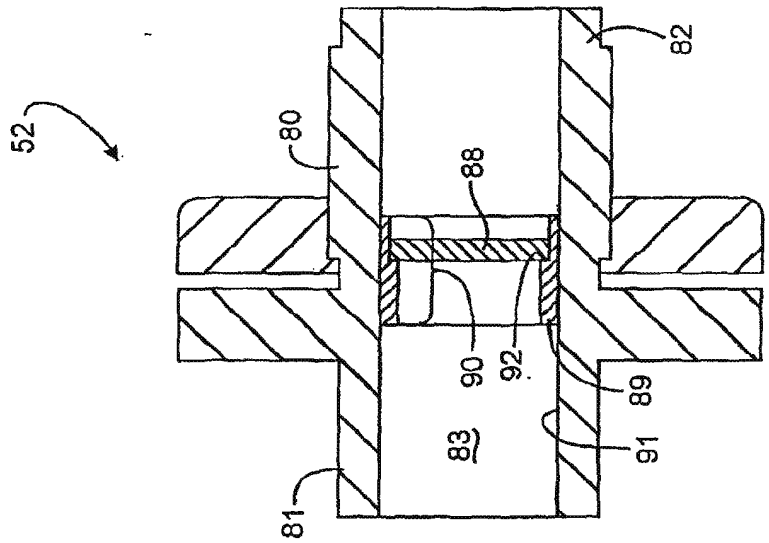


FIG.9

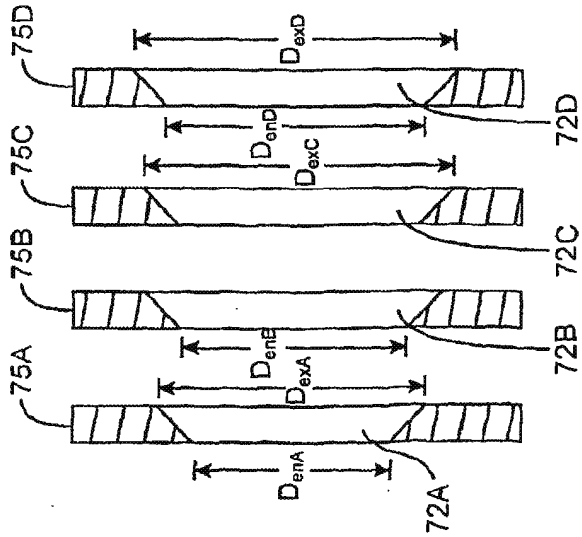


FIG. 7

SPECTRAL 0000292

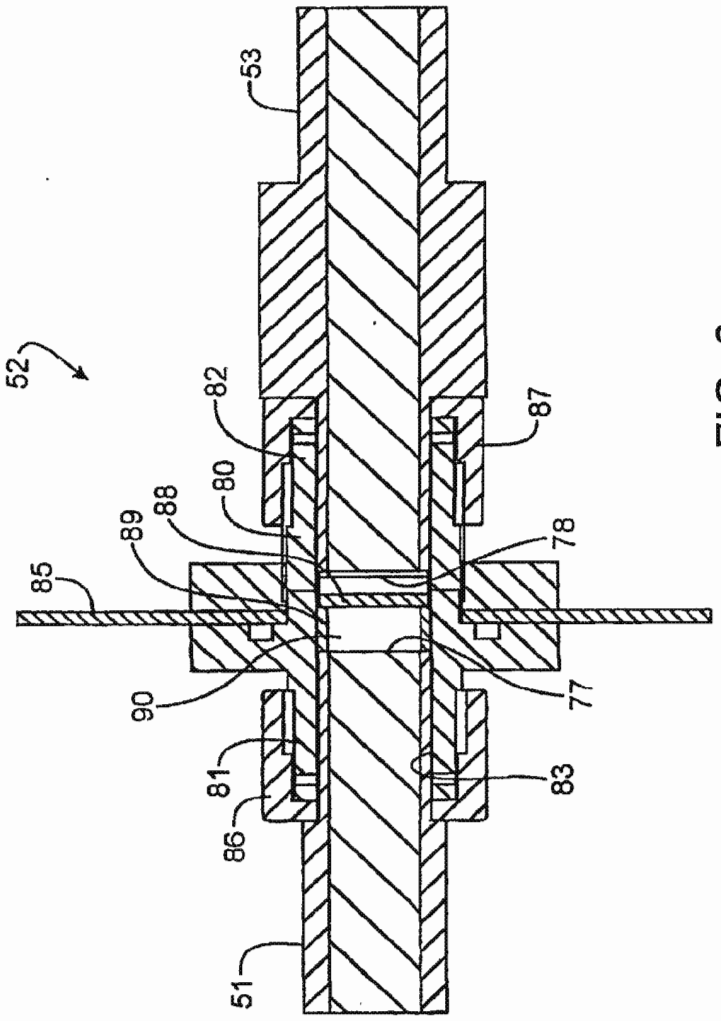


FIG. 8

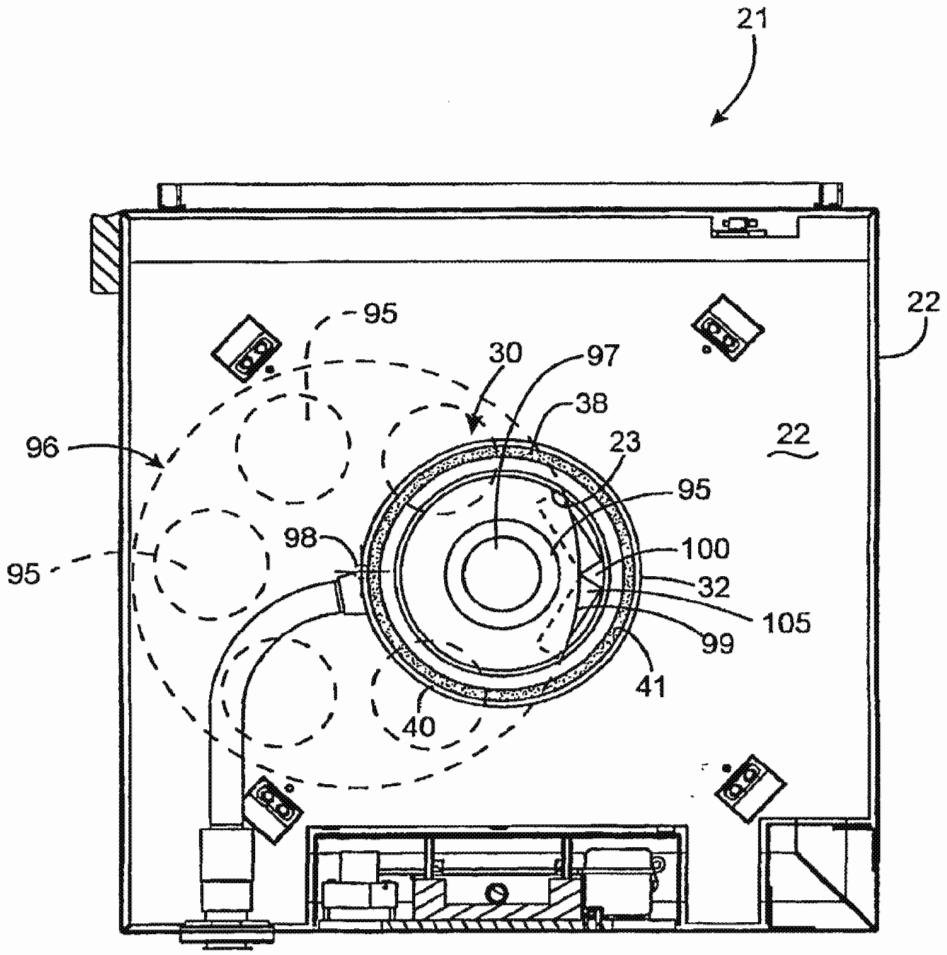


FIG. 10

SPECTRAL_0000294

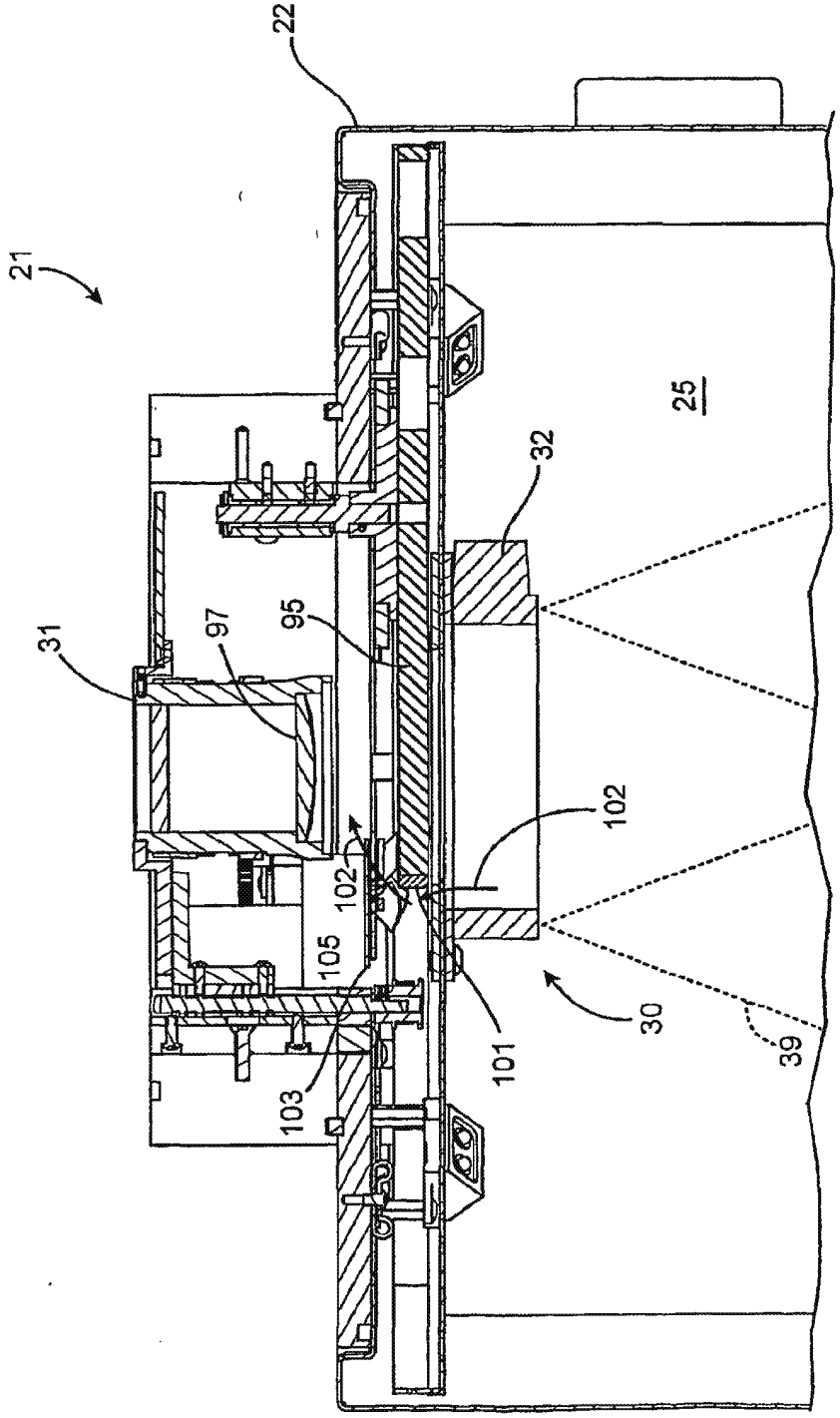


FIG. 11

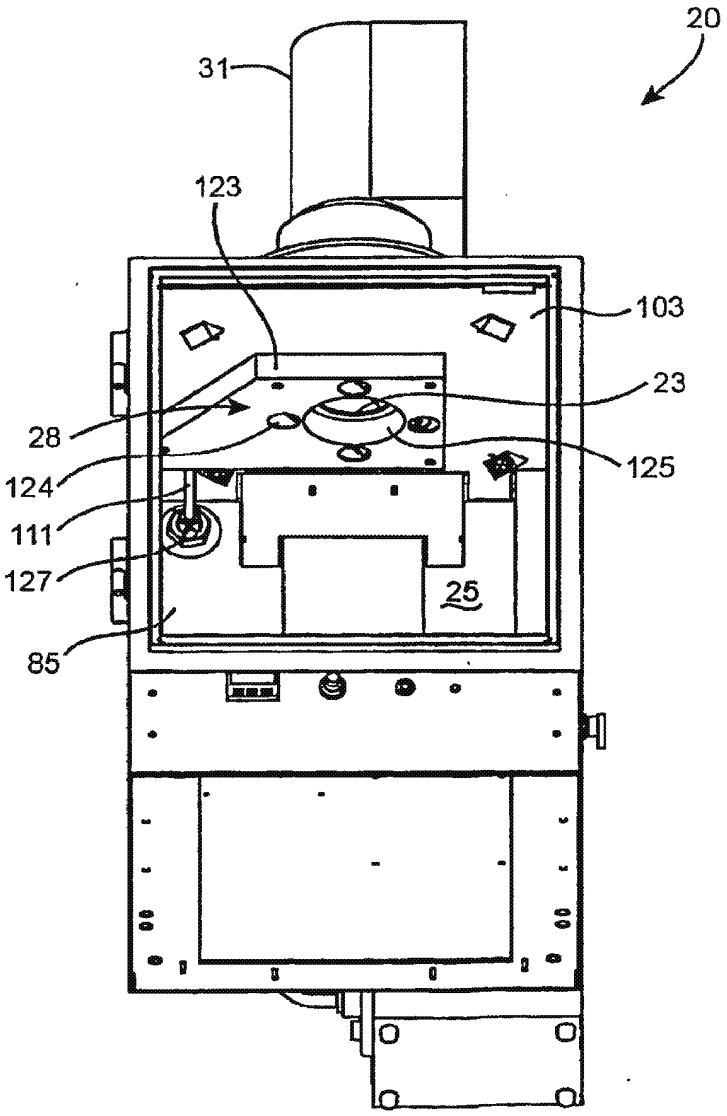


FIG. 12

SPECTRAL_0000296

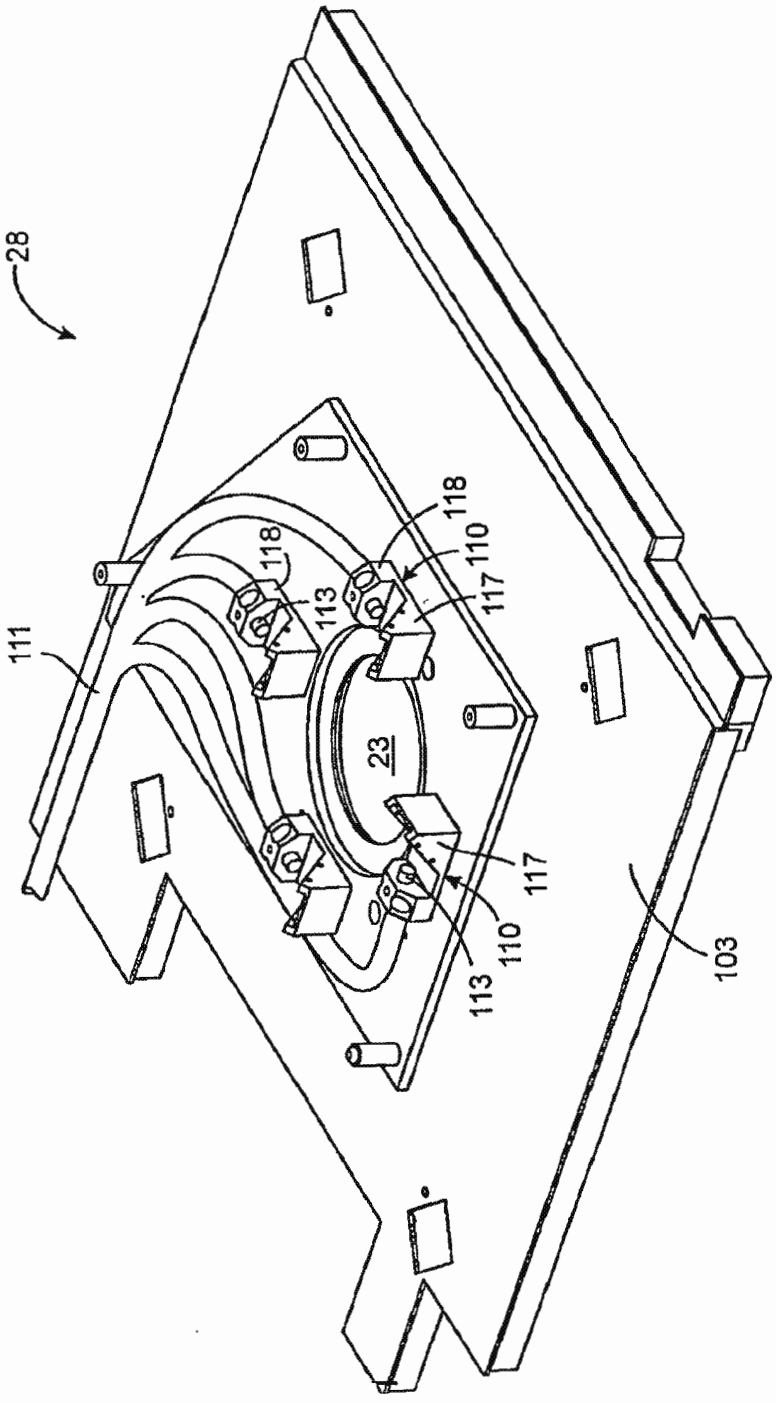


FIG. 13

SPECTRAL_0000297

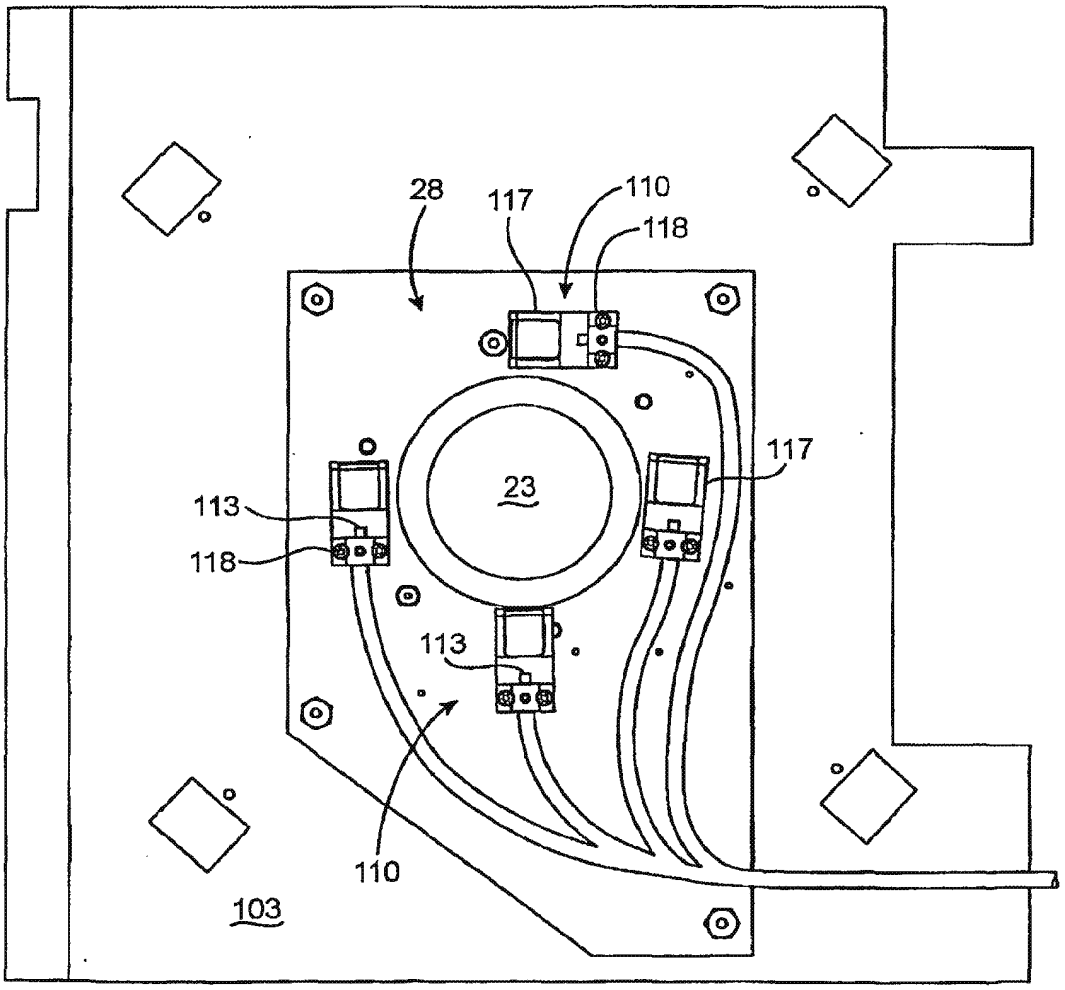


FIG. 14

SPECTRAL_0000298

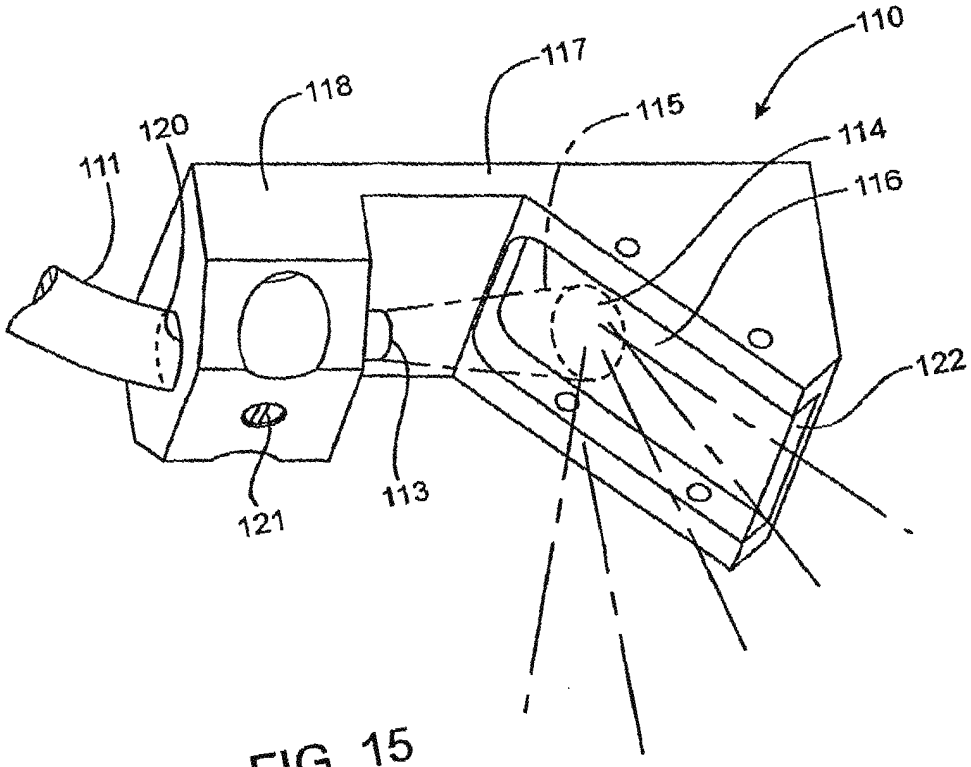


FIG. 15

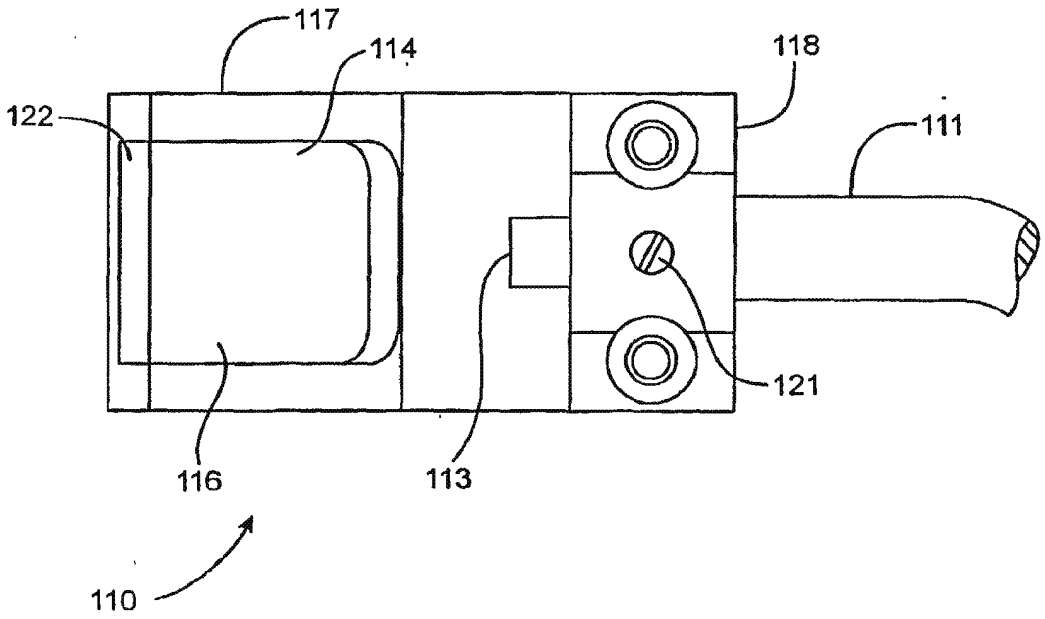


FIG. 16

300

SPECTRAL_0000300

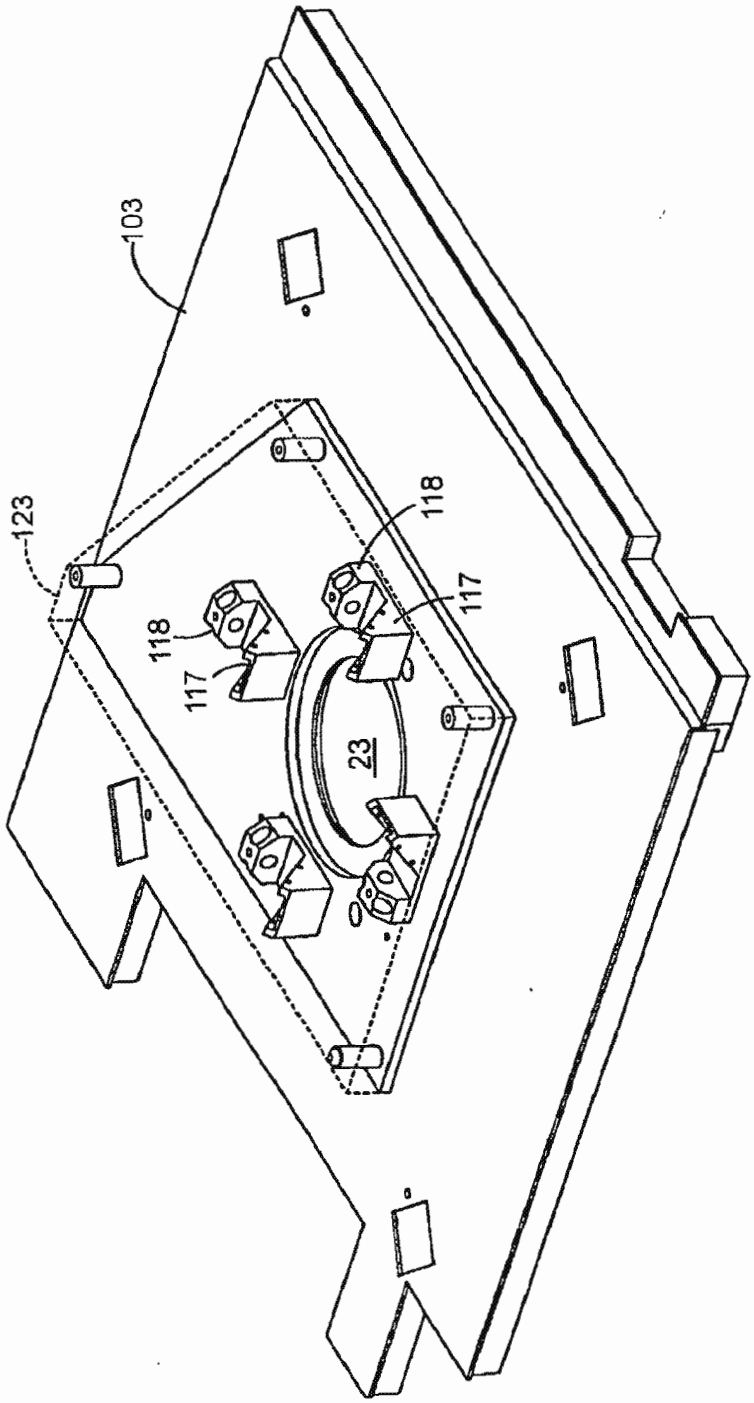


FIG. 17

SPECTRAL_0000301

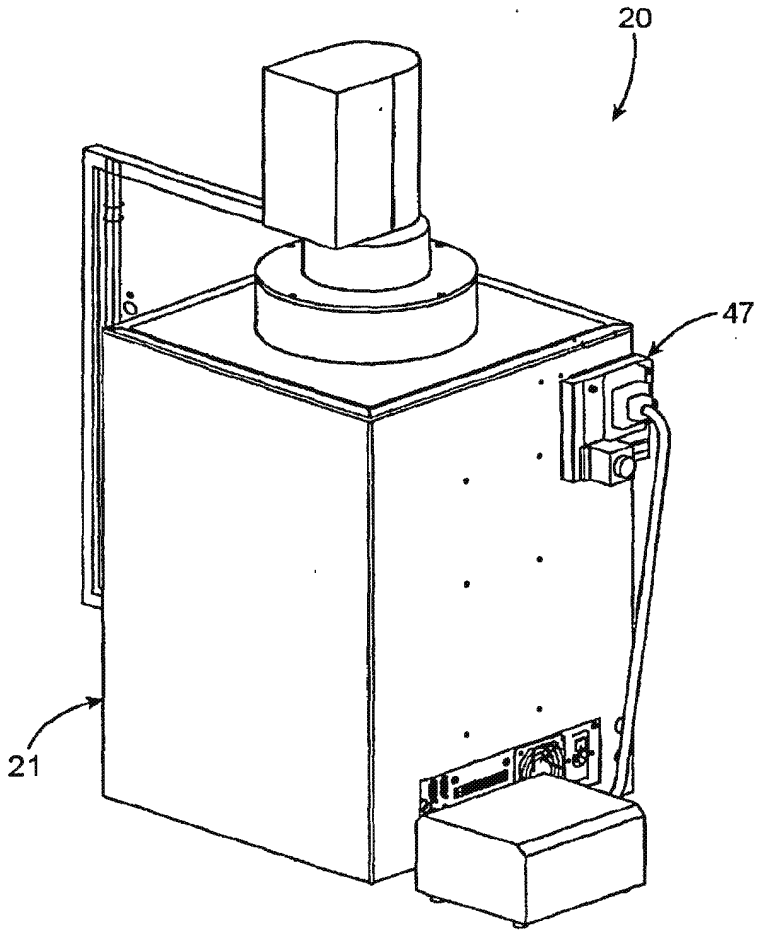


FIG. 18

SPECTRAL 0000302

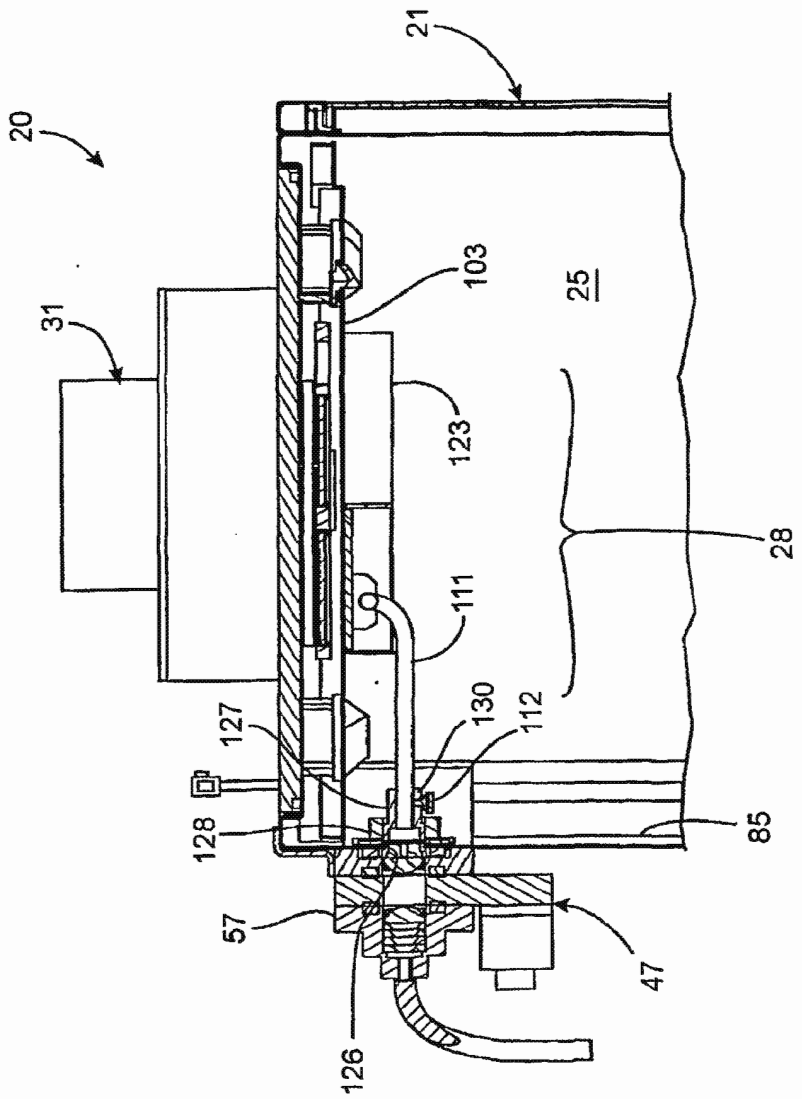


FIG. 19

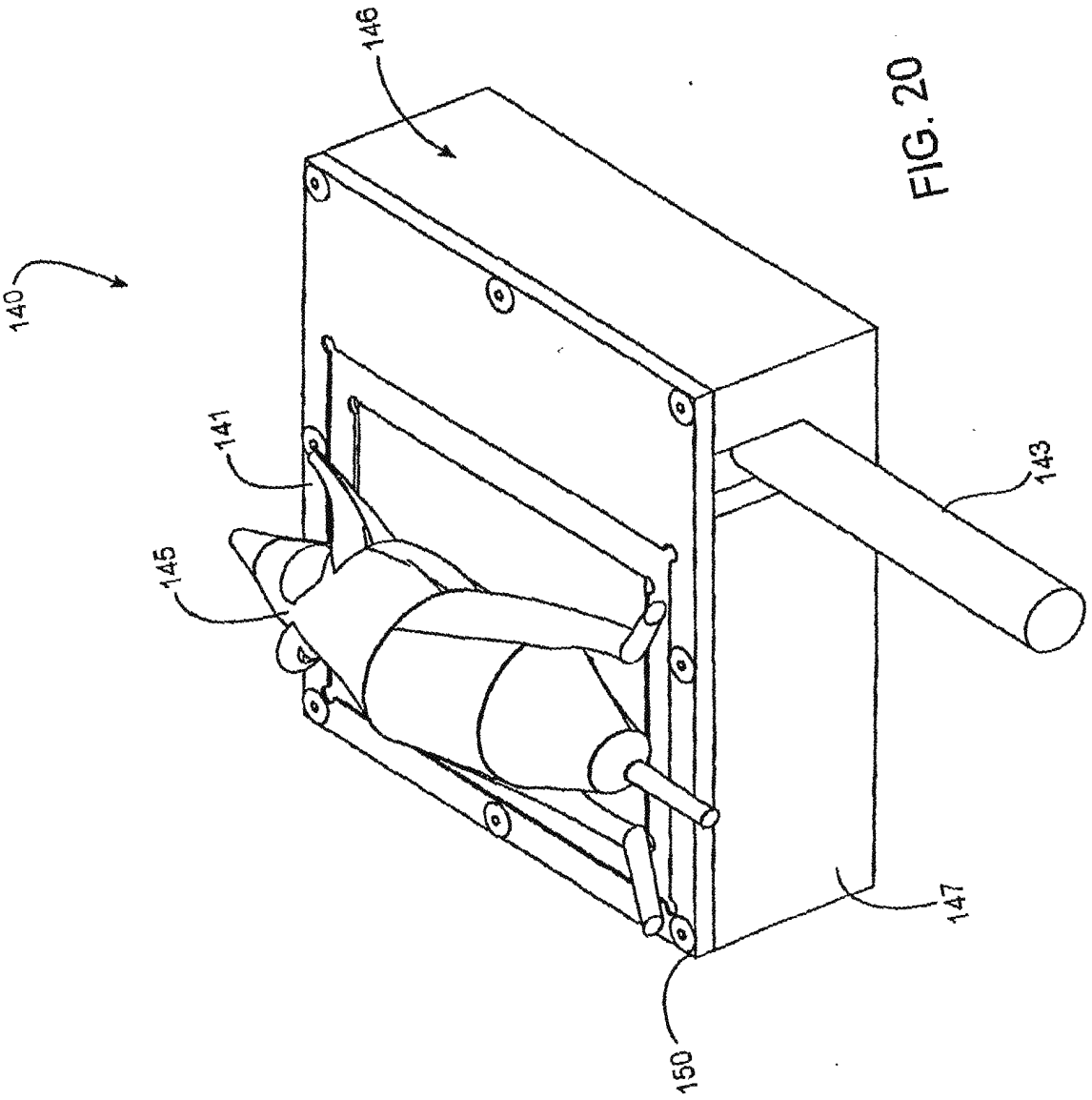


FIG. 20

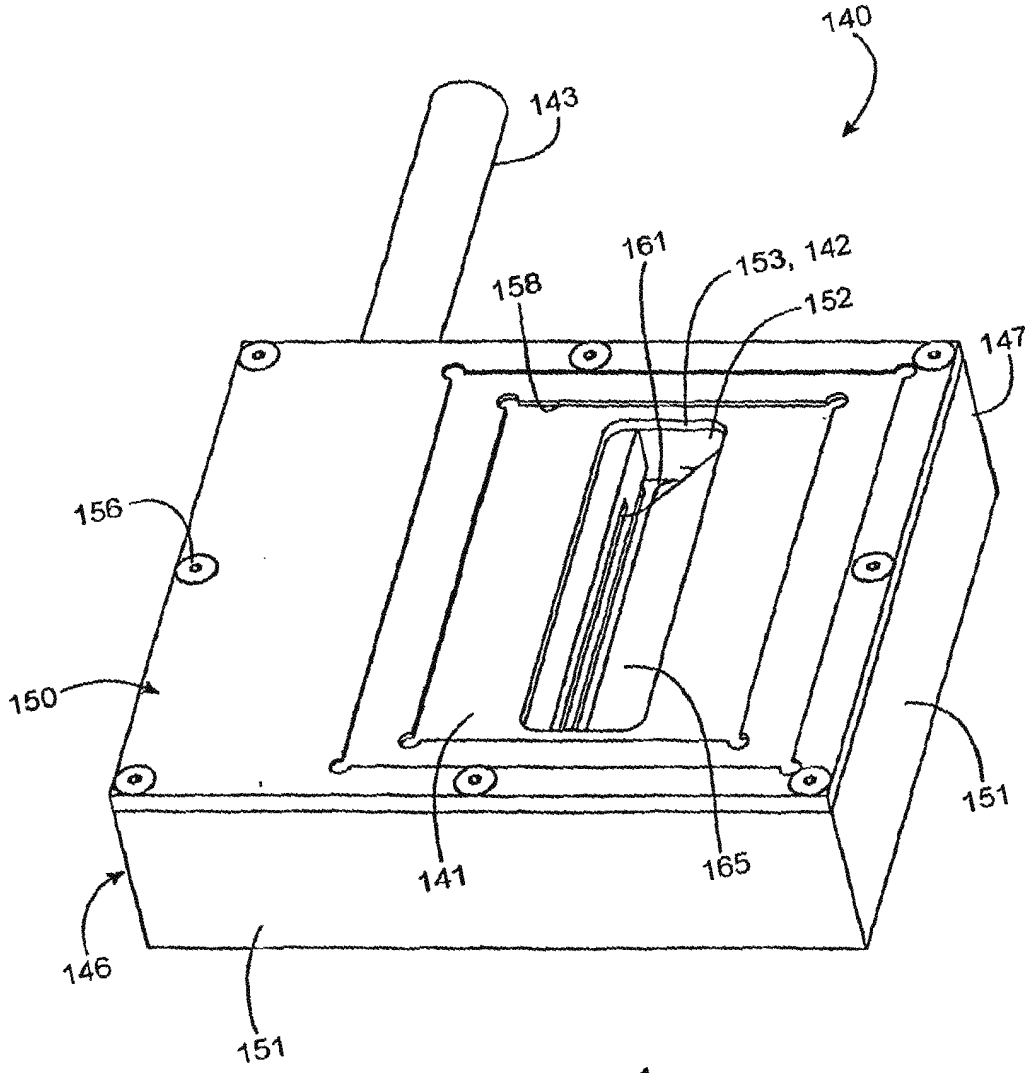


FIG. 21

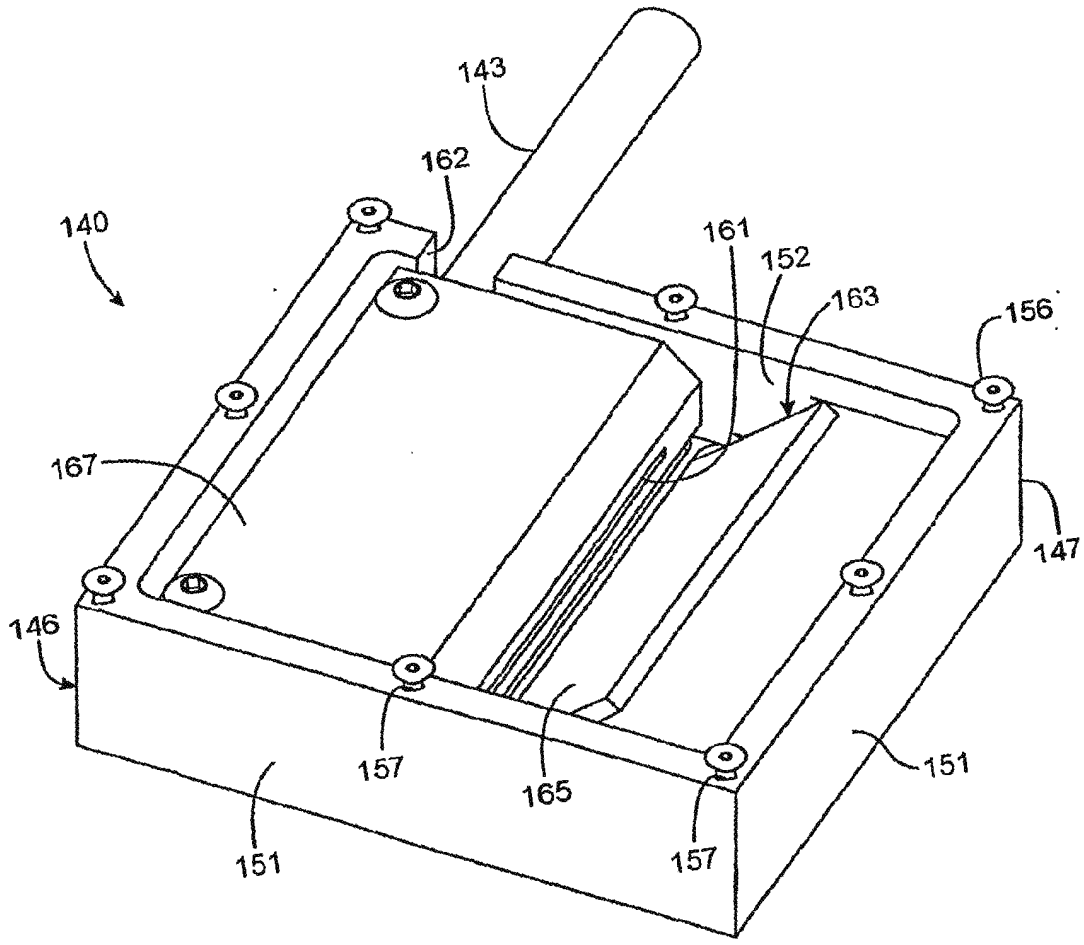


FIG. 22

SPECTRAL_0
306

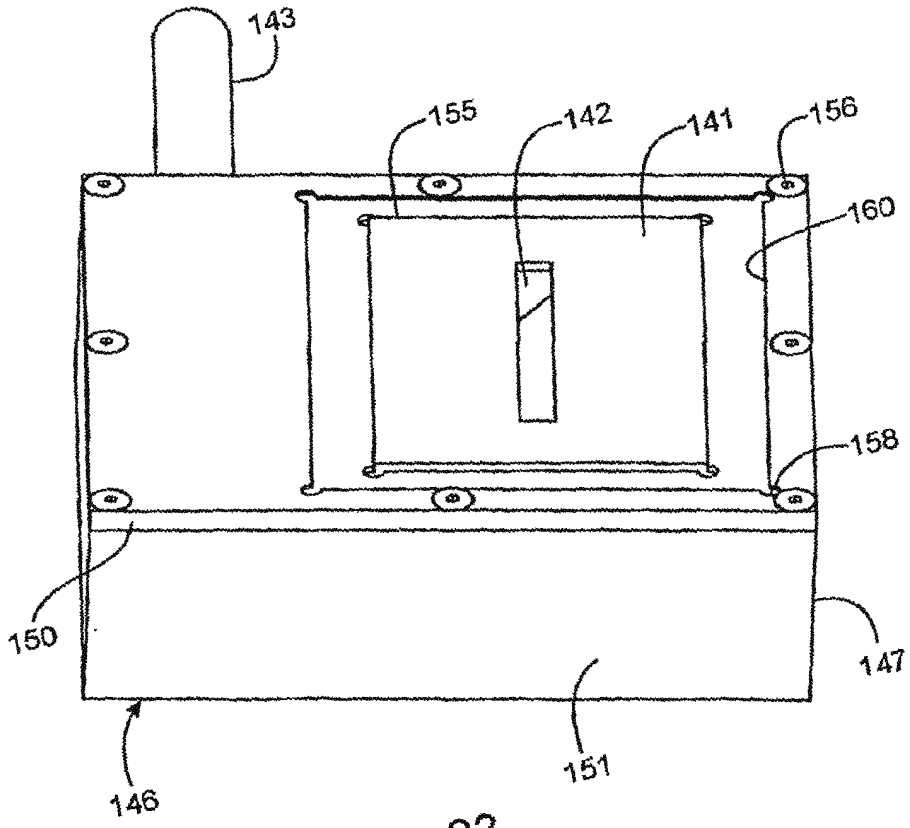


FIG. 23

SPECTRA

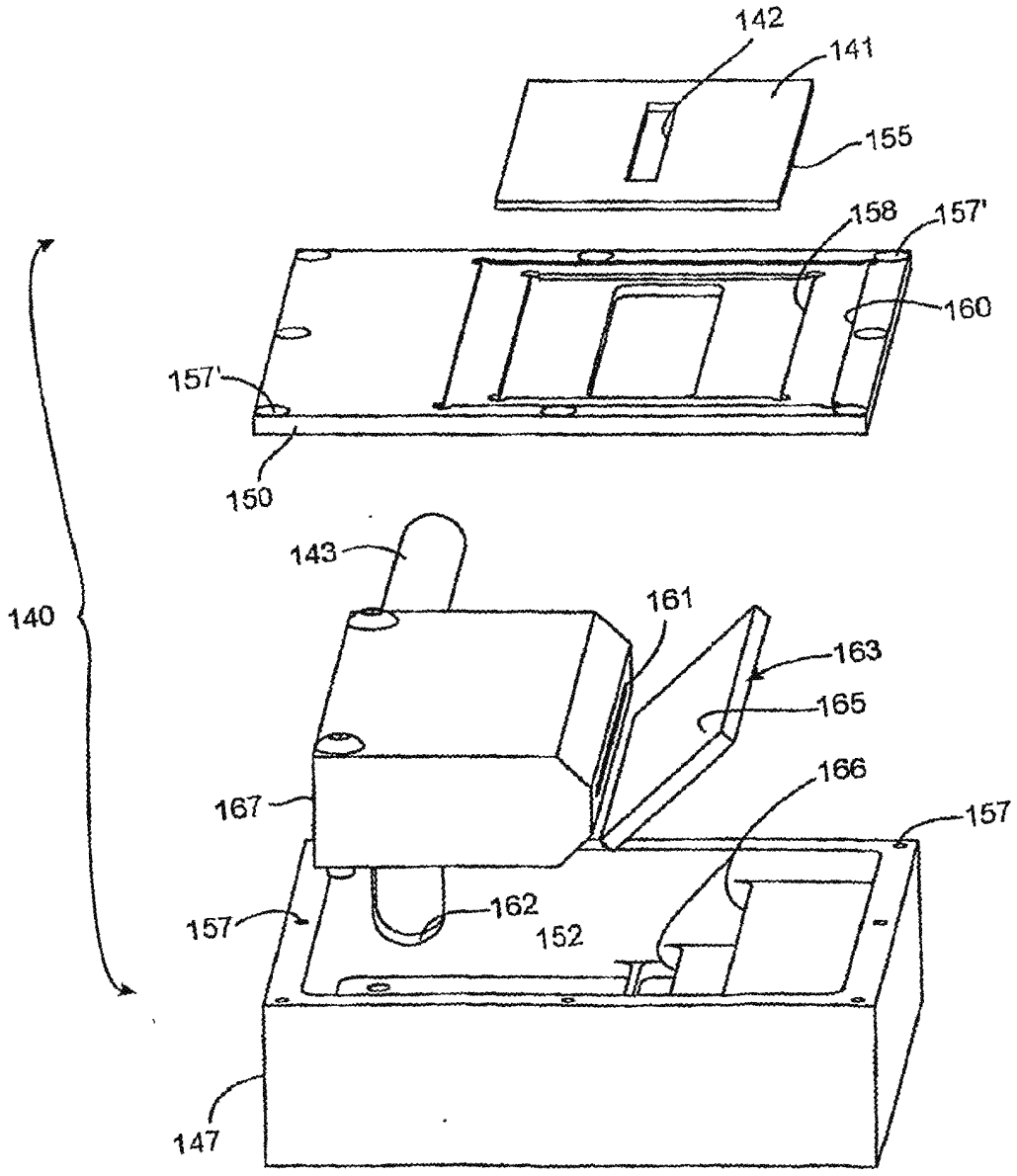

142
141
155
158
157'
160
157'
150
143
161
163
165
140
167
166
157
162
157
152
147

FIG. 24

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 08104002 A, Nilson **[0001]**
- US 35966302 P **[0001]**
- US 20010028510 A **[0010]**
- WO 0163247 A **[0011]**
- WO 9908233 A **[0012]**

Non-patent literature cited in the description

- **JAY RICHMAN.** the Handbook of Optical Filters for Fluorescence Microscopy. the Chroma Technology Corp, June 2000 **[0073]**

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61B 1/00, 1/04, G02B 6/40

A1

(11) International Publication Number: **WO 00/49938**

(43) International Publication Date: 31 August 2000 (31.08.00)

(21) International Application Number: PCT/US00/05076

(22) International Filing Date: 24 February 2000 (24.02.00)

(30) Priority Data:
09/257,897 25 February 1999 (25.02.99) US

(71) Applicant: MICRO MEDICAL DEVICES, INC. [US/US]; 11000 Cedar Avenue, Cleveland, OH 44124 (US).

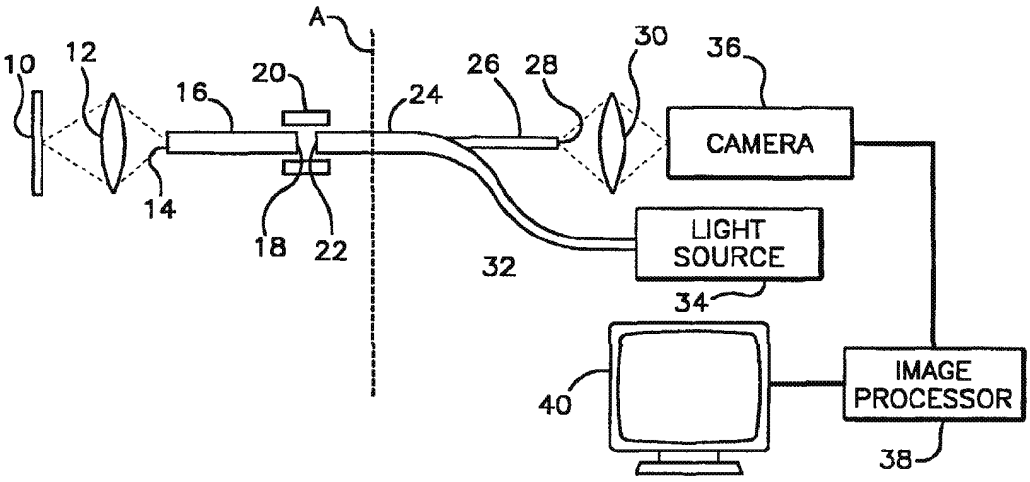
(72) Inventors: GUST, Gary, R.; 16341 Maruffa Circle, Huntington Beach, CA 92649 (US). SMITH, B., Thomas; 2383 Cambridge Boulevard, Columbus, OH 43221 (US). LOVERING, Steven, B.; 117 W. Center College, Yellow Springs, OH 45387 (US).

(74) Agents: SCANLON, Stephen, D. et al.; Jones, Day, Reavis & Pogue, North Point, 901 Lakeside Avenue, Cleveland, OH 44114 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, 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, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ENDOSCOPIC APPARATUS COMPRISING A FIBER OPTIC CABLE CONNECTOR



(57) Abstract

Images from a surgical area may be relayed to a camera (36) outside the sterile field by means of a fiber optic cable (16, 26). Cost issues associated with the long fiber optic cable are avoided by breaking it into two segments, only one of which is likely to be damaged during use. An image preserving connection is formed between the cable segments (16, 26) by a connector (20) that fixes the ends of the cables in close proximity to one another, but a certain fixed distance apart. This configuration allows an image preserving connection to be made between two fiber optic cables without the aid of a lens.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yngoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

SPECTRAL_0000311

ENDOSCOPIC APPARATUS COMPRISING A FIBER OPTIC CABLE CONNECTOR

Field Of The Invention

This invention arose in the field of endoscopic surgical devices but also pertains to the field of fiber optics.

Background

There is great incentive to make endoscopes smaller. Smaller endoscopes make endoscopic surgeries less invasive and healing times shorter. If the endoscopes are small enough, the entire procedure can be carried out in a doctor's office, without general anesthetic, on an outpatient basis, resulting in a substantial cost savings. Today, most endoscopes in use are rigid endoscopes, comprising chains of lenses. An introducer at least 6 millimeters in diameter must be inserted in the patient's body to conduct surgery with the smallest of these. Applicants are working to develop flexible, serviceable, and affordable fiber optic endoscopes by means of which surgeries may be conducted through an introducer 2 millimeters or less in diameter.

In a typical endoscopic surgery, the introducer is a cannula with a trocar through its center, which is inserted into the patient's joint. The trocar is then withdrawn and the endoscope inserted. The endoscope is connected to a camera through an adapter that clips on to the base of the endoscope. A wire from the camera carries the image to a video processor.

The person conducting the surgery holds the camera while manipulating the endoscope. Although orthopedic surgeons have become used to holding cameras while conducting these surgeries, relieving them of this burden could only enhance their ability to conduct them.

An important issue in conducting surgeries of this type is maintaining sterile conditions. For this purpose, a sterile field is created. A sterile

SPECTRAL_0000312

field is an area around the point of incision that is prepared before a surgical procedure and maintained during the procedure. The preparation and maintenance of a sterile field is described in Atkinson and Fortunato, Berry & Kohn's Operating Room Technique (8th ed. 1996). All items within the sterile field must be sterilized. In prior art endoscopic surgeries, the camera, wire, and adapter are within the sterile field. Federal regulations and good practice therefore require these parts to be sterilized between uses. This is inconvenient, time consuming, and reduces the availability of the apparatus for use in other surgeries. Furthermore, the sterilization procedure can damage the electronic components.

Summary of The Invention

In one respect, the present invention comprises a method of conducting endoscopic surgery in which the camera is outside the sterile field. According to this method, the camera is placed outside the sterile field and the image is transmitted to the camera by a fiber optic cable. Preferably, the apparatus for conducting the surgery is organized so that the person conducting the surgery does not have to hold the camera while manipulating the endoscope.

The inventors observed that use of a single long fiber optic cable to carry the image outside the sterile field would makes this procedure expensive. The initial cost of such a cable would be high and even with care it is inevitable that it would be damaged during use. The cable would probably not last through ten surgeries.

To ameliorate this expense, the inventors developed a segmented fiber optic cable. By a segmented fiber optic cable, applicants mean an image transmitting fiber optic cable formed by the union of two or more separate fiber optic cables. Applicants observed that a short segment of the cable nearest the patient is the portion most likely to be damaged during use. In a segmented fiber optic cable, this portion (called the "disposable cable portion", although it may be used several times) may be replaced while the longer portion of the cable (the "reusable cable portion") continues to be reused. The reusable cable portion can

SPECTRAL_0000313

also be armored. While such armoring on the disposable cable portion might interfere with the operation of the endoscope and would, in any case, excessively increase the diameter of the introducer, armoring on the reusable cable portion presents no disadvantages. An armored reusable cable portion is expected to withstand upwards of a hundred surgeries.

In developing a segmented fiber optic cable, the inventors were faced with the problem of connecting the segments in a way that would preserve the image quality required for surgical procedures. Fiber optic cables comprise bundles of optical fibers. Thousands of fibers are required to form a satisfactory image. Image preserving connections between such bundles of image transmitting fibers appear to have been unknown prior to applicant's invention. By an image preserving connection, applicants mean a connection across which simple images, such as those of grids, that do not push the resolution limits of the image conveying apparatus, are not degraded beyond recognition. Applicants have invented an image preserving fiber optic cable connector that preserves images to the degree required for endoscopic surgery.

Applicants' preferred connector functions by holding the tips of the fibers a certain small distance apart. It was unexpected that two fiber optic cables could be connected in this way. If the fibers were butted against one another, the image would suffer from fixed pattern noise, giving the image a mottled appearance and making it essentially useless. If the fibers were too far apart, the image would be blurred. Applicants unexpectedly discovered that there is a fiber tip spacing that allows the image to be transferred without noticeable fixed pattern noise, substantial loss of resolution, or excessive loss of image quality.

Applicants endoscopic surgical apparatus is expected to change the economics that affect the way endoscopic surgeries are performed. By placing the camera outside the sterile field, the expense and damage associated with sterilizing the camera can be avoided. By using a segmented fiber optic cable, the expenses associated with the cable itself can be kept under control. If the connection between the cable segments is made, according to applicants' preferred

embodiment, using a connector that functions by holding the ends of the cables a small distance apart, rather than by means of a lens, problems associated with connector itself will not present problems comparable to those that were associated with a camera within the sterile field.

Applicants' invention is also expected to have a direct effect on the mechanics of endoscopic surgeries. The person conducting the procedure will no longer have to hold the camera, allowing greater control of the endoscope. In conjunction with other advances made by applicants, the invention is expected to contribute to the adoption of small diameter fiber optic endoscopes, which will mean less invasive surgeries, surgeries that can be performed on smaller joints, and surgeries that no longer have to be performed in a hospital.

Brief Description Of The Drawings

Figure 1 is a schematic of a preferred endoscopic surgical apparatus comprising a segmented fibre optic cable.

Figure 2 is a plot showing how the width and intensity of an image of a line vary with the gap between the ends of two fiber optic cables.

Figure 3 is an exploded isometric view of a preferred fiber optic cable connector that connects both image transmitting fibers and illumination transmitting fibers.

Figure 4 shows a preferred fiber optic cable image transmitting sub-connector with the two portions disconnected.

Figure 5 shows the fiber optic cable image transmitting sub-connector of Figure 4 with the two portions connected.

Figure 6 shows another example of a fiber optic cable image transmitting sub-connector.

Figure 7 demonstrates a method of setting the gap during assembly of the sub-connector of Figure 6.

Detailed Description

In one embodiment, applicants invention is an endoscopic surgical technique that places the camera outside the sterile field. Preferably the image is transmitted to the camera using a segmented fiber optic cable. Preferably, the fiber optic cable, with sheath, is four millimeters in diameter or less, more preferably 1.4 millimeters in diameter or less. In another embodiment, the invention is a method of conducting surgeries using a segmented fiber optic cable to transmit an image from within a patient's body to where it can be viewed by a person conducting the surgery.

A method of conducting multiple surgeries in accordance with the present invention treats one end of the cable, the one nearest the patient, as being disposable or interchangeable. For example, the end of the cable nearest the patient may be separated from the rest of the cable and sterilized between one surgery and the next. Alternatively, the end of the cable nearest the patient may be disposed of and replaced between one surgery and the next.

A preferred apparatus for conducting endoscope surgeries in accordance with the invention is shown schematically in Figure 1. It comprises a lens **12**, a disposable fiber optic cable **16**, a connecter **20**, a reusable fiber optic cable **24**, a camera **36**, an image processor **38**, a monitor **40**, and an illumination light source **34**. The illuminating light is generally transmitted to the target area **10** through a set of fibers **32** that are bundled with, but separate from, the image transmitting fibers **26**. As described in co-pending application serial number 08/890,803, which is incorporated herein by reference, it is also possible to combine the functions of channeling illuminating light and image light in one set of fibers thereby further reducing the size of the device that must be inserted in the patient.

In operation, the lens **12** gathers an image from a target area **10** illuminated by light channeled from light source **34**. The lens **12** directs the image into the polished ends of the fibers at one end **14** of disposable fiber optic cable **16**.

SPECTRAL_0000316

Light is transmitted down the length of the fibers to the other end **18** of the disposable fiber optic cable **16**. The light within each fiber of the cable comprises a single point of image data. The data from a group of fibers must be in proper spacial relation for an image from one cable end to form at the other end The disposable fiber optic cable **16** is a coherent optical fiber bundle, in that the spacial relationship between the fibers is the same at both ends of the cable. Thus an image of the target area **10** appears at the end **18** of the disposable fiber optic cable **16**.

In the preferred embodiment, the image from the end **18** of the disposable fiber optic cable **16** falls directly upon the end **22** of the reusable fiber optic cable **24**. Alternatively, the connector **20** could comprise a lens that directs the image from the end **18** onto the end **22**. Such a lens would need to be somewhat out of focus to avoid fixed pattern noise. The reusable fiber optic cable **24** channels the image to the lens **30** associated with camera **36**.

The camera **36** generates a signal coding the image. The signal coding the image is transferred from the camera **36** to the image processor **38**. The image processor **38** contains software and operates to remove artifacts from the image and enhance it. The output of the image processor **38** is displayed on the monitor **40**. The delay in processing the image is minimal, so that the image displayed is current with the image being gathered by the lens **12**. In other words, the image displayed is a real time image.

When, as in the preferred embodiment, the apparatus comprises a segmented fiber optic cable, a fiber optic cable connector is necessary. For surgical applications, the connector is preferably small, easy to operate, and essentially fool proof. Ideally, the connector should operate to form an image transmitting connection between the cables when the two ends of the connector are pushed together with a minimum of force. If the connector is of the type that requires the ends to be oriented correctly in terms of rotations about the cable axes, the two ends of the connector should provide both visual and tactile indications as to the correct orientation.

SPECTRAL_0000317

The preferred connector is one that can hold the ends of the cables a fixed distance apart. Theoretically, the connection could be made using a lens. Lenses have a number of drawbacks however, which drawbacks relate to the logistics of aligning the lens and the fibers with sufficient precision and the trade-offs that must be made between size of the lens, its precision, and its cost.

Applicants have experimentally determined the positioning required to form an image preserving connection between the ends of two 1.0 millimeter diameter fiber optical cables each having 30,000 close packed fibers, each fiber having a diameter of approximately 4 micrometers and a numerical aperture of approximately 0.35. The solid line **56** in Figure 2 plots the variation of intensity **50** of an image, which is that of a line, with a gap **52** between the cable ends. By cable ends, the applicants mean the surfaces formed by the ends of the fibers of the cables when the ends of the cables have been polished. The dashed line **58** plots the variation of the image width **54** with the gap **52**. The width of the line's image is a measure of the image resolution. Resolution was deemed adequate for medical diagnostic purposes when the gap was 20 micrometers or less, although resolution was noticeably better when the gap was 10 micrometers or less. When the gap was essentially zero, the image suffered from severe fixed pattern noise. This fixed pattern noise decreased to a satisfactorily low level when the gap was increased to 4 micrometers. The optimal gap was 4 to 8 micrometers. The offset of the axes of the two cables is preferably also controlled to some degree. Preferably, the offset is not more than 10 micrometers.

The preferred gap between cable ends is expected to vary proportionally with fiber diameter and inversely proportionally with numerical aperture, at least for small numerical apertures. As a first approximation, the applicants suggest that, when working with fibers having substantially different diameters and/or numerical apertures from the ones used in the examples given here, the gap be selected in the range:

SPECTRAL_0000318

$$.35\frac{d}{NA}<g<.70\frac{d}{NA}$$

where g is the gap, d is the mean fiber diameter for the two cables, and NA is the numerical aperture of the cable from which the image is to be transmitted. Ultimately, however, the ideal gap may need to be determined empirically.

A preferred connector in accordance with the present invention is shown disassembled in Figure 3. The connector **60** comprises two housing ends **62** and **64**. The end **62** shown on the left hand side of Figure 3 comprises connector housing portions **66** and **68**, which contain sub-connector ends **74** and **76**. The other end **64** comprises connector housing portions **70** and **72**, which contain sub-connector ends **78** and **80**. Sub-connector ends **74** and **80** form and an image transmitting sub-connector. Sub-connector ends **76** and **78** form an illumination transmitting sub-connector. Connector end **62** slides within connector end **64**. Shaped corners **61** and **63** key the two connector ends so they cannot be joined unless they are properly oriented. The ends are reversibly held together by protrusions (not shown) that snap into notches **82**.

A detailed example of a preferred image transmitting sub-connector is shown in Figures 4 and 5. The female portion **74** comprises a fiber bundle **94** surrounded by a stainless steel ferrule **96**. The fiber bundle **94** is bound to the ferrule **96** by epoxy **98**. The male portion comprises a cable fiber bundle **102** surrounded by ferrule **104** and bound to it by epoxy **106**. The ends **108** and **112** of the ferrules **96** and **104** are flush with the polished ends **110** and **114** of the fiber bundles **94** and **102**.

The female end fiber ferrule **96** is positioned concentrically within an alignment tube **100**. A tack **116** prevents the alignment tube **100** from sliding relative to the ferrule **96**. The alignment tube **100** helps guide and axially align the fiber bundle ends **100** and **114** together when the male and female portions of the connector are being joined.

SPECTRAL_0000319

The alignment tube **100** is itself positioned within an elastic member **90**, which fits tightly within the connector housing **62** in the region **86**. The elastic member **90** functions to allow the fiber bundle **94** to move freely in the direction of its axis relative to the connector housing **62**. The elastic member **90** also exerts a biasing force tending to press the fiber bundle ends **110** and **114** together when the two ends **62** and **64** of the connector **60** are joined. Displacement of the end **114** of the male fiber bundle **102** is resisted by the male end ferrule **104**, which rests against the housing end **64** at the point **84**.

The male end ferrule **104** is positioned concentrically within elastic member **92**. Elastic member **92** fits tightly within connector housing **64** in the region **88**. The elastic member **92** allows the fiber bundle **102** to move in directions perpendicular to its axis.

A spacer **106** is formed by electroplating on the end **112** of the male end ferrule **104**. The spacer **106** fixes the gap between the fiber bundle ends **110** and **114** when the ends of the connector are joined. One of the advantages of forming a spacer by electroplating is that an electroplating-formed spacer compensates for the rounding that tends to occur around the perimeters **113** and **115** of the ferrule ends. Figures 4, 5, 6, and 7 are not drawn to scale in that the gap is much narrower, and the spacer much thinner, than shown in these illustrations.

In the preferred embodiment, the illumination transmitting sub-connector is the same as the image transmitting sub-connector, except that the illumination transmitting sub-connector does not have a spacer.

Another example of a sub-connector in accordance with the invention is shown in Figure 6. In this example, there is an extension sleeve **120** between the female end ferrule **96** and the alignment tube **122**. When the male and female ends of this sub-connector are joined, the extension sleeve **120** meets the male end ferrule **124** and maintains the gap between the fiber bundle ends **110** and **114**. In this embodiment, the male end ferrule **124** has a larger diameter than the female end ferrule **96**. In assembling the connector with the extension sleeve, the gap can be set, as illustrated in Figure 7, by pressing the female end ferrule **96** and

SPECTRAL_0000320

the extension sleeve **120** against a shaped rubber surface **130** and sliding the extension sleeve to conform to the surface. When the extension sleeve is correctly positioned, a tack weld **126** can be used to fix it in place.

When a segmented fiber optic cable is used, the portion of the cable which does not have to fit in the cannula can be armored. A preferred method of armoring is by enclosing the cable in a relatively thick metal sheath.

A surgical apparatus constructed and operated in conformity with one or more of the preferred embodiments described above will provide convenient and cost effective means with which surgical procedures can be conducted in accordance with the applicants invention. These procedures solve important sterilization problems and contribute to the practicability of minimally invasive surgeries using fiber optics.

SPECTRAL_0000321

Claims

We claim:

1. An apparatus for conducting endoscopic surgeries, comprising:

a distal fiber optic cable;

a lens positioned to direct images into one end of the distal fiber optic cable;

a proximal fiber optic cable;

a connector, with ends attached to the distal and proximal fiber optic cables, the connector being capable of forming an image preserving connection between the distal and proximal fiber optic cables;

a camera for receiving images from the proximal fiber optic cable; and

a monitor for displaying images from the camera.

2. An apparatus as in claim 1, wherein:

the connector functions without the aid of a lens by maintaining the ends of fibers at one end of the proximal fiber optic cable in proximity to, but some distance apart from, the ends of fibers at one end of the distal fiber optic cable.

3. An apparatus as in claim 2, wherein:

the connector comprises a spacer that is positioned such that when the connector is operating to form a connection, the spacer resides between ferrules on the distal and proximal fiber optic cables and maintains the spacing between the fiber ends.

4. An apparatus as in claim 2, wherein:

each connector end comprises a ferrule that surrounds fibers of the cable to which the connector end is attached, each ferrule having an end that is flush with the ends of the fibers it surrounds, one of the ferrules being surrounded by an extension sleeve that extends slightly beyond the flush end of the ferrule, the

SPECTRAL_0000322

extension sleeve functioning to maintain the spacing between the fiber ends when the two ends of the connector are joined.

5. A fiber optic cable connector, comprising:

a first connector end attached to one fiber optic cable and a second connector end attached to a second fiber optic cable;

wherein these first and second ends, when joined together, are capable of maintaining fiber ends of the two cables a determinate small distance apart, which distance is at least about equal to the median fiber diameter for the fibers of the two cables;

the connector being capable of forming an image preserving connection between the two fiber optic cables; and

the connector operating without requiring a lens disposed between the fibers of the two cables.

6. A fiber optic cable connector as in claim 5, further comprising:

a spacer;

wherein the first and second connector ends comprise ferrules surrounding fibers of the cables, the ferrules having ends that are flush with the ends of the fibers the ferrules surround, the spacer being positioned such that when the first and second connector ends are joined, the spacer is disposed between the ferrules and maintains the distance between the fiber ends.

7. A fiber optic cable connector as in claim 5, wherein

at least one of the connector ends comprises an alignment tube, the alignment tube functioning to help guide the fiber ends into proximity with one another as the two ends of the connector are joined.

8. A fiber optic cable connector as in claim 5, wherein:

SPECTRAL_0000323

at least one of the connector ends comprises a housing and an elastic member, the elastic member being positioned between the cable and the housing, the elastic member allowing freedom of movement of the cable relative to the connector housing.

9. A fiber optic cable connector as in claim 5, wherein:

the first and second connector ends comprise ferrules surrounding fibers of the cables, the ferrules having ends that are flush with the ends of the fibers the ferrules surround, one of the ferrules being surrounded by an extension sleeve that extends slightly beyond the flush end of the ferrule, the extension sleeve functioning to maintain the spacing between the fiber ends when the two ends of the connector are joined.

10. A method of connecting two fiber optic cables, comprising:

maintaining fiber ends of the two cables a determinate small distance apart, which distance is at least about equal to the median fiber diameter for the fibers of the two cables, the distance and positioning being such that an image preserving connection between the two fiber optic cables is formed without the aide of a lens disposed between the ends of the fibers of the two cables.

11. A method of conducting endoscopic surgeries using an apparatus comprising a segmented fiber optic cable, the method comprising the steps of:

performing a first surgery on one patient using the apparatus;

disconnecting part of the segmented fiber optic cable from the rest of the segmented fiber optic cable to form a disposable cable portion and a reusable cable portion;

connecting the reusable cable portion to another fiber optic cable portion; and

performing a second surgery using the reusable cable portion.

12. A method of conducting endoscopic surgeries using an apparatus comprising a segmented fiber optic cable as in claim 11, wherein:

during the first surgery, a camera is connected to the reusable cable portion, wherein the camera is outside the sterile field.

13. A method of conducting surgery, comprising:

using an endoscope to obtain an image from within a patient's body;

transmitting the image to a monitor that displays the image as the surgery progresses;

wherein the monitor is outside the sterile field;

wherein a person conducting the surgery views the monitor while conducting the surgery.

14. A method of conducting surgery as in claim 13, wherein:

the image is transmitted by a fiber optic cable.

15. A method of conducting surgery as in claim 14, wherein:

the fiber optic cable, with sheath, is less than 2 millimeters in diameter where it enters the patients body.

16. A method of conducting surgery as in claim 15, wherein:

the fiber optic cable is segmented.

17. A method of conducting surgery as in claim 16, wherein

two segments of the segmented cable are connected in a manner that maintains the ends of the two cables close to each other, but a small distance apart, and wherein images are transmitted between the two segments without passing through a lens.

18. A method of conducting surgery, comprising:

SPECTRAL_0000325

using a segmented fiber optic cable to transmit an image from within a patient's body to where it can be viewed by a person conducting the surgery.

19. A method of conducting surgery as in claim 18, wherein:

at least one segment of the cable is armored to a greater extent than at least one other segment of the cable.

20. A method of conducting surgery as in claim 21, wherein:

the cable has at least one connector that functions to maintain fibers at the end of one segment of the cable between four and twenty micrometers from fibers at the end of another segment of the cable.

SPECTRAL_0000326

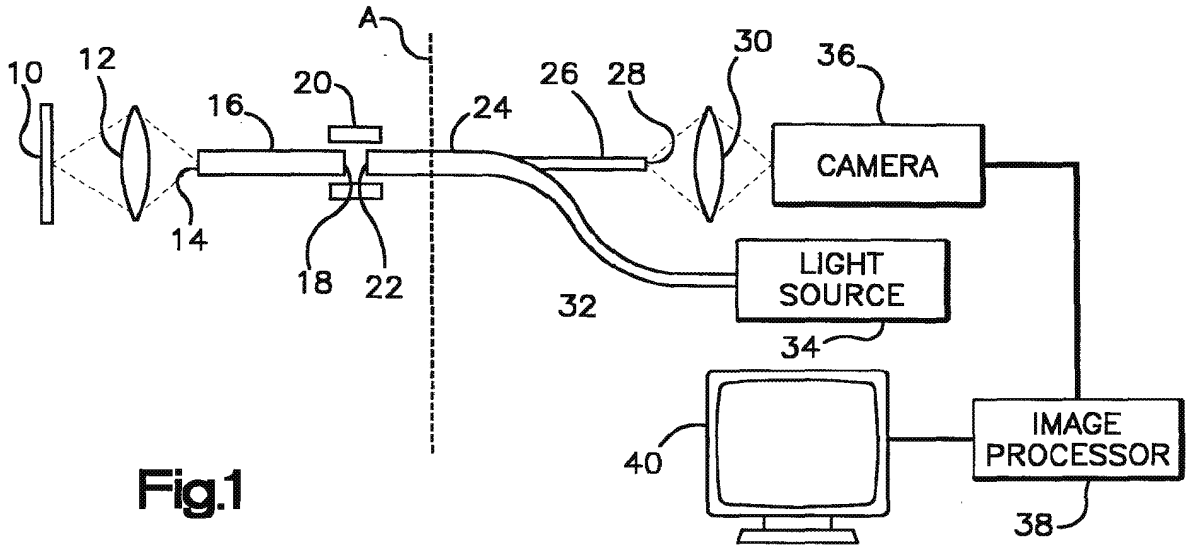

A
10
12
16
20
24
26
28
30
36
CAMERA
14
18
22
32
LIGHT SOURCE
34
40
IMAGE PROCESSOR
38

Fig.1

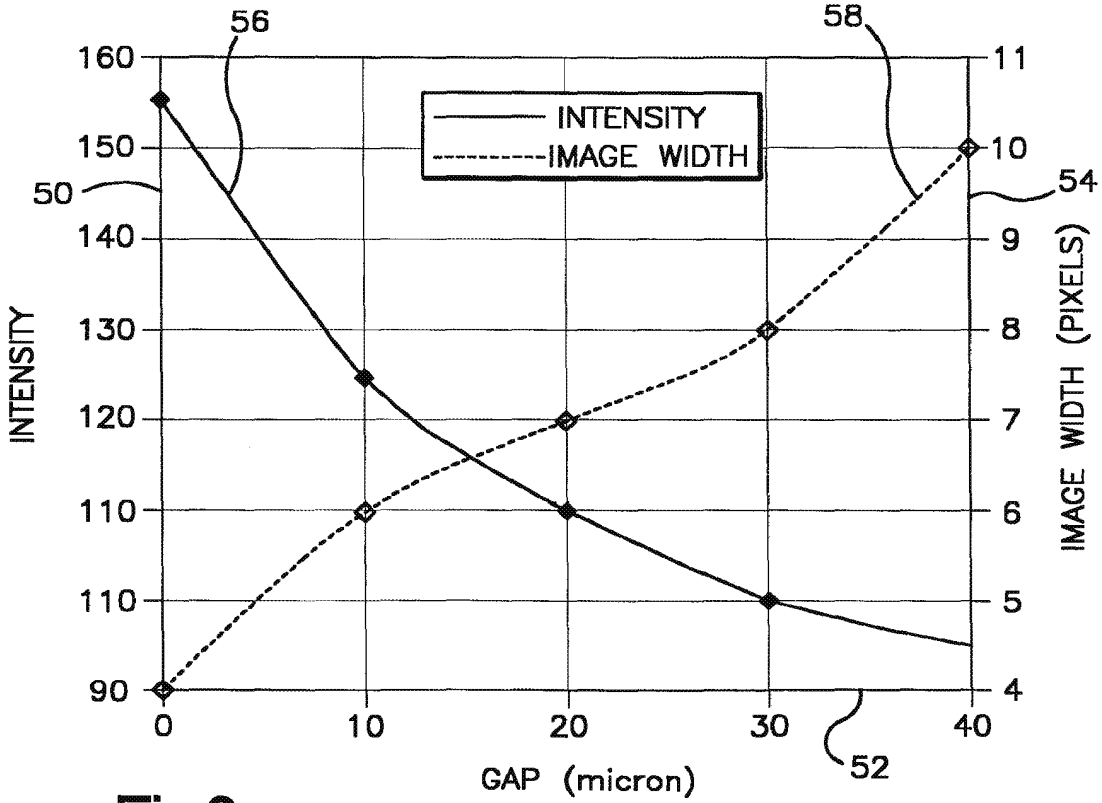

56
58
160
150
50
140
130
120
110
110
90
11
10
54
9
8
7
6
5
4
INTENSITY
IMAGE WIDTH
INTENSITY
IMAGE WIDTH (PIXELS)
0
10
20
30
40
GAP (micron)
52

Fig.2

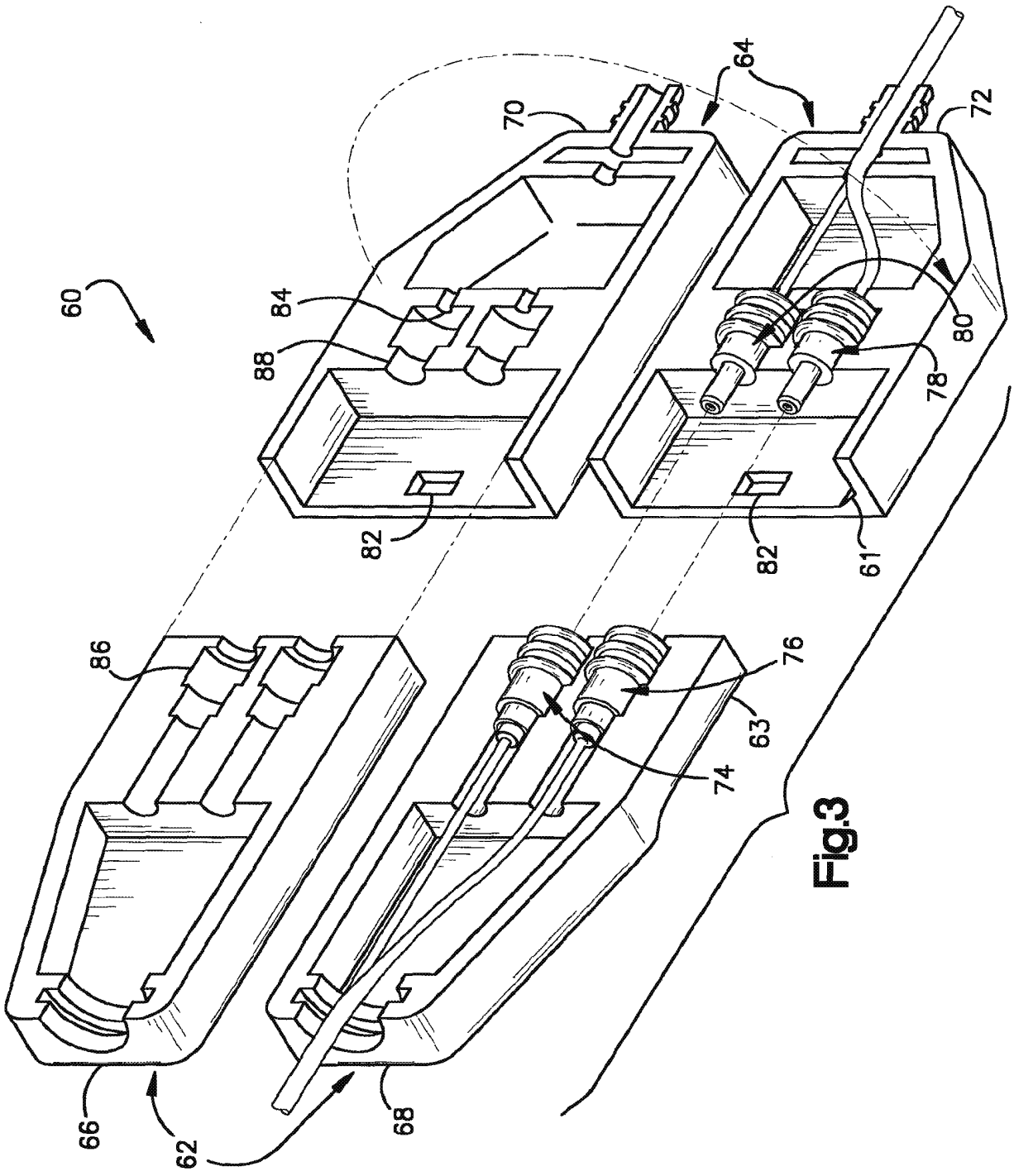


Fig.3

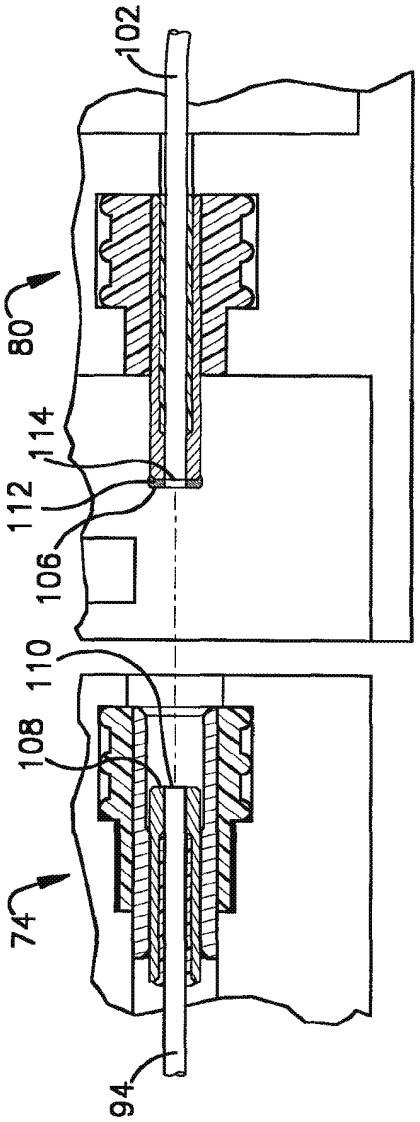

102
80
114
112
106
110
108
74
94

Fig.4

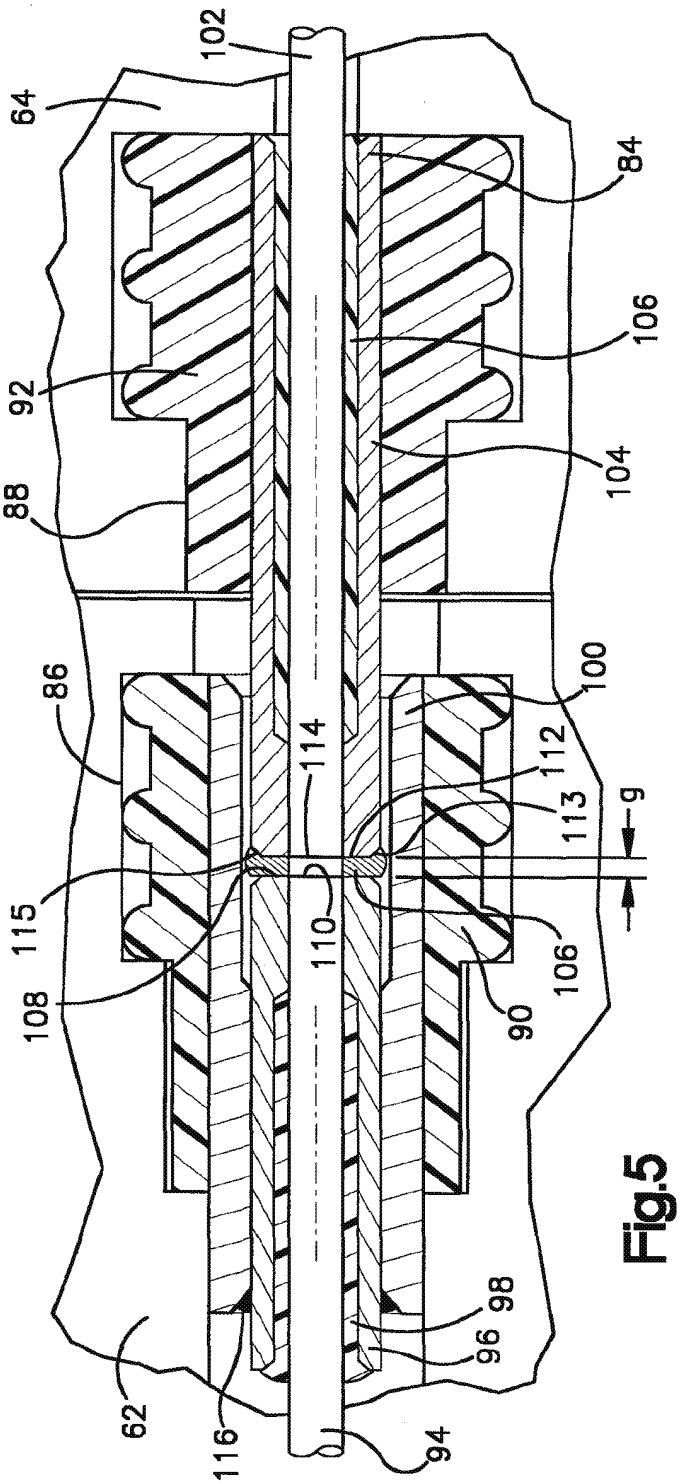

102
64
84
106
92
104
88
86
100
114
112
113
g
115
110
108
106
90
98
96
62
116
94

Fig.5

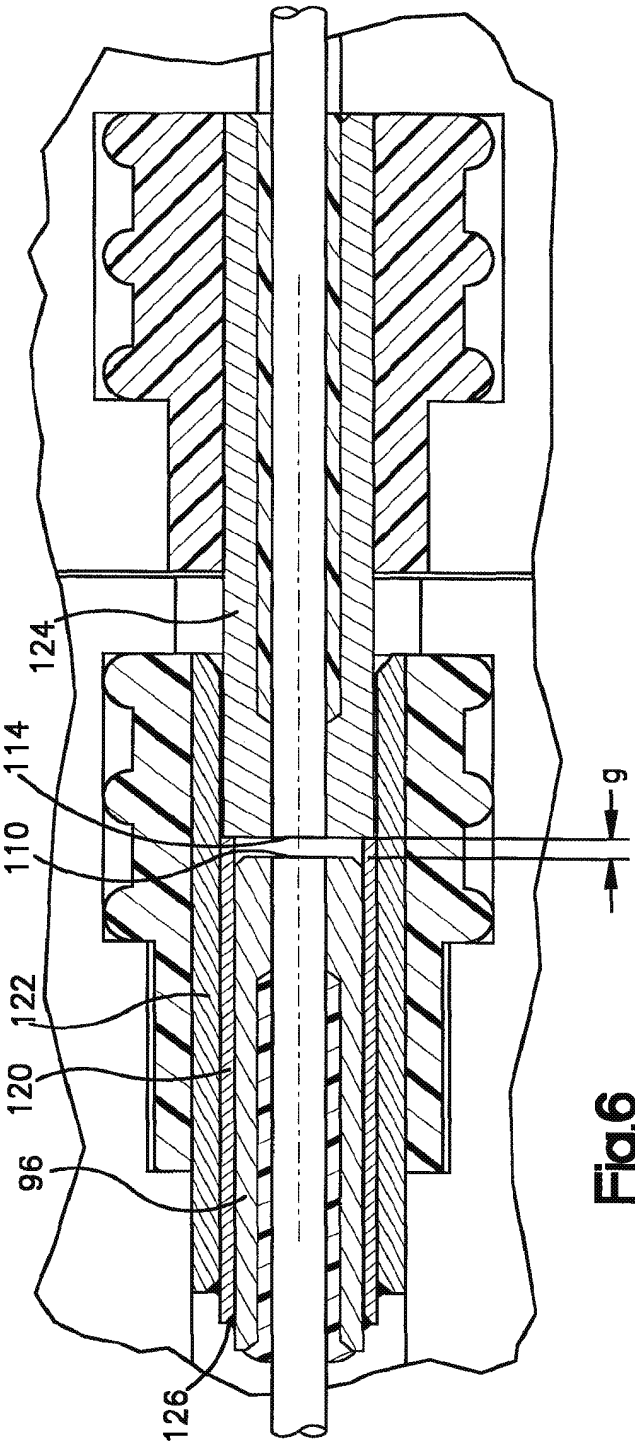


Fig.6

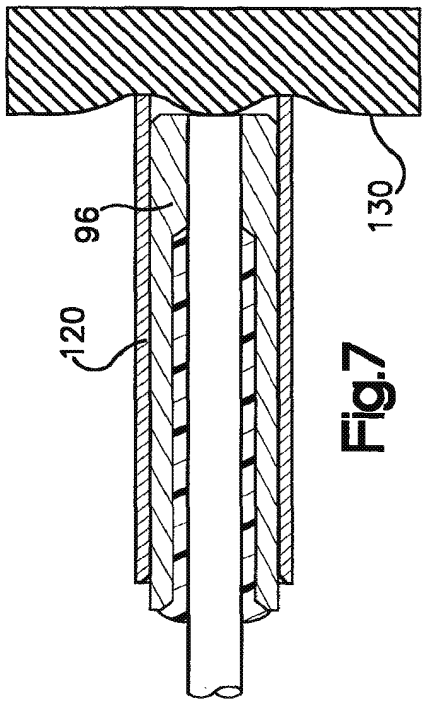


Fig.7

INTERNATIONAL SEARCH REPORT

Inte. .onal Application No
PCT/US 00/05076

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61B1/00 A61B1/04 G02B6/40

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 A61B 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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 39 14 825 C (AESCULAP A.G.) 13 September 1990 (1990-09-13) column 2, line 4 -column 3, line 15	1,2,5,7
A	US 5 101 468 A (D. CHIU) 31 March 1992 (1992-03-31) column 2, line 49 -column 3, line 3 column 4, line 53 -column 5, line 5	1-7,10
A	FR 1 426 646 A (R. WOLF) 15 April 1966 (1966-04-15) page 2, column 2, line 8 - line 43	1-7,9,10
A	WO 98 14812 A (COGENT LIGHT TECHNOLOGIES, INC.) 9 April 1998 (1998-04-09) page 5, line 26 -page 7, line 29	1-10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 July 2000	13/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Rieb, K.D.

1

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

INTERNATIONAL SEARCH REPORT

Inter. ...nal Application No
PCT/US 00/05076

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 173 110 A (WESTINGHOUSE CORP.) 5 March 1986 (1986-03-05) page 5, line 24 -page 7, line 35	1,2,5,7, 9,10

1

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

INTERNATIONAL SEARCH REPORT

..nformation on patent family members

Inter ..nal Application No
PCT/US 00/05076

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 3914825 C	13-09-1990	AT 96991 T	15-11-1993
		WO 9013254 A	15-11-1990
		EP 0470983 A	19-02-1992
		ES 2045916 T	16-01-1994
US 5101468 A	31-03-1992	NONE	
FR 1426646 A	15-04-1966	NONE	
WO 9814812 A	09-04-1998	US 5881192 A	09-03-1999
		AU 4490697 A	24-04-1998
		CN 1232548 A	20-10-1999
		EP 0929832 A	21-07-1999
		US 5905830 A	18-05-1999
EP 173110 A	05-03-1986	ES 546267 D	16-10-1986
		ES 8700760 A	16-01-1987
		JP 61061112 A	28-03-1986
		ZA 8506049 A	26-03-1986

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

SPECTRAL_0000333

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 August 2001 (23.08.2001)

PCT

(10) International Publication Number
WO 01/61324 A1

(51) International Patent Classification[7]: G01N 21/64

(21) International Application Number: PCT/US01/05107

(22) International Filing Date: 16 February 2001 (16.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2000104231 17 February 2000 (17.02.2000) RU

(71) Applicant *(for all designated States except US)*: **THE UNIVERSITY OF CHICAGO** [US/US]; 5801 South Ellis Avenue, Chicago, IL 60637 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BARSKY, Victor** [RU/RU]; Petrovka, 26, Apt. 117, Moscow, 10351 (RU). **MIRZABEKOV, Andrei** [RU/US]; 1005 Village Court, Darien, IL 60516 (US). **VENGEROV, Yuri** [RU/RU]; Englehardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str. 32, Moscow, 117984 (RU). **YERGOROV, Yegor** [RU/RU]; 7 Pyatniskoe Shosse, 77, Moscow, 123627 (RU).

(74) Agent: **CHERSKOV, Michael, J.**; Cherskov & Flaynik, The Civic Opera Building, 20 North Wacker Drive, 1447, Chicago, IL 60606 (US).

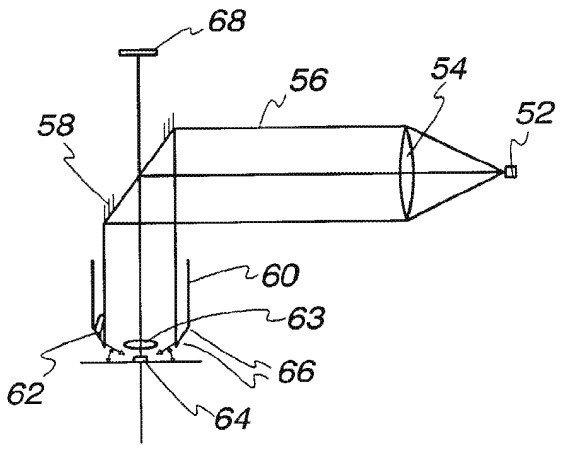
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

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: FLUORESCENCE MICROSCOPY METHODS AND DEVICES USING LIGHT EMISSION DIODES



(57) Abstract: A device for illuminating a sample (64) undergoing microscopic examination is provided, the device comprising a fluorescing radiation detector (68) and a light emitting diode (52) juxtaposed to the sample so as to irradiate the sample and prevent the detecting means from detecting non-fluorescing radiation from the sample.

WO 01/61324 A1

SPECTRAL_0000334

FLUORESCENCE MICROSCOPY METHODS AND DEVICES USING LIGHT EMISSION DIODES

CONTRACTUAL ORIGIN OF THE INVENTION

The United States Government has rights in this invention under Contract No. W-31-109-ENG-38 between the U.S. Department of Energy and the University of Chicago representing Argonne National Laboratory.

BACKGROUND OF THE INVENTION

1. Field of the Invention.

The present invention relates to a device and a method for illuminating samples in microscopy, and more particularly, the present invention relates to a device and a method for incorporating light emitting diodes in fluorescence microscopy procedures.

2. Background of the Invention

Fluorescence microscopy has many uses related to detection of target molecules. Target molecules when illuminated with excitation light may either fluoresce spontaneously or they may fluoresce after a tag is attached thereto. In the latter situation, a fluorescence tag is attached onto a moiety which itself is complimentary to the topography or chemistry of the target molecule. When the moiety finds this target, the fluorescent tag attached to the moiety indicates the existence of the duplex thus formed. One can then analyze the topography or

structure of the molecules or their relative abundance.

At present, two light sources are used for fluorescent microscopy, namely lasers and high power lamps (such as tungsten-, mercury-, and xenon-lamps). Lasers are employed mainly with scanning devices to provide a high power output in a narrow spectral region on a small surface. Lamps provide powerful illumination of large fields and also provide different wavelength capabilities. There are draw backs to both types of light sources. These drawbacks are discussed below.

Present microscope designs used in carrying out routine detection of fluorescent molecules incorporate epi-fluorescence microscopy. Epi-fluorescence microscopy most frequently employs high power tungsten or arc lamps in combination with light filters to excite the fluorescence in the sample. "Epi-" is a reference to the so-called epi-illumination scheme wherein the same objective lens is used for both illuminating the sample and collecting the fluorescence. These systems require the use of high-powered mercury or xenon lamps to ensure that after narrow-band filtering (monochro-matization), there is still enough light to provide a measurable signal at the detector. However, these sources are inefficient in that they produce a large amount of heat and light in excess of that required to illuminate the sample. Also, the large power and size requirements of these lamps makes systems incorporating them bulky. So, portability is a problem. Costs for these systems range from $12,000 to $100,000.

Lasers have been utilized in fluorescence microscopy processes. Elaborate confocal microscopy methods (i.e., confocal laser scanners) and devices exist (e.g. U.S. Patent Nos. 5,631,734 and 5,578,832) whereby sharply focused laser beams scan a sample point by point. Lasers emit light in a narrow spectral region, and as such, obviate the need for polarization filters. However, when fluorochromes are used which excite at different wave lengths, different lasers must be employed, leading to higher costs. Also, lasers do not provide enough power to illuminate the whole microscope field.

Confocal laser systems rely on complex, and therefore expensive, electronics (e.g., photomultiplier detectors and digitizing devices) to reconstruct the image as a whole. Indeed, confocal laser scanning systems cost from $500,000 to $1 million. Unlike epi-fluorescence microscopes, confocal configurations do not record the fluorescing image in parallel (i.e., all points at once).

An exemplary state of the art confocal configuration is depicted in FIG. 1 as numeral 10. Briefly, a stationary laser light source 12 supplies a light beam 14 for illuminating an objective 16. The beam is required to traverse a complex mirror- and piezo quartz-configuration 20 before arriving at the objective.

To facilitate detection of the fluorescence emanating from the now illuminated objective, the fluorescence energy must traverse through the dichroic mirror 20 to reach a detector 22 such as a photo multiplier tube. In an effort to eliminate the possibility of reflected light being detected by the detector (as opposed to just the desired fluorescent energy) a confocal pinhole 24 is juxtaposed intermediate to the objective and the detector. A signal processing unit 26 delivers the fluorescence signal to a computer 28 for processing. This configuration requires that the sample be able to move with respect to the laser (or, conversely, that the laser be able to move with respect to the sample).

The drawbacks of the above system are as follows: (a) the necessity of high precision mechanical means to move either sample or laser; (b) the impossibility of direct visual observation of the sample as the image must be processed by a computer using specially programmed software; (c) analysis takes considerable time because the sample is analyzed point by point; and (d) the instrument operator must be familiar with the fundamental features of the software.

In light of the foregoing, confocal methods are too expensive for routine fluoroscopic detection procedures.

A need exists in the art for an efficient fluorescence detection device and method which utilizes conventional microscope configurations without the need for complex signal processing units or lasers. The device and method should eliminate the energy inefficiencies and sample-desiccating heat associated with high power sources such as tungsten or arc lamps. The device and method also should utilize inexpensive light sources which are easily replaced and substituted.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a device and method for use in fluorescence microscopy that overcomes many of the disadvantages of the prior art.

Another object of the present invention is to provide an efficient fluorescence microscopy device. A feature of the invention is the incorporation of interchangeable

light emission diodes (LEDs) in the device. An advantage of the invention is enabling the device to provide fluorescence data at different excitation wavelengths. Another advantage is that the diodes can be adapted for use on a typical microscope, thereby conferring portability heretofore not seen in fluorescence microscopy systems, and at minimal cost.

Still another object of the present invention is to provide a low-cost alternative to typical fluorescence microscopy configurations. A feature of the device is the illumination of an entire sample and at an angle, or by some other means, which precludes exposure of the objective to incident or reflected illuminating radiation. An advantage of the device is that exposure of the detector to reflected light is eliminated without the need for dichroic mirrors, confocal pinholes, or other means to shield the detector.

Yet another object of the present invention is to provide an efficient, compact, and portable fluorescence microscopy method. A feature of the invention is illuminating the sample with modular, low-energy (and therefore low-heat) LEDs that can be battery powered. Another feature of the invention is that the LED provides a swath of light that can be positioned to illuminate the entire sample at once. An advantage of the method is that the simultaneous illumination of the entire sample facilitates parallel analysis of the sample located in different regions of the field of view (FOV). Another advantage is the ability to substitute, or use simultaneously, different LEDs so as to either serially- or simultaneously-illuminate the same sample with different radiation.

Briefly, the invention provides for a device for fluorescence microscopy comprising a means for illuminating a sample undergoing microscopic examination by juxtaposing a appropriately filtered light from one or more light emitting diode to the sample so as to irradiate the sample, a means for filtering and detecting radiation fluorescing from the sample and visually monitoring or permanently imaging the radiation from the sample, and a means for preventing said detecting means from detecting non-fluorescing radiation from the sample or fluorescent radiation that does not originate from the sample.

The invention also provides a fluorescence microscope comprising a means for supporting a sample; an objective lens opposing said support means; a means for simultaneously illuminating all points of the sample with incident radiation; and a means for preventing reflected radiation and the incident radiation from passing through said lens.

The invention also provides for a method for illuminating a microscopic construct, the method comprising providing an illumination detector capable of simultaneously monitoring all points of the construct; and simultaneously directing illuminating radiation to all regions of the construct and in a manner that prevents exposure of the detector to the illuminating radiation.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other objects and advantages of the present invention will become readily apparent upon consideration of the following detailed description and attached drawings, wherein:

FIG. 1 is a schematic depiction of a typical state-of-the-art confocal fluorescence microscope configuration;

FIG. 2 is a schematic depiction of an exemplary fluorescence microscope to provide dark-field epi-illumination using LEDs in combination with a ring mirror, in accordance with features of the present invention;

FIG. 3 is a schematic depiction of another fluorescence microscope but without a ring mirror, in accordance with features of the present invention;

FIG. 4a is a schematic depiction of a means for providing sample illumination using a fiber optic cable, in accordance with features of the present invention;

FIG. 4b is a schematic depiction of a means for providing uniform sample illumination using fiber optic cables, in accordance with features of the present invention;

FIG. 4c is a schematic depiction of a means for providing uniform sample illumination using fiber optic cables embedded in the housing of the objective, in accordance with features of the present invention;

FIG. 5 is a schematic depiction of illuminating microscopic samples from a non-perpendicular transmitted angle, in accordance with features of the present invention;

FIG. 6 is a perspective view of an LED-objective construct, in accordance with features of the present invention;

FIG. 7 is a cross sectional view of LEDs embedded into the housing for an objective, in accordance with features of the present invention;

FIG. 8 is a cross sectional view of an LED embedded into an objective, in accordance with features of the present invention;

FIG. 9 is a schematic view of a sample illumination configuration utilizing two parabolic mirrors, in accordance with features of the present invention; and

FIG. 10 is a schematic view of a sample illumination configuration utilizing two parabolic mirrors and a fiber optic cable, in accordance with features of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

A low-cost, highly optimized fluorescence microscope configuration is provided. Salient features of the invented device include the minimization of exposure of detectors to the excitation light (for example, reflected light) due to the off-specular-reflection angle placement of illuminating light emitting diodes. As such, the invented configuration resembles conventional epi-fluorescent microscope illumination wherein reflected light is directed away from the detector via a dichroic mirror.

The LEDs may be located inside the microscope, between the objective lens and the image detector, one possible configuration being a ring of LEDs coaxial with the microscope, the LEDs themselves being surrounded by an annular mirror that focuses the LED light onto the sample. Or the LED's light may be transmitted to the sample by means of optical fibers terminating in the region between the objective lens and the detector.

Also, one may have the LEDs encased in a container the inner walls of which are coated with a substrate that would reflect the LEDs' radiation onto the sample. Several other variations of LED placement and of means to reflect the LED light onto the sample can also be effected. All of these configurations minimize specular reflection from the sample of the fluorescing radiation into the microscope eyepiece and detector as well as direct incidence of the fluorescing radiation into the microscope eyepiece and detector. This eliminates signal-masking background that obscures the effect to be observed.

Furthermore, provision can be made for easy substitution of LEDs so that by using LEDs emitting light of different frequencies, one may excite different sample

resonances or different tag molecules and thus observe different features of the sample. Similarly, provision can be made for the interchange of different types of lens or mirror objectives or combinations thereof.

The LEDs small size, high power output, low heat output, high quantum yield, and their interchangeability allows one to design devices that cannot be built with other light sources. The use of LEDs results in fluorescence microscopes of smaller dimensions, simpler construction, better signal to noise ratio and higher sample illumination, $3mW/mm^2$ as compared to $1mW/mm^2$ with other light sources.

An exemplary configuration of the invented device is depicted in FIG. 2. Briefly, light from a light emitting diode 52 impinges upon a lens 54. The lens 54 transforms the light into a parallel beam 56. The beam 56 is directed to a ring mirror 58 which serves to redirect or bend the beam into a sheath 60. Generally cylindrical in form, the sheath 60 defines longitudinally varying inside surfaces 62 comprised of reflective material so as to facilitate rerouting of the light beam to a sample 64.

To enhance illumination of the sample, a portion of the reflective surface is formed into a frusto-conical configuration region 66 which converges toward the sample area. Alternatively, instead of a frusto-conical configuration, the sheath defines a parabolic section. As with the frusto-conical section described supra, the parabolic section defines inwardly facing surfaces converging toward the sample.

Generally, the sheath is configured to direct light to the sample so as to prevent any light reflecting off of the sample from being read by the detector 68. For example, the reflective surface of the sheath is configured so that light incident upon the reflective surface is directed at an angle toward the sample so as to prevent reflection of un-utilized excitation light into the field of view of the detector. Thus, only light emanating from the sample as a result of fluorescence of the sample is detected.

Utilizing the sheath also eliminates the state-of-the art requirement of illuminating the sample through an objective 63. As such, no optics fluorescence occurs and therefore a further decrease in background radiation is realized. Increased sensitivity and contrast results from this invented arrangement. This embodiment has become practical now that high output LEDs have become available.

The fluorescence emanating from the illuminated sample 64 is observed via an

eye piece 68 or recorded via a detector, such as photographic film, CCD camera, etc.. The light beam 56 impinges on the sample 64 for a time sufficient to induce the excitation of fluorochromes located in the sample

It may be noted that the embodiment illustrated in FIG. 2 may be simplified by omitting the sheaf 60 and by using a plane partially silvered or dichroic disk mirror instead of the ring mirror 58.

Instead of an LED situated outside of the sheath 60, one or a plurality of LEDs 52 are optionally situated inside the sheath, as depicted in FIG. 3. This configuration obviates the need for a ring mirror or a lens.

In either configuration, the reflective surface is configured to direct light to the sample 64 at an angle α so as to prevent any of the directed light, or any reflecting portion of the directed light to be read by a fluorescence-recording or -detecting means 68. Generally, an impinging light beam or reflected light beam which does not travel in parallel with the focal path of the microscope indicates that the LED and reflective surfaces are suitably oriented, relative to each other. A typical configuration therefore is side illumination of the sample, i.e, where an angularly and medially-directed illumination light is applied to the sample from a point lateral from the longitudinal axis of the microscope. Such a configuration obviates the need for dichroic mirrors, confocal pinholes or other filtering devices.

Depending on the configuration of the light emitting diode, and to provide even illumination to all fluorochrome-containing entities in the sample, the light radiation from the LED may first be directed to an optofiber system comprising an axial optical fiber 70 as depicted in FIG. 4a or a plurality of optical fibers 71 as depicted in FIG. 4b. Such optofibers disperse the light from an LED or from several LEDs, thereby providing more homogenous illumination of the sample 64. FIG. 4c is a schematic depiction of a means for providing uniform sample illumination using fiber optic cables embedded in a housing 61 of the objective 63.

Suitable optical fibers are available from a myriad of commercial suppliers, including Optical Cable Corporation of Roanoke, Virginia, USA, and Litkarino, of Moscow, Russia.

FIG. 5 depicts the utilization of LEDs in a dark-field configuration. Generally,

this configuration provides impinging radiation 57 at a non-perpendicular angle to the sample. The sample is illuminated in a dark field wherein the transmitted light beams impinge upon the sample at a point lateral from the midline 65 of the field of view.

That the impinging radiation contacts the sample 64 at an angle not perpendicular to the plane 72 upon which the sample rests is achieved via a cardioid, parabolic, or dark-centered condenser 73 juxtaposed intermediate the LED 52 and the sample 64. As such, the LED and the condenser are located distal from the detector relative to the sample 64 and below the plane 72 of the sample.

The arrangement depicted in FIG. 5 is particularly suited for viewing transparent samples mounted on transparent supports. This arrangement has the advantage that the exciting radiation does not impinge on the objective 63 and thus does not contribute to the background. Best results are obtained when the excitation beam is formed with the help of a parabolic condenser positioned between the LED 52 and the sample 64.

The inventors have utilized the invented fluorescence microscopy configuration to illuminate tagged moieties residing in nearly microscopic acrylamide substrates (0.1 x 0.1 x 0.02 mm). In such scenarios, wherein as little as 5×10^5 molecules of Texas red may be detected with average excitation intensities of between 0.5 and 10 mW/mm^2. Therefore, LEDs suitable for use in the instant invention should have light intensities ranging from approximately 0.5 to 50 milliwatts per square millimeter, depending on the spectral region targeted. Average exposure time is less than or equal to one minute. More typically, exposure times range from approximately 0.5 to 30 seconds.

LED Detail

A myriad of types of light emitting diodes lasers are utilized in the invented device. A suitable laser must emit a radiation suitable to induce fluorescence from typical chromophores used in tagging processes. Typical chromophores include, but are not limited to, Texas Red, Cy dyes, Naphthofluorescein, Fluorescein, BODIPY dyes and others. Table 1 shows the various types of fluorochromes that can be utilized with various LEDs currently available. A myriad of LED suppliers exist, including, but not limited to Agilent Technologies, Palo Alto, CA, Arachs Tech of

Hsinchu, Taiwan, Coombs Associates, Inc., of Arlington Heights, IL, Hewlett Packard, and Toshiba.

Table 1.

Source	LED Emitting λ	Labels used	Label's abs. max.
HPWL-BD01 Hewlett-Packard	629	CY 5	650 nm
		BODIPY 650	634 nm
LEAAA-100YE Arachs Technology	600	Naphthofluorescein	580-620 nm
		Texas Red	594 nm
LEAAA-100GN Arachs Technology	525	CY3	560 nm
		Lissamine	575 nm
		BODIPY TMR	545 nm
		Alexa	555 nm
LEAAA-100 BL Arachs Technology	470	Fluorescein	495 nm
MCDPG-3X0S Coombs Assoc.	450	Porphyrins	650 nm

Example

Sixteen blue-light LEDs 52 were installed along the periphery of a Zeiss objective 20 x 0.5". No additional lenses were utilized, inasmuch as the "effective illumination" area of each of the LEDs were approximately 10 mm in diameter. A perspective view of the LED-illuminated objective, is depicted in FIG. 6. Also depicted in FIG. 6 is a power-source 75 removably attached to a region of the objective's housing 76. The power source is provided for energizing the LEDs. For the sake of convenience, the LEDs typically are removably, and circumferentially arranged along the periphery of the objective's housing 76. The LEDs can be removably attached in a myriad of ways, including but not limited to a hook-and pile arrangement (e.g. VELCRO®), and a male-female configuration, whereby clips (not shown) arranged along the periphery are adapted to receive the LED's in a proper configuration for illuminating a sample juxtaposed to the objective. In this manner, LEDs can be substituted and the same objective can be utilized for various wavelength experiments.

Alternatively, as shown in FIG 7, the LEDs can be permanently affixed to the

objective housing with adhesive, mechanical fasteners, or integrally molded to the material forming said housing. This scheme allows design and use of specialized objectives which can be incorporated into an ordinary microscope and transforming it into a fluorescence microscope. The fluorescent lighting may be used instead of or in conjunction with ordinary microscope lights.

Upon energizing the LEDs, a total illuminated area of approximately 10 mm in diameter resulted, which was larger than the objective's field of view. As such, any illuminated area at least as large as the objective's field of view is suitable.

The intensity of illumination inside the field of view of the 20 x 0.5 objective was approximately 3 mW/sq. mm. This intensity is used to excite FITC, which is an activated derivative of fluorescein. With a standard emission filter for FITC and utilizing Polaroid® ISO-3000 film as the detector recording medium, exposure time for tubercle bacilli immersed with fluorescein, and washed, was approximately 2-3 seconds. This exposure time is comparable with those provided by commercially-available fluorescence microscopes.

A variation of the LED-objective configuration depicted in FIG. 6 is illustrated in FIG. 8. In FIG. 8, the LED 52, or a plurality of LEDs contact a region 67 of the objective 63 which defines the field of view. In the depicted embodiment, the LED is partially imbedded into the surface of the lens which is proximal to the sample 64 and distal from the detector (not shown in FIG. 7). In the embedded configuration, the LED is positioned at a distance "D" within 300 μm of the sample so as to obviate the need for additional focusing means.

To prevent background radiation from reaching the detector, the region of the lens which receives the LED is treated with an opaque material 69 or otherwise "blacked out" to the detector. Furthermore, inasmuch as the electrical conductors (for example, wires) energizing the LED are not located in the object plane, the conductors are not visible in the field of view. As such, their presence will not obstruct the field of view but may instead slightly decrease the intensity of illumination or spatial resolution. This scheme is most promising but it requires adapting present-day objectives.

FIG. 9 depicts an illumination configuration utilizing two parabolic mirrors. First, the sample, 64 is directly illuminated by a light source 52. The light source directs energizing radiation away from the detector 68. This radiation from the light source

first passes through a lens 54.

Fluorescing radiation 55 emanating from the sample impinges upon a first parabolic reflective surface 80 and is then redirected to a second parabolic surface 82. The twice reflected radiation 64 is finally observed through an eye piece 68 or other detection means.

In the embodiment depicted in FIG. 9, the first parabolic mirror 80 is concentrically arranged about the longitudinal axis α of the microscope with the surface of the mirror deviating medially, or inwardly toward the axis. The parabolic configuration of the first reflective surface depends downwardly toward a plane 86 supporting the sample.

The second reflective surface 82 also is of a parabolic-shaped substrate concentrically arranged relative to the longitudinal axis α to converge medially therewith. However, this second surface is directed away from the sample so as to oppose the first reflective surface. As such, the second surface is directed upwardly to receive first reflected radiation 83 from the first reflective surface.

The first and second reflective surfaces are optimized in albedo and configuration to maximize capture of fluorescing radiation emanating from the sample.

An advantage of the dual reflectance configuration of FIG. 9 is the complete isolation of the detector 68 from non-fluorescing radiation emanating from the light source 52. To assure complete isolation of the detector 68 to unutilized energizing radiation, a backstop 88 of the light source, a backstop 90 of the first reflective surface 80 and a backstop 92 of the second reflective surface 82 are configured to be opaque to the energizing radiation utilized in the device depicted in FIG. 9. This scheme is made possible by the present availability of small-size LEDs together with large size mirror objectives.

As a further alteration to FIG. 9, the light source 52 need not be incorporated into the dual mirror configuration. Instead, as shown in FIG. 10, one or a plurality of optical fibers 70 can illuminate the sample 64 by directing light to the sample from an LED 52 or plurality of LEDs not embodied by the device. As shown in FIG. 4b, significant increase in the illumination intensity may be achieved by having the fluorescence radiation be brought to the sample by optical cables and by placing the end of the cables near the sample.

While the invention has been described with reference to details of the illustrated embodiments, these details are not intended to limit the scope of the invention as defined in the appended claims. For example, the utilization of CCD cameras in the invented device would obviate the need for changing the objectives or other magnification means, inasmuch as CCD cameras confer the ability to change the range of magnification digitally.

CLAIMS

The embodiment of the invention in which an exclusive property or privilege is claimed is defined as follows:

1. A device for illuminating a sample undergoing microscopic examination, the device comprising:
   a) a means for detecting radiation;
   b) a light emitting diode juxtaposed to the sample so as to irradiate the sample; and
   c) and means to prevent said detecting means from detecting radiation not emanating from the sample.

2. The device as recited in claim 1 wherein the light emitting diode is embedded in an objective lens.

3. The device as recited in claim 1 wherein radiation from said light emitting diode impinges upon the sample at an angle that is not perpendicular to a plane upon which the sample rests.

4. The device as recited in claim 1 wherein radiation from said light emitting diode impinges directly upon the sample.

5. The device as recited in claim 1 wherein radiation from said light emitting diode is reflected onto the sample.

6. A fluorescence microscope comprising:

a) a means adapted to support a sample;

b) an objective lens opposing said support means;

c) a means for simultaneously scrutinizing all points of the support means; and

d) means for illuminating said support means with radiation which does not first pass through said lens.

7. The microscope as recited in claim 6 wherein the illuminating means is one or more light emitting diodes (LEDs).

8. The microscope as recited in claim 6 wherein the scrutinizing means is photographic film.

9. The microscope as recited in claim 6 wherein the radiation contacts the support means from a point lateral to a line defined by the lens and an opposing point on the support means.

10. The microscope as recited in claim 7 wherein the LEDs rotate around their longitudinal axis.

11. The microscope as recited in claim 6 wherein the radiation is filtered before impinging on the sample.

12. The microscope as recited in claim 6 wherein the radiation is filtered before impinging on the scrutinizing means.

13. The microscope as recited in claim 6 wherein the illuminating means comprises an LED that is mobile relative to the support means.

14. The microscope as recited in claim 6 wherein the sample support means are movable.

15. The microscope as recited in claim 6 wherein the illuminating means is one or more LEDs that are stationary relative to the support means.

16. The microscope as recited in claim 7 further comprising a ring mirror positioned between the objective lens and the scrutinizing means, said mirror adapted to reflect light from the LEDs onto the sample.

17. The microscope as recited in claim 7 wherein said LEDs are embedded in the objective lens.

18. The microscope as recited in claim 16 wherein the ends of the fiber optic cables are located between the objective lens and the scrutinizing means.

19. The microscope as recited in claim 7 further comprising a sheath encircling the objective lens, said sheath adapted to reflect light from the LEDs onto the sample.

20. The microscope as recited in claim 19 wherein the LEDs are positioned between said sheath and said objective lens.

21. The microscope as recited in claim 6 wherein the illuminating means comprise fiber optic cables

22. The microscope as recited in claim 21 wherein the ends of the fiber optic cables are located between the objective lens and the sample.

23. The microscope as recited in claim 21 wherein the ends of the fiber optic cables are juxtaposed to the sample.

24. The microscope as recited in claim 21 wherein the ends of the fiber optic cables are embedded in the objective lens.

25. The microscope as recited in claim 6 wherein the sample support means are movable.

26. The microscope as recited in claim 7 wherein the LEDs are located intermediate said sample and said objective lens.

27. The microscope as recited in claim 7 wherein the LEDs are located around said objective lens.

28. The microscope as recited in claim 7 wherein the objective lens is replaced by an objective assembly comprising one or more mirrors.

29. The microscope as recited in claim 28 wherein the LEDS are positioned between the objective assembly and the sample.

30. The microscope as recited in claim 28 wherein the sample is illuminated by fiber optic cables located between the sample and the objective assembly.

31. The microscope as recited in claim 7 wherein the LEDS all emit light are positioned between the objective assembly and the sample.

32. The microscope as recited in claim 28 wherein the LEDS each have a specific light spectrum.

33. The microscope as recited in claim 32 wherein the LEDS have the same specific light spectrum.

34. The microscope as recited in claim 32 wherein one or more of the LEDS

have a different specific light spectrum from one or more LEDs.

35 The microscope as recited in claim 7 wherein one or more of the LEDS may be rotated around an axis of the microscope.

36 The microscope as recited in claim 7 wherein one or more of the LEDS are adapted to be quickly replaced.

37. A method for illuminating a microscopic construct, the method comprising:

a) providing an illumination detector capable of simultaneously monitoring all points of the construct; and

b) directing illuminating radiation to the construct in a manner that prevents exposure of the detector to the illuminating radiation.

38. The method as recited in claim 37 wherein the source of illuminating radiation is an LED.

39. The method as recited in claim 37 wherein the illuminating radiation is derived from a plurality of LEDs.

40. The method as recited in claim 39 wherein each of the LEDs have a different wavelength and the beams impinge on the construct serially.

41. The method as recited in claim 39 wherein each of the LEDs have the same wavelength and impinge on the construct simultaneously.

42. The method as recited in claim 38 wherein the LED is in motion relative to the construct.

43. The method as recited in claim 38 wherein the LED is rotating about its longitudinal axis.

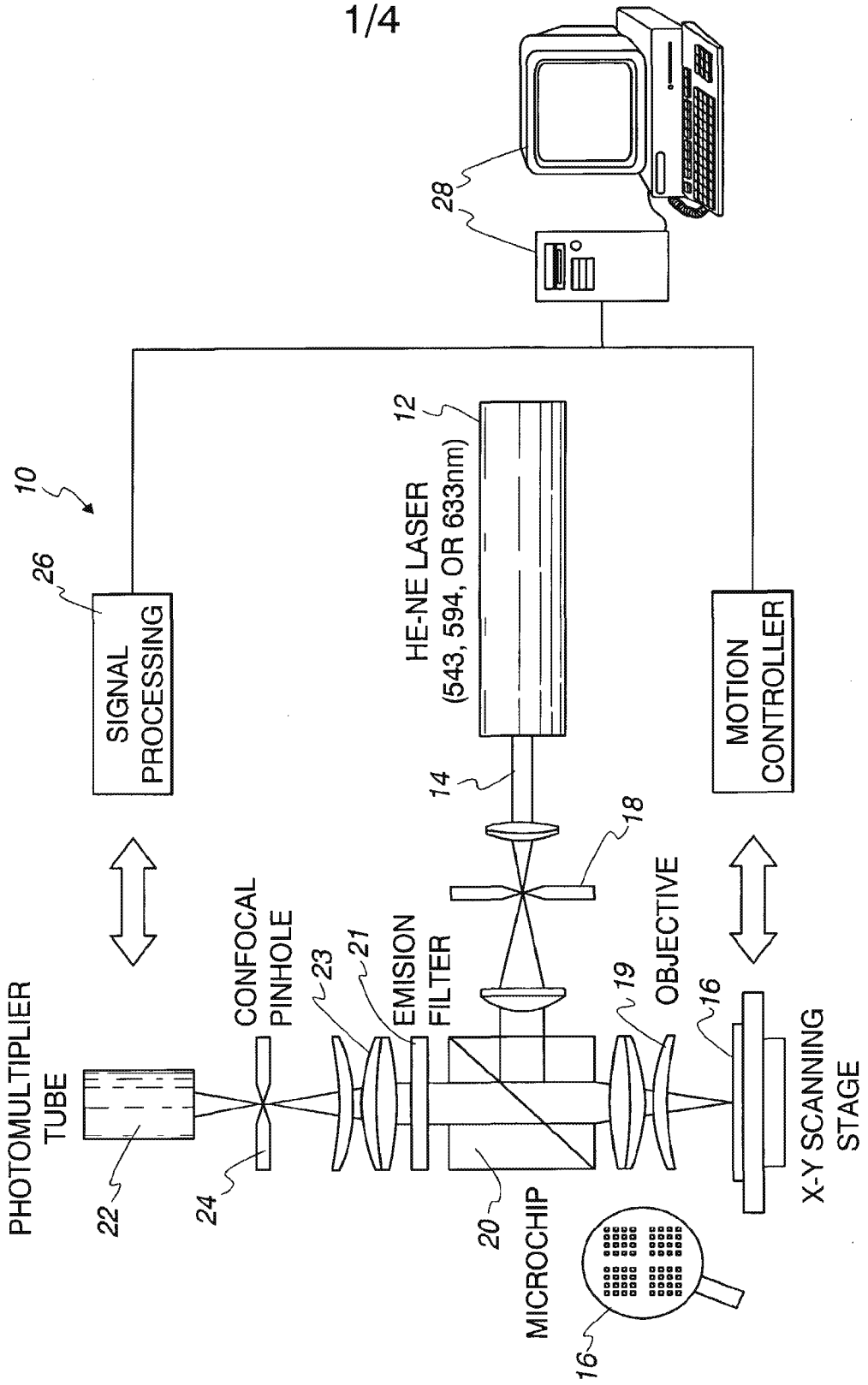

28
10
26
SIGNAL PROCESSING
HE-NE LASER (543, 594, OR 633nm)
12
MOTION CONTROLLER
14
18
PHOTOMULTIPLIER TUBE
CONFOCAL PINHOLE
23
21
EMISION FILTER
19
OBJECTIVE
16
X-Y SCANNING STAGE
22
24
20
MICROCHIP
16

Fig. 1

Fig. 2

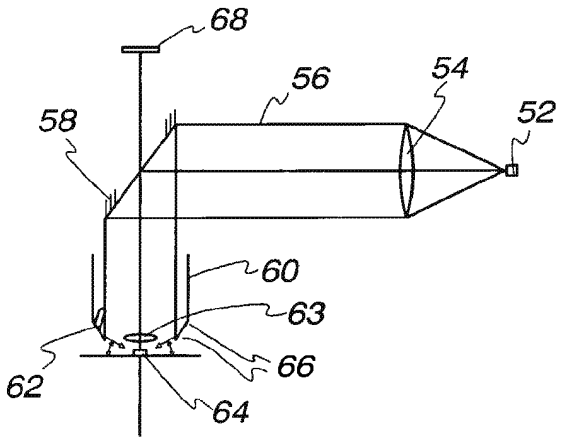


Fig. 3

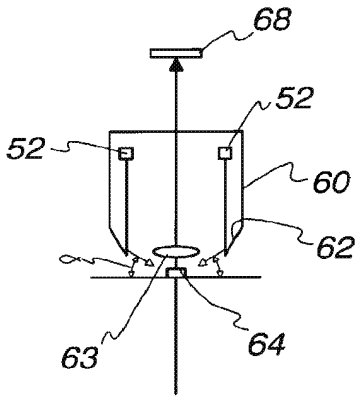


Fig. 5

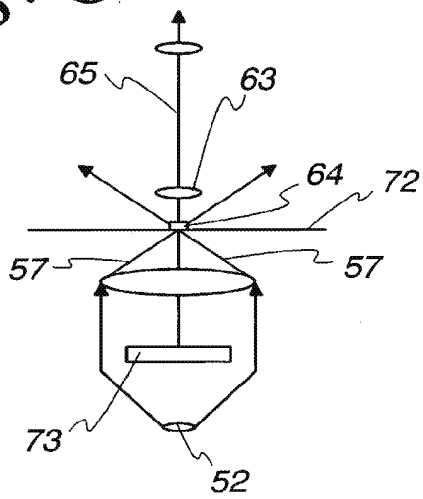


Fig. 6

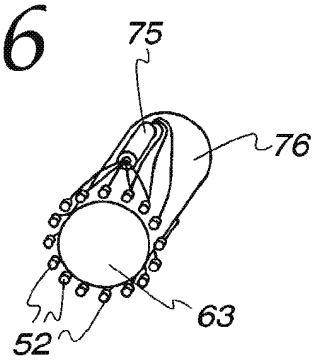


Fig. 7

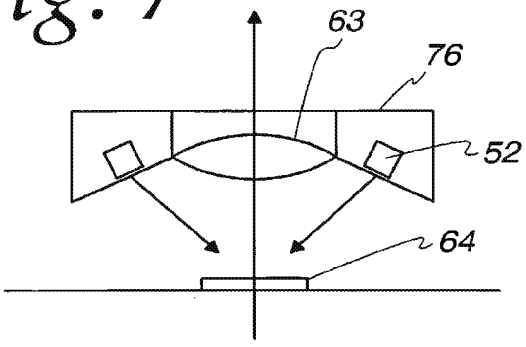
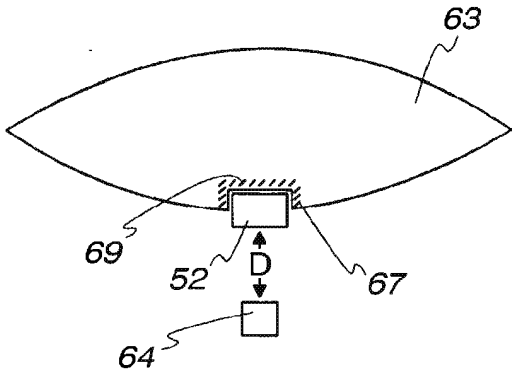


Fig. 8



SPECTRAL_0000354

Fig. 4a

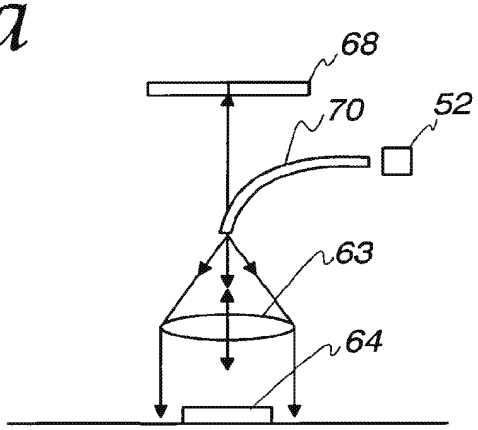


Fig. 4b

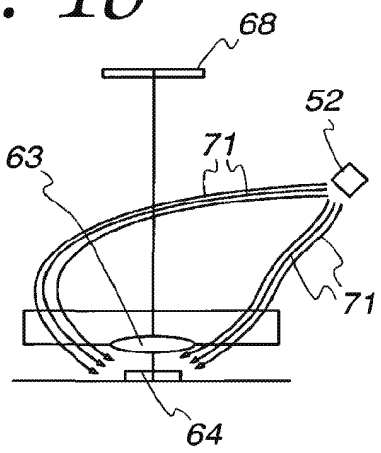


Fig. 4c

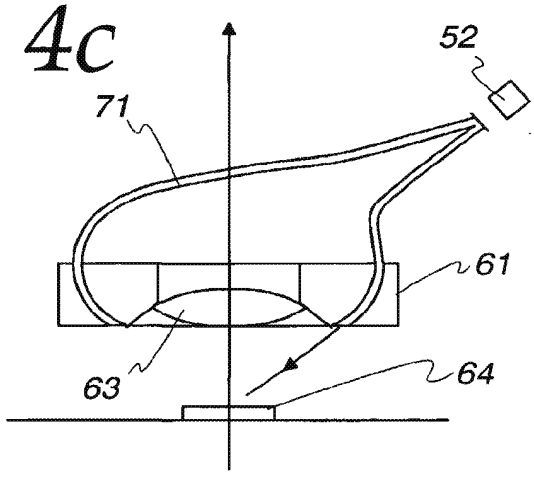


Fig. 9

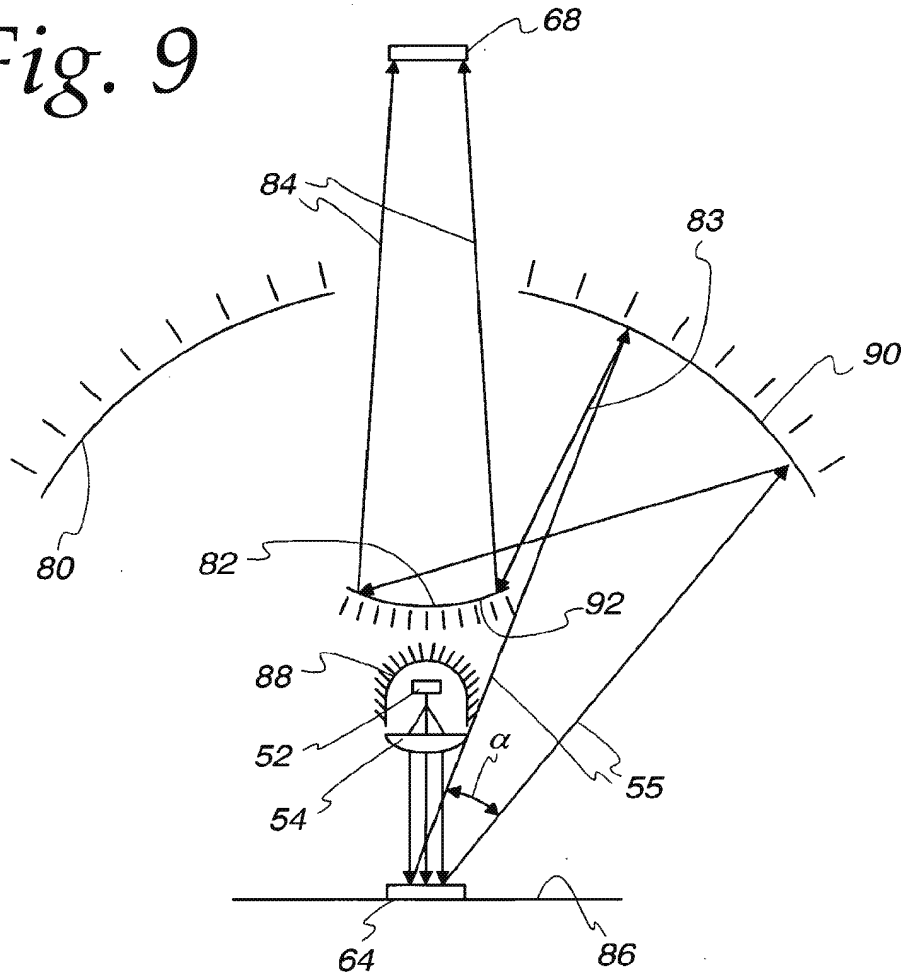
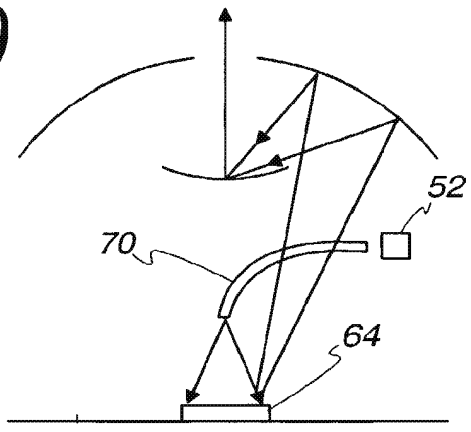


Fig. 10



SPECTRAL_0000356

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/05107

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :G0IN 21/64
US CL :356/417, 317, 318; 250/458.1, 461.1
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. : 356/417, 317, 318; 250/458.1, 461.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

EAST
search terms: microscope, fluorescence, light emitting diode, scanning, LED

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 3,705,755 A (BAER) 12 DECEMBER 1972 (12-12-72), see entire document.	1, 5, 6, 7, 15, 32 --- 28, 33, 34, 37, 38, 39
X --- Y	US 5,646,411 A (KAIN et al.) 08 JULY 1997 (08-07-97), see entire document.	1, 3-7, 14-15, 25-26, 31, 33-35, 36-40 --- 9-13, 16, 21-3, 42

[X] 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 document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 APRIL 2001	08 MAY 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ZANDRA V. SMITH Telephone No. (703) 305-7776

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

SPECTRAL_0000357

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/05107

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 6,008,892 A (KAIN et al.) 28 DECEMBER 1999 (28-12/99), see entire document.	1, 3-8, 14-15, 25-26, 31, 33-34, 36-40 ---------- 9-13, 16, 21-23, 42
X,P ---- Y	US 6,154,282 A (LILGE et al.) 28 NOVEMBER 2000 (28-11-00), see entire document.	1, 4, 5 ---------- 6-7, 9, 14-15, 25, 28, 32-34, 37-39

Form PCT/ISA/210 (continuation of second sheet) (July 1998) ★

SPECTRAL_0000358

WO 01/63247 PCT/US01/06078
(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 August 2001 (30.08.2001)

PCT

(10) International Publication Number
WO 01/63247 A2

(51) International Patent Classification[7]: G01N 21/00

(21) International Application Number: PCT/US01/06078

(22) International Filing Date: 23 February 2001 (23.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/184,859 25 February 2000 (25.02.2000) US
09/795,056 21 February 2001 (21.02.2001) US

(71) Applicant *(for all designated States except US)*: **XENOGEN CORPORATION** [US/US]; 860 Atlantic Avenue, Alameda, CA 94501 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CABLE, Michael, D.** [US/US]; 1090 Shady Creek Place, Danville, CA 94526 (US). **NELSON, Michael, B.** [US/US]; 2240 Lombard Street, #303, San Francisco, CA 94123 (US). **RICE, Bradley, W.** [US/US]; 93 Woodranch Circle, Danville, CA 94506 (US). **RASCO, Christopher, A.** [US/US]; 906 Carmel Avenue, Albany, CA 94706 (US). **SPRAGUE, David, N.** [US/US]; 34635 Pueblo Terrace, Fremont, CA 94555 (US). **NILSON, David** [US/US]; 21 Garron Court, Walnut Creek, CA 94596 (US). **DALGETTY, Rick** [US/US]; 550 Westfield Way, Oakland, CA 94619 (US).

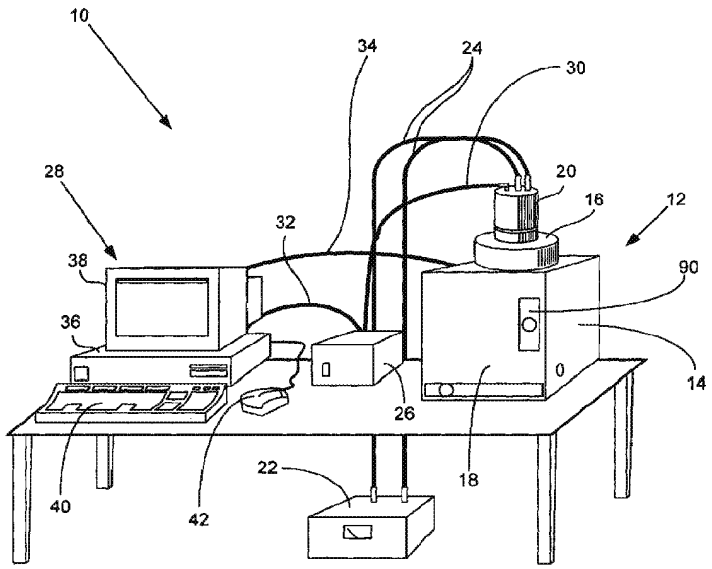
(74) Agent: **THOMAS, C., Douglass**; Beyer Weaver & Thomas, LLP, 2030 Addison Street, 7th floor, Berkeley, CA 94704 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, 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.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European

[Continued on next page]

(54) Title: IMPROVED IMAGING APPARATUS



(57) Abstract: An improved imaging apparatus is disclosed that allows a user to perform numerous imaging operations. The imaging apparatus may include one or more improvements to imaging box design to improve illumination control within the imaging box, such as improved door seal arrangements, improved door closing mechanisms, and improved light seals. The present invention may also include one or more improvements to imaging apparatus design to facilitate image capture, such as: an automated filter select device, a moveable stage, automated focus control, f-stop adjustment and stage height, and improved internal illumination for capturing photographic images.

WO 01/63247 A2

SPECTRAL_0000359

patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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.

Published:

— *without international search report and to be republished upon receipt of that report*

SPECTRAL_0000360

IMPROVED IMAGING APPARATUS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority under 35 U.S.C. §119(e) from co-pending U.S. Provisional Patent Application No. 60/184,859 filed February 25, 2000, naming M.D. Cable et al. as inventors, and titled "Light-Tight Specimen Chamber", which is incorporated by reference herein for all purposes.

FIELD OF THE INVENTION:

The present invention relates generally to imaging systems. More specifically, the present invention relates to an imaging box which forms part of an imaging system used for imaging low intensity light sources, and also relates to numerous improvements to various components of an imaging box.

BACKGROUND OF THE INVENTION:

One specialized type of imaging involves the capture of low intensity light - on the order of individual photons - from a light emitting sample. The source of the light indicates portions of the sample where an activity of interest may be taking place. For example, specialized *in-vivo* imaging applications may include analysis of one or more representations of emissions from internal portions of a specimen superimposed on a photographic representation of the specimen. The luminescence representation indicates portions of the specimen where an activity of interest may be taking place. The photographic representation provides the user with a pictorial reference of the specimen. Such imaging applications present particular challenges to the design of a box or chamber in which the sample is contained during imaging.

One particular challenge to imaging box design is the diverse lighting needs required during image capture. Photographic image capture typically requires the sample to be illuminated. Luminescent image capture requires substantially no light other than minute amounts produced within the sample. Conventional "light boxes", or

"specimen chambers" have thus been developed to maintain the sample being imaged in relative darkness during luminescent image capture.

Fig. 1A is a fragmentary, rear elevation view of the inside of a latchable door 1 of a conventional light box, as seen from the interior of the box, showing a current latch mechanism 2. Figs. 1B and 1C illustrate a seal 4 situated between the door 1 of Fig. 1A and the front wall of the box that the door is attached to. Collectively, the latch mechanism 2 and seal 4 allow a significant amount of light to enter the light box.

Thus, conventional imaging boxes or specimen chambers may not be adequate for many imaging applications, e.g., when the imaging involves the capture of low intensity light on the order of individual photons. In view of the foregoing, improved imaging apparatus would be desirable.

SUMMARY OF THE INVENTION

The present invention relates to an improved imaging apparatus that allow a user to perform numerous imaging operations. The present invention may include one or more improvements to imaging box design to improve illumination control within the imaging box, such as improved door seal arrangements, improved door closing mechanisms, and improved light seals between housing surfaces. The present invention may also include one or more improvements to imaging apparatus design to facilitate image capture, such as: an automated filter select device, automated focus control, f-stop adjustment and stage height, and improved internal illumination for capturing photographic images.

In one aspect, the present invention provides a box or chamber device that is substantially more "light-tight" than conventional light boxes, and thereby allows for more sensitive and accurate detection and imaging of low intensity light sources. The present invention also provides a variety of new features and improvements to the light box and accompanying imaging components to make the imaging process more convenient and accurate than was possible with "prior art" light boxes.

In another aspect, the present invention relates to an imaging box for capturing an image of a sample. The imaging box comprises a body including an interior cavity for receiving the sample and having a front wall defining an opening into the cavity. The imaging box also comprises a door having a rear wall, the door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning the rear wall substantially adjacent the body front wall to prevent access through the cavity opening. The door rear wall is adapted to cooperate with the front wall of the body, in the closed condition, to define a capture space extending substantially perimetrically about the cavity opening. The imaging box further comprises a compressible material disposed at least partially within the capture space, the compressible material having, when the door is in the closed position, a first side compressed substantially uniformly by the door rear wall and a second side compressed substantially uniformly by the front wall.

In still another aspect, the present invention relates to an imaging box for capturing an image of a sample. The imaging box comprises a body including an interior cavity for receiving the sample, and having a front wall defining an opening into the cavity, the front wall also including a first wall extending outward from the front wall. The imaging box also comprises a door having a rear wall, the door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning the rear wall substantially adjacent the body front wall to prevent access through the cavity opening. The door also including a second wall skewed toward the front wall, the second wall adapted in a manner such that the second wall and the first wall substantially overlap, relative their respective depth, when the door is in the closed condition. The imaging box further comprises a compressible material disposed at least partially between the first wall and the second wall.

In yet another aspect, the present invention relates to an imaging box for capturing an image of a sample. The image box comprises a body including an interior cavity for receiving the sample, and having a front wall defining an opening into the cavity. The image box also comprises a door having a rear wall, the door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning the rear wall substantially adjacent the body

front wall to prevent access through the cavity opening. The image box further comprises one of the body front wall and the door rear wall including a pair of generally parallel channel walls extending outwardly therefrom to form a channel extending substantially around the perimeter of the interior cavity opening when the door is in the closed condition. The image box additionally comprises a compressible material disposed in the channel. The image box also comprises the other of door rear wall and body front wall having an interengaging wall extending outwardly therefrom and adapted to extend into the channel in a manner such that the interengaging wall and the channel walls substantially overlap, relative their respective depth, when the door is in the closed condition, the interengaging wall further engaging the compressible material such that light entering the channel from the exterior of the body is intercepted by compressible material.

In another aspect, the present invention relates to an imaging box for capturing an image of a sample. The image box comprises a body including an interior cavity for receiving the sample, and having a front wall defining an opening into the cavity. The image box also comprises a door having a rear wall and an exterior face. The door movable is between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning the rear wall substantially adjacent the body front wall to prevent access through the cavity opening. The image box further comprises a compressible material disposed on one of the rear wall and the front wall. The image box additionally comprises a first magnetic element attached to one of the rear wall and the front wall, the first magnetic element providing a first securing force between the door and the front wall when the door is in the closed condition.

In still another aspect, the present invention relates to an imaging box for capturing an image of a sample. The image box comprises a body including an interior cavity for receiving the sample and having a front wall defining an opening into the cavity. The image box also comprises a door having a rear wall and an exterior face, the door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning the rear wall substantially adjacent the body front wall to prevent access through the cavity opening. The image box further comprises a compressible material disposed on one of the rear

wall and the front wall. The image box additionally comprises a user handle on the exterior face of the door. The image box also comprises a first latch operably positioned by the user handle and providing a securing force between the door and the front wall at a first location. The image box additionally comprises a second latch providing a securing force between the door and the front wall at a second location.

In yet another aspect, the present invention relates to an imaging system for capturing an image of a sample. The imaging system comprises an imaging box having a body including an interior cavity for receiving the sample and having a front wall defining an opening into the cavity. The imaging box also having a door with a rear wall and an exterior face, the door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning the rear wall substantially adjacent the body front wall to prevent access through the cavity opening. The imaging system further comprises an optical filter select device adapted to carry a plurality of optical filters, the filter select device capable of selectively positioning one of the plurality of optical filters to intersect light emitted from the sample.

In another aspect, the present invention relates to an imaging system for capturing an image of a sample. The imaging system comprises an imaging box having a body including an interior cavity for receiving the sample and a front wall defining an opening into the cavity. The imaging system also comprises a door having a rear wall and an exterior face. The door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning the rear wall substantially adjacent the body front wall to prevent access through the cavity opening. The imaging system further comprises a moveable stage in the cavity interior that supports the sample, the moveable stage having a first vertical position and a second vertical position in the interior cavity, wherein the first vertical position and the second vertical position have the substantially same horizontal position in the interior cavity.

In still another aspect, the present invention relates to an imaging system for capturing an image of a sample. The imaging system comprises an imaging box having a body including an interior cavity for receiving the sample, and having a front wall defining an opening into the cavity. The imaging system also comprises a door having a rear wall

and an exterior face, the door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning the rear wall substantially adjacent the body front wall to prevent access through the cavity opening. The imaging system further comprises a stage in the cavity interior configured to support the sample. The imaging system additionally comprises a gas manifold in the cavity interior and detachably coupled to the stage, the manifold including a first interface adapted to provide a gas to the sample. The imaging system also comprises a tube configured to transport the gas from outside the imaging box to the gas manifold.

In yet another aspect, the present invention relates to an imaging box for capturing an image of a sample. The image box comprises a body including an interior cavity for receiving the sample, and having a front wall defining an opening into the cavity. The image box also comprises a door having a rear wall, the door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning the rear wall substantially adjacent the body front wall to prevent access through the cavity opening. The image box further comprises at least one light tight seal, the seal comprising a first surface including a first channel, a second surface including a second channel, the first surface opposing the second surface such that the first channel and second channel at least partially face each other, and a compressible gasket disposed in the first and second channel, the gasket configured to contact opposing edges of the first and second channel when the first and second surfaces are in contact.

These and other features of the present invention will be described in more detail below in the detailed description of the invention and in conjunction with the following figures.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is illustrated by way of example, and not by way of limitation, in the figures of the accompanying drawings and in which like reference numerals refer to similar elements and in which:

Fig. 1A is a rear elevation view of the inside of a latchable door of a conventional light box, as seen from the interior of the box, showing a current latch mechanism.

Fig. 1B is an enlarged perspective view, partially broken away, of a conventional seal used in the light box of Fig. 1A.

Fig. 1C is a top plan view, in cross-section of the conventional seal of Fig. 1B.

Fig. 1D is an enlarged, cross-sectional view of a conventional seal between two surfaces.

Fig. 2 is a perspective view of an imaging system including an imaging box, in accordance with one embodiment of the present invention.

Fig. 3 is a perspective view of the imaging box and some attached imaging components of Fig. 2, with the imaging box door open and its electronics drawer pulled out.

Fig. 4 is a front perspective view of part of the imaging box of Fig. 2 with a portion of the door cut away, illustrating a sealing arrangement in accordance with one embodiment of the invention.

Figs. 5A, 5B, 5C and 5D are cross-sectional views of parts of the door and front wall of the imaging box of Fig. 2, illustrating sealing arrangements in accordance with various embodiments of the invention.

Figs. 6A, 6B, 6C and 6D are fragmentary rear elevation views that illustrate the inside of the door, as seen from the interior of the imaging box of Fig. 2, showing latch mechanisms in accordance with various embodiments of the present invention.

Fig. 6E illustrates a magnetic latch mechanism for securing the door of Fig. 2 in accordance with another embodiment of the present invention.

Fig. 7 is a perspective view of the imaging box of Fig. 2 with its door open, showing a uniform pressure applying mechanism, in accordance with another embodiment of the present invention.

Fig. 8 is a fragmentary cross-sectional view of the door of the imaging box of Fig. 7, illustrating a portion of the uniform pressure applying mechanism in accordance with one embodiment of the present invention.

Fig. 9 is a bottom plan view, cut-away, of the imaging box of Fig. 2, illustrating various imaging components in accordance with one embodiment of the present invention.

Fig. 10 is a perspective view of selected imaging components included in the imaging box of Fig. 2 in accordance with one embodiment of the present invention.

Fig. 11 is a front elevation view, in cross-section, of the interior of the imaging box of Fig. 2 and associated imaging components.

Fig. 12 is a bottom perspective view of the interior of the imaging box of Fig. 2, with portions cut away, showing various features of the box and associated imaging components.

Fig. 13 is a perspective view of the moveable stage of the imaging box of Fig. 2 showing an integrated heating element in accordance with one embodiment of the present invention.

Figs. 13B-C are side and top views, respectively, of a gas delivery system comprising a gas manifold detachably coupled to the moveable stage of the imaging box of Fig. 2 in in accordance with one embodiment of the present invention.

Fig. 14A is a top view of a light source mounted in the imaging box of Fig. 2 according to one embodiment of the invention.

Fig. 14B is a top view of a light source mounted in the imaging box of Fig. 2 according to another embodiment of the invention.

Fig. 15 is a cross-sectional view of an "o-ring" light-seal according to another embodiment of the invention.

Fig. 16 is a perspective view of the electronic component drawer of the imaging box of Fig. 2 in accordance with one embodiment of the present invention.

Fig. 17 is a flow chart illustrating a method of capturing photographic and luminescence images using the imaging box of Fig. 2 in accordance with embodiments of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following detailed description of the present invention, numerous specific embodiments are set forth in order to provide a thorough understanding of the invention. However, as will be apparent to those skilled in the art, the present invention may be practiced without these specific details or by using alternate elements or processes. In other instances well known processes, components, and designs have not been described in detail so as not to unnecessarily obscure aspects of the present invention.

I. Imaging System

In one aspect, the present invention relates generally to improved imaging systems. Fig. 2 illustrates an imaging system 10 configured to capture photographic and luminescence images in accordance with one embodiment of the present invention. The imaging system 10 may be used for imaging a low intensity light source, such as luminescence from luciferase-expressing cells, fluorescence from fluorescing molecules, and the like. The low intensity light source may be emitted from any of a variety of light-emitting samples which may include, for example, tissue culture plates, multi-well plates (including 96, 384 and 864 well plates), and animals or plants containing light-emitting molecules, such as various mammalian subjects such as mice containing luciferase expressing cells.

The imaging system 10 comprises an imaging box 12 adapted to receive a light-emitting sample in which low intensity light, e.g., luciferase-based luminescence, is to be

detected. The imaging box 12 includes an upper housing 16 in which a camera lens is mounted. A high sensitivity camera, e.g., an intensified or a charge-coupled device (CCD) camera 20 is positioned on top of the imaging box 13 and positioned above, the upper housing 16. The CCD camera 20 is capable of capturing luminescent and photographic (i.e., reflection based images) images of the sample within the imaging box 12. The CCD camera 20 is cooled by a suitable source such as a refrigeration device 22 that cycles a cryogenic fluid through the CCD camera via conduits 24. A suitable refrigeration device is the "CRYOTIGER" compressor, which can be obtained from IGC-APD Cryogenics Inc., Allentown, PA. Other methods, such as liquid nitrogen, may be used to cool the CCD camera 20.

An image processing unit 26 optionally interfaces between camera 20 and a computer 28 through cables 30 and 32 respectively. The computer 28, which may be of any suitable type, typically comprises a main unit 36 that typically contains hardware including a processor, memory components such as random-access memory (RAM) and read-only memory (ROM), and disk drive components (e.g., hard drive, CD, floppy drive, etc.). The computer 28 also includes a display 38 and input devices such as a keyboard 40 and mouse 42. The computer 28 is in communication with various components in the imaging box 12 via cable 34. To provide communication and control for these components, the computer 28 includes suitable processing hardware and software configured to provide output for controlling any of the devices in the imaging box 12. The processing hardware and software may include an I/O card, control logic for controlling any of the components of the imaging system 10, and a suitable graphical user interface for the imaging system 10. The computer 28 may also includes suitable processing hardware and software for the camera 20 such as additional imaging hardware, software, and image processing logic for processing information obtained by the camera 20. Components controlled by the computer 28 may include the camera 20, the motors responsible for camera 20 focus, the motors responsible for position control of a platform supporting the sample, the camera lens, f-stop, etc. The logic in computer 28 may take the form of software, hardware or a combination thereof. The computer 28 also communicates with a display 38 for presenting imaging information to the user. By way of example, the display 38 may be a monitor, which presents an image

measurement graphical user interface (GUI) that allows the user to view imaging results and also acts an interface to control the imaging system 10.

A. Imaging Box

In one aspect, the present invention relates to an imaging box suitable for various imaging operations. Fig. 3 illustrates the imaging box 12 of Fig. 2 in accordance with one embodiment of the present invention. The imaging box 12 is suitable for imaging including the capture of low intensity light on the order of individual photons, for example. The imaging box 12 substantially improves the control of imaging performed therein and is designed to improve the quality of the images generated from extremely low levels of light. In one embodiment, the imaging box 12 the quality of imaging by preventing the entry by light external to the imaging box in the ambient room. The imaging box 12 is referred to as "light-tight", e.g., it seals out essentially all of the external light from the ambient room from entering the box 12. The term "light-tight box" as used herein means a box or chamber device that seals out essentially all of the external light that would otherwise enter the box.

As shown in Fig. 3, the imaging box 12 includes a number of adaptations in accordance with the present invention. The imaging box 12 is illustrated with its door 18 open, showing an interior cavity 44 for receiving the sample. The interior cavity 44 is defined by opposing side enclosure panels 45a and 45b (45b not visible in Fig. 3), a light-tight partition 52 on the bottom, a top partition 103 (Fig. 10), a back enclosure panel 47, and a front wall 48 defining a cavity opening 49 into the interior cavity 44. Below the cavity 44 is a smaller compartment separated therefrom by the light-tight partition 52, the upper surface of which serves as a floor for the cavity 44. In one embodiment, the smaller compartment acts as a housing space and is adapted to slideably receive a drawer 54 though a front opening 55 formed in the body 14. The drawer 54 houses electronic components 56 which are in electrical communication with the computer 28 (Fig. 2) and control various components and functions of the box 14. In

another embodiment, the imaging box 12 has a body 14 made of a suitable metal such as steel.

A latchable door 18 is pivotally attached to box body 14 by way of hinges 46 which permit the door 18 to be moved from the closed position as shown in Fig. 2 to the open position as shown in Fig. 3. In the open position, the door 18 enables user access to the cavity 44 through the opening 55. In the closed position, where an inside wall of the door 18 is substantially adjacent to the body front wall 48, the door 18 prevent access to the cavity interior 44 through the cavity opening 55. Although the hinges 46 may be of any suitable design, they are generally designed, made and installed to enable the door 18 to close properly to provide the required sealing characteristics, as will be explained below. In addition, although the imaging box is illustrated and discussed with only one door 18 for sake of brevity, the imaging box may comprise two or more doors for access to the interior cavity 44.

The body front wall 48 defines the cavity opening 49 to the interior cavity 44. Around the perimeter of the cavity opening 49, extending outwardly, generally perpendicular to front wall 48, is a second wall 50. In one embodiment, the second wall 50 extends substantially perimetrically around the cavity opening 49. The second wall 50 includes a distal edge portion positioned substantially adjacent the door rear wall when the door 18 is in the closed position. As will be explained in several embodiments below, the walls 48 and 50 cooperate with walls on door 18 to form a capture space around the perimeter of the cavity opening 49. This capture space substantially minimizes the amount of external light that can enter the cavity 44 when the door 18 is closed.

As shown in Fig. 3, the door 18 carries a latch mechanism 60 for securing the door 18 in the closed position. The door 18 also includes a compressible material 62 attached thereto for preventing light penetration by light in the ambient room. Thus, when the door 18 is closed and secured, a seal formed by cooperation between the door 18 and the body 14 creates a substantially "light-tight" seal for the cavity 44.

Figs. 4, 5A, 5B, 5C and 5D illustrate different embodiments of a light-tight seal formed by cooperation between the door 18 and the body 14 in accordance with various

embodiments of the present invention. Each of the seals 61a, 61b, 61c and 61d in Figs. 5A, 5B, 5C and 5D, respectively, may include the compressible material 62. In one embodiment, the compressible material 62 is non-transparent, preferably black, and made from a resiliently deformable material. In a specific embodiment, the compressible material 62 is an elastomer having a modulus of elasticity of less than about 1000 psi. Preferably, the compressible material's modulus of elasticity is less than about 200 psi, and more preferably is less than about 100 psi. In another embodiment, the material has a durometer rating of between about 10 and about 50, and preferably between about 20 and 30.

Figs. 4 and 5A illustrate a light-tight seal 61a between the door 18 and the body 14 walls 48 and 50 in accordance with one embodiment of the present invention. As shown, door 18 comprises a front wall 64, a rear wall 70, a side wall 66 and a second wall segment 68. The rear wall 70 is attached to the inner surface of the door front wall 64. In a specific embodiment, door rear wall 70 has a wall section 71 that extends substantially perimetrically around the cavity opening 49 when the door 18 is in the closed position. In another embodiment, the wall section 71 extends toward the body front wall 48 at an orientation engaging a fourth side portion 63d of the compressible material 62 between a second side portion 63b thereof engaged by the body front wall 48 and a first side portion 63a of the compressible material 62 engaged by the door rear wall 70.

As shown in Figs. 4 and 5A, door walls 66, 68 and 70 cooperate with external surfaces on box walls 48 and 50 to define a capture space 72. Generally, the door 18 and box walls 48 and 50 may be arranged to form the capture space 72 having any polygonal cross-section. In this case, the capture space 72 has a rectangular cross-sectional area, as shown in Figs. 4 and 5A, where a vertex A formed by box walls 48 and 50 opposes a vertex B formed by door walls 64 and 66.

The compressible material 62 is preferably disposed at least partially in the capture space 72 and compressed therein. In one embodiment, the compressible material 62 has a transverse cross-sectional dimension compressed at least partially by the body front wall 48 and at least partially by the door rear wall 70 when the door 18 is in the closed position. The door rear wall 70 is adapted to compress the compressible material

62 substantially along the entire first side portion 63a of the compressible material 62, and the body front wall 48 is adapted to compress the compressible material 62 substantially along the entire second side portion 63b, when the door 18 is in the closed position. Consequently, light entering the capture space from the exterior of the box 12 is intercepted by compressible material 62. In this case, the sides of the compressible material 62 being compressed by the door 18 and body 14 are positioned on opposite sides of the compressible material 62. It should be noted that the walls defining the capture space 72 may compress more than two sides of the compressible material 62. By way of example, the second wall 50 extending from the body front wall 48 is adapted to engage a third side portion 63c of the compressible material 62 extending between the first side portion 63a and the second side portion 63b.

In one embodiment, the capture space 72 and compressible material 62 both extend substantially perimetrically about the cavity opening 49. For example, in Fig. 5A, the dimensions and compressibility of the compressible material 62 are selected such that, when the door is closed, (i) each portion of the material 62 that contacts a door or box wall (i.e., box wall 48, second wall 50, and door rear wall 70) is compressed substantially uniformly along its contact portion, and (ii) there is a relatively small gap G between door wall 68 and front wall 48, such that the force exerted by the latch mechanism to retain the door 18 in the closed position is a consequence of contact with the compressible material 62 and not by contact between the door 18 and the box 12. The small gap G is provided by the compressible material 62 which is sized and dimensioned to prevent the door rear wall 70 from contacting the front wall 50 when the door 18 is in the closed condition.

The light seal 61 is thus formed by the material 62 pressing against one or more planar surfaces of the door 18 and body 14, thereby compressing substantially the entire perimetric sealing surface between the door 18 and the front wall 48 of the box 12. Accordingly, the door rear wall 70 and the body front wall 48 cooperate with the compressible material 62 to provide a light seal that causes light entering the capture space 72 to traverse the transverse the cross section of the material 62 for interception of the light thereof. The seal 61a thus greatly minimizes light penetration resulting from any

interruptions in perimeter contact between the wall 50 and material 62a when the door 18 is closed.

Referring back to Fig. 1C, experimentation performed by the applicants suggests that the seal 4 can be traversed by light making only slight deflections from a straight path. Accordingly, any defects in the seal 4 along its length can result in a significant amount of light entering the box.

To further reduce light penetration, the capture space 72 of FIG. 5A introduces two right angles that light would need to negotiate before it could enter the interior cavity 44 from the outside. Any light passing between front wall 48 and second wall segment 68 would thus be required to traverse at least two right angle turns before reaching the interior side of wall 50. Moreover, since the compressible material 62 is also preferably disposed at these right angle turns, such light would be intercepted by the disposed material. This design therefore advantageously improves the light barrier between the cavity 44 and the ambient room.

In the embodiment illustrated in Figs. 4 and 5A, the compressible material 62a is rectangular-shaped in cross-section (e.g., about 0.75 in. by 0.5 in.) and has a solid core. By way of example, the compressible material 62 may be made of an adhesive backed closed cell foam having a density of between about 7 to about 9 lbs./cu. ft. and a compressibility of about 25% deflection at 5 to 9 psi. Typical of such materials is produced by Rogers of Decatur, Ill. In another embodiment, the material 62b is opaque with a light absorbing surface.

In addition to the light barrier provided by the capture space 72 and the compressible material 62a, the door side wall 66 and second wall segment 68 also function to prevent light penetration into the interior cavity 44. The side wall 66 is attached to the front wall 64 of the door and extends outwardly therefrom toward the body front wall 48. The side wall 66 is skewed toward the front wall 48 of the box 12 and is adapted such that the side wall 66 substantially overlaps the second wall 50 relative their respective depths when the door 18 is closed. Thus, a distal edge portion of the second wall 50 is positioned substantially adjacent the door rear wall 70, and a distal

edge portion of the door side wall 66 is positioned substantially adjacent the body front wall 48. Preferably, the side wall 66 extends substantially perimetrically around the opening 49 and substantially perimetrically outside and generally parallel to the second wall 50 when the door 18 is in the closed position. In another embodiment, the side wall 66 extends toward and substantially perpendicular to the box front wall 48 when the door 18 is closed.

Extending from the distal portion of the side wall 66 is the second wall segment 68 which is also substantially adjacent and preferably parallel to the body front wall 48 as shown in FIG. 5A As previously indicated, the compressible material 62 is sized to create a relatively small gap G between the distal edge portion of side wall 66 and front wall 48 which prevents the door rear wall 70 from contacting the front wall 50 when the door 18 is in the closed condition. In one embodiment, this gap G is in the range of about 1/1000 inches to about 1/2 inches. In a more specific embodiment, the small gap G is in the range of about 1/1000 inches to about 1/8 inches.

Referring now to Fig. 5B, a light-tight seal 61b is formed between the door 18 and the body 14 walls 48 and 50 in accordance with another embodiment of the present invention. In this embodiment, the compressible material 62b is also disposed in the capture space 72 (formed by walls 48, 50, 70 and 71). Similar to the embodiment of Fig. 5A, the compressible material is compressed along its opposite sides 63b and 63a by walls 48 and 70, respectively. In addition, the compressible material 62b includes a ledge portion 76 which protrudes between the distal portion of box second wall 50 and the door wall 70 to extend into the box cavity 44 when the door 18 is in the closed position. In this case, the second wall 50 is shortened and its distal portion compresses the ledge portion 76 outside the capture space 72 to provide additional light sealing. More specifically, upon closing the door 18, the ledge portion 76 is compressed by the distal portion of box wall 50 and door wall 70. The material 62b with its custom profile may be custom-made by a suitable seal or gasket manufacturer such as EPM, Inc., Stockbridge, GA. In one embodiment, the material 62b is made of solid rubber having a durometer between about 20 and about 30, such as ethylene propylene diene monomer (EPDM) or styrene-butadiene rubber (SBR).

Fig. 5C illustrates yet another light-tight seal 61c in accordance with another embodiment of the present invention. The seal 61c includes a channel 75 formed by generally parallel channel walls 78 and 79 which extend outwardly from the door 18 toward the box front wall 48, and further extend perimetrically about the cavity opening 49 when the door 18 is in the closed condition. A compressible material 62c is disposed in the channel 75. The box second wall 50, , extending outwardly from the front wall 48, is adapted to extend into the channel 75 in a manner such that the second wall 50 and the channel walls 78 and 79 substantially overlap, relative their respective depth, when the door 18 is in the closed condition. In addition, , the channel walls 78 and 79 and the interengaging second wall 50 are, preferably substantially parallel to one another when the door 18 is in the closed condition.

In one embodiment, the dimensions and compressibility of the compressible material 62c of FIG. 5C are selected such that, when the door 18 is closed and latched, the material 62c is relatively uniformly compressed around the perimeter of the cavity opening 49 when the distal portion of the box second wall 50 contacts the material. Further, the compressible material is sized to form the small gap G between door wall 68 and front wall 48, such that the force exerted by the latch mechanism to retain the door 18 in the closed position is dispersed by the material 62c. In this case, the light seal 61c is formed when the distal portion of the box wall 50 compresses the material against the door wall 70. As a result, the geometry of the overlap requires light entering the channel 75 from the exterior of the body 14 to be intercepted by the compressible material 62c. In one embodiment, the compressible material 62c has a thickness of at most 3/4 of the depth of the channel 75, and preferably at most 1/2 of the depth of the channel 75.

It should be noted that in the structural arrangement of FIG. 5C, a light-tight seal may still be formed without the compressible material 62c. This is due to the geometry of the spaced-apart channel walls 78, 79 and the interengaged second wall 50, and their spatial relationships, in the closed position. Any ambient light entering through Gap G must negotiate the maze formed between these interengaged walls. As viewed in FIG. 5C, such diffused light must traverse at least three right angle turns created by the walls before it can enter the interior cavity 44.

Although the channel walls 78 and 79 are on the door 18 and the interengaging second wall 50 is on the opposing front wall 48, it will be understood that the channel walls may be placed on the front wall 48 and the opposing interengaging wall situated on the door 18. Further, it will be appreciated that the seal 61 according to the present invention may contain two or more such channels and interengaging walls to improve light protection for the interior cavity 44.

Fig. 5D illustrates such a light-tight seal 61d which includes first and second compressible materials 62d and 62e, retained inside two channels 77a and 77b, respectively. The channel 77a is formed by channel walls 78 and 79, both of which extend from the door 18 similar to the light-tight seal 61c. The channel 77b is formed by generally parallel channel walls 50a and 50b, both of which extend from the front wall 48 of the box 12 and substantially perimetrically around the cavity opening 49. In this case, the channel wall 79 is received in the channel 77b when the door 18 is in the closed condition. Thus, the channel wall 79 engages the second compressible material 62e such that light entering the second channel 77b from the exterior of the body 14 is intercepted by the second compressible material 62e.

The materials 62d and 62e fill only a portion of the channels 77a and 77b respectively, e.g., at most about ¾ of the channel 75 depth as measured from the walls 70 and 48 respectively, preferably at most about ½ the channel depth, and more preferably at most about ¼ the channel depth. Thus, the interengaged walls 50a and 50b and channel walls 78 and 79 substantial overlap, relative their respective depth, when the door 18 is closed. Any light attempting to penetrate the light barrier would require at least six right angle turns (including four through the compressible materials 62d and 62e) before it can enter the interior cavity 44 from the ambient room.

Similar to the embodiments illustrated in Figs. 5A and 5B, the door 18 of Figs. 5C and 5D further includes door side wall 66 extending outwardly from the door 18 toward the front wall 48 when the door is in the closed position. In this case, the door side wall 66 is positioned perimetrically outside and generally parallel to the channel walls 78 and 79 and the interengaging front wall 50. The door 18 further includes the second wall segment 68 extending from the door side wall 66, and oriented substantially adjacent to

the front wall 48 when the door 18 is in the closed condition. As shown, the second wall segment 68 extends in the inward direction toward interengaging front wall 50 and the channel walls 78 and 79.

In another aspect of the present invention, several designs for securing the door 18 are provided that minimize light penetration from the ambient room. Referring back to Figs. 1A-C, experimentation performed by the applicants suggests that the securing force provided by the latch mechanism 2 when the door 1 is closed may also lead to additional undesired light entering the box. When the door 1 is closed, the latch mechanism 2 provides a localized securing force at a single point along the door 1 (i.e., at the point of contact between the latch mechanism 2 and the door 1). This localized force provides an inconsistent pressure along the seal 4 and may lead to gaps in the seal 4 and other light sealing inconsistencies around the perimeter of the box 12.

As shown in Figs. 6A-6D, latch mechanisms 60a-d for securing the door 18 in accordance with various embodiments of the present invention. The latch mechanisms 60a-d include multiple points of contact between the door 18 and the box 12 to provide more uniform force distribution across for the seal 61. This more uniform distribution minimizes light penetration resulting from any inconsistencies in perimeter contact between the wall 50 and material 62 when the door 18 is closed.

Figs. 3 and 6A illustrate the latch mechanism 60a in accordance with one embodiment of the present invention. The latch mechanism 60a includes a plurality of latches 86 located on the inside of the door 18. Each latch 86 is moveable between a first position, disengaged with the front wall 48, and a second position, engaged with the front wall 48 in which a securing force is applied between the door 18 and the front wall 48 at a strategic location. Together, the latches 86 provide a multi-point seal between the compressible material 62 in the door 18 and box walls 48 and 50 and distribute the applied compressive closing force along the vertical non-hinged edge 81 of the door. This multiple contact provides a substantially uniform securing and compression force against the compressible material 62 and along the vertical non-hinged edge 81 between the latches 86.

The latch mechanism 60a includes a main latch 80 and two dependent latches 82, each of which is moveably linked to the main latch 80 by rods 84. The rods 84 are each slideably supported by stays 86 which are mounted to the rear wall 70 of the door 18. The main latch includes a latch element 88 rotatably mounted on the inside of the door 18 for applying a force against the box 12. A handle 90 (see Fig. 2) extends from the exterior face of the door 18 to enable user manipulation. The handle 90 permits a user to operably position the latch element 88 and each of the dependent latches 82 between the first position, where the latches are disengaged with the inner surface of wall 48 (solid lines in Fig. 6A), and the second position, where the latches are engaged (broken lines in Fig. 6A). In one embodiment, the latch element 88 and dependent latches 82 may have a tapered engaging surface that contacts the wall 48 and provides a controllable amount of force which increases as the rotation of the handle 90 increases. In another embodiment, the main latch does not include a latch element 88 and the dependent latches 82 each comprise a rectangular element that applies the securing force between the door 18 and the box 12. Such a configuration is commercially available from Doortronics Systems, Inc. of Sag Harbor, NY.

Fig. 6B illustrates another latch mechanism 60b in accordance with one embodiment of the present invention. The latch mechanism 60b includes a plurality of spaced-apart latches 82 disposed along the inside of the door 18 to provide a multi-point seal against the compressible material 62 between the door 18 and box walls 48 and 50. This arrangement uniformly distributes the compressive force when the door 18 is closed substantially about the perimeter of the box opening 49. This is performed by providing four dependent latches 82a distributed on four different edges of the door. Each dependent latch 82a is moveably linked by individual rods 84a to a cam device 80a which is rotatably mounted to the center of the door 18, . These rods 84a are each slideably supported by stays 86a mounted to the inside of the door 18. With this arrangement, the cam device 80a does not have a latch element that engages to the box 12, such as the latch element 88 of the embodiment of FIG. 6A An external knob, however, is included for user manipulation and simultaneously actuating the four dependent latches 82 between engaged and disengaged positions. In one embodiment,

the dependent latches 82 may have a tapered engaging surface that contacts the wall 48 in order to provide a controllable amount of force when securing the door 18.

Referring now to Figs. 6C and 6D, latch mechanisms 60c and 60d are illustrated in accordance with other embodiments of the present invention. In both embodiments, at least three latches 80b and 80c, respectively, are provided, each with their own independently controllable external handle. These latches are spaced-apart at strategic locations about perimeter of the door 18 and box opening 49. Each latch 88b, 88c is moveable between a first position, disengaged with the front wall 48 (solid lines in FIGS. 6C and 6D), and a second position, engaged with the front wall 48 in which a securing force is applied between the door 18 and the front wall 48 (broken lines in FIGS 6C and 6D). In the latch mechanism 60c embodiment, the latches 88b are spaced-apart along the vertical edge 81 of the door opposite the door hinges. Collectively, the latches 88 provide securing forces which uniformly compress the compressible material 62 substantially between each pair of latches 88 along the vertical edge 81 of the door 18. In the latch mechanism 60d embodiment, in contrast, the latches 88c are spaced-apart along different door 18 edges. Each latch 88c is equipped with its own user handle to operably position its corresponding latch 88 between the disengaged first position (solid lines in FIG. 6D), and the engaged second position (broken lines in FIG. 6D). Similarly, the latch element may have a tapered engaging surface which provides a controllable amount of force which increases with increased rotation of its external knob.

Fig. 6E illustrates a latch mechanism 180 for securing the door 18 of Fig. 2 in accordance with another embodiment of the present invention. The latch mechanism 180 includes a magnetic element 182 attached to the box 12 front wall. The magnetic element 182 provides a securing force between the metal door 18 and the front wall when the door 18 is in the closed condition. In a specific embodiment, the magnetic element 182 is a permanent magnet that provides a securing force large enough to compress the compressible material 62 and to keep the door from inadvertently opening when someone brushes the door 18 or other similar small forces that may open the door 18. In a specific embodiment, the magnetic element 182 is a permanent magnet that generates a securing force in the range of 25- 30lbf. In another embodiment, the latch mechanism 180 includes a second magnetic element, similar to the magnetic element

182, attached to the box 12 front wall at another point on the box 12 face. Together, the two magnetic elements provide a multi-location closing mechanism that distributes the applied compressive closing forces along the perimeter of the door 18. This multi-location magnetic latch provides a substantially uniform securing and compression force against the compressible material 62 and along the door 18 between the magnetic elements.

In another embodiment, the latch mechanism 180 includes an electromagnet 183a and 183b attached on facing surfaces of the door 18 and the box 12 front wall. The electromagnet 183 provides a securing force between the metal door 18 and the front wall when the door 18 is in the closed condition. The securing force provided by the electromagnet 183 is large enough to keep a user from opening the door 18. In a specific embodiment, the electromagnet is only powered during luminescent image capture of the sample, thus preventing the door 18 to be accidentally or inadvertently opened during luminescent image capture.

Figs. 7 and 8 illustrate a mechanism for securing the door 18 in accordance with another embodiment of the present invention. A plurality of screw latches 92 are spaced about the perimeter of the door 18 and configured to engage with corresponding threads 94 in wall 48 of the box 12. When the door 18 is in the closed position, the screw latches 92 contact their corresponding threads 94. Each screw 92 is then driven by an individual worm-drive stepper motor 96 to apply a predetermined amount of pressure to the compressive material 62 disposed between the door 18 and the wall 48A pressure transducer 98 communicates with each motor 96 and is programmed to stop the motor when the predetermined amount of pressure is reached. The transducers 98 may be coupled to the computer 28, and may be, programmed to be conveniently adjust the properties thereof through keyboard 40 and/or mouse 42.

Advantageously, the design of the seal 61 and the distributed manner in which the seal 61 is engaged when the door 18 is closed provide a substantially more light-tight imaging box than was previously available, as evidenced by the comparison testing described below. Having briefly discussed various illumination control improvements of

the imaging box 12, numerous other aspects of the imaging box 12 will now be discussed.

Referring now primarily to Figs. 9, 10 and 11, various components housed in the upper portion of the box 12 will now be detailed. Fig. 9 is a bottom view of the components in the upper portion of the box 12 with the top face of the box 12 cut away. Fig. 10 is a perspective view of selected imaging components in the upper portion of the box 12. Fig. 11 is a cross-sectional side view of the box 12 looking into the cavity 44 with various components in the upper portion of the box 12 shown in cross-section.

The system 10 provides user automated control of image capture in the box 12. Referring to Figs. 9 and 11, a camera lens 100 is mounted in upper housing 16, with the lens 100 in view of the interior cavity 44 through a hole 101 formed in a top plate 103 of the box 12. The camera lens 100 is optically coupled to the camera 20 of Fig. 3. and includes a user controlled aperture or F-stop ring 102 for adjusting the F-stop or aperture of the lens 100, thereby modulating the amount of light passing through the lens. By way of example, a Navitar, f 0.95, 50 mm TV lens is suitable for use as the camera lens 100. The F-stop ring 102 includes circumferentially disposed teeth that engage a gear 104 driven by an F-stop motor 106. The F-stop motor 106 is in electrical communication with the electrical components 56 and controlled by computer 28. Together, the motor 106 and processor in computer 28 act to position the f-stop of the lens 100.

Also associated with the camera lens 100 is a focusing mechanism including lens support 107 for supporting and focusing the lens 100 to provide reciprocal movement thereof. The lens support 107 includes a stationary portion mounted to upper housing 16 and a movable portion that includes a threaded bore 109. A bolt 108, operably engageable with the bore 109, includes a wheel that is driven by a toothed belt 110 through a corresponding drive wheel 112 of a camera lens focus motor 114 to move the camera lens 100 into focus. The camera lens focus motor 114 is in electrical communication with the electrical components 56 and controlled by a processor included in computer 28.

In addition to automated focus control of the camera lens 100, the system 10 also includes an automated filter select device 117 capable of selectively providing multiple filters 118 at least partially between the camera 20 and the sample. The filters 118 may each facilitate image capture for one or more particular imaging applications. As shown in Figs. 9 and 11, the optical filter select device 117 includes a circular filter select wheel 116 adapted to carry a plurality of optical filters 118 around its perimeter. The filter select wheel 116 is capable of selectively positioning one of the plurality of optical filters 118 to intersect light emitted from a sample within the cavity interior 44. The wheel 116 is rotatably mounted at its center to a mounting bracket 120 attached to upper housing 16. The filter wheel 116 is mounted off-center from the lens 100 such that the individual filters 118 can each be rotated into position to intersect light emitted from the sample before reaching the camera lens 100. The filter wheel 116 has a groove along its perimeter edge in which a toothed belt 122 is seated. The toothed belt 122 is also engaged with a drive wheel 124 on a filter wheel motor 126. The filter wheel motor 126 is in electrical communication with the electrical components 56 and controlled by a processor included in computer 28. The plurality of optical filters 118 carried by the filter wheel 116 may include any of a variety of optical filters for facilitating image capture such as a neutral density filter for bright samples, one or more wavelength cutoff filters for restricting specific wavelengths, a fluorescent filter for fluorescence applications in which the excitation light differs from the detected light, etc.

In another embodiment, the filter select device 117 comprises a two filter wheel 116 system. In this case, the filter select device 117 includes a first optical filter select wheel 116 and a second optical filter select wheel 116, both of which are rotatably mounted in parallel at their center to the mounting bracket 120 attached to upper housing 16. The first filter select wheel 116 is adapted to position a first set of optical filters included in the plurality of optical filters. The second filter select wheel 116 is adapted to position a second set of optical filters included in the plurality of optical filters. The filter select device 117 may then selectively position a combination of optical filters from the first and second wheels such that light emitted from the sample must pass through two optical filters. In a specific embodiment, the first and second filter select wheels 116 are

each adapted to carry seven optical filters. In another specific embodiment, the first and second filter select wheels 116 are each adapted to carry twelve optical filters.

The F-stop motor 106, lens focus motor 114, and filter wheel motor 126 are each stepper motors capable of suitable position control of their respective components. By way of example, a model number SST 39D 1010 (1.8 deg/step, 4.3V, 0.85A), manufactured by Shinano Kenshi Co., Ltd, Japan, is suitable for use with any of the motors 106, 114 and 126. As shown in Fig. 12, each of the motors 106, 114 and 126 is in electrical communication with one or more electronic components 56 housed in drawer 54 via wires 134. The electronic components 56 are, in turn, in communication with the computer 28 where the motors 106, 114 and 126 may be controlled by appropriate software and/or by user input.

The box 12 also includes a movable stage 58 on which the light-emitting sample is supported. The movable stage 58 is capable of linear, reciprocal movement between the partition 52 and the top enclosure panel 41, and may be retained at any position therebetween for image capture. Thus, the moveable stage 58 has a multiple vertical positions in the interior cavity having the substantially same horizontal position. As shown in Fig. 12, the movable stage 58 has a threaded bore that is operably engaged with a worm gear 136. The worm gear 136 provides vertical translation of the moveable stage 58. A motor 138 (e.g., model number SST 42D 2120 from Shinano Kenshi Co. (1.8 deg/step, 3.7V, 1.2A)) drives the worm gear 136 to move the stage 58 up and down along a pair of guides 140. In another embodiment, the stage 58 is driven vertically using a belt driven system that provides a faster response than the worm gear 136.

In one embodiment, the movable stage 58 supports a removable vertical wall placed on the upper surface of the movable stage 58. The vertical wall acts as a light shield that prevents light emitting from a sample to translate horizontally across the stage 58 surface to a portion of the moveable stage 58 that does not support the sample. For image capture of multiple samples in which one sample producing excessive light, the vertical wall may then be useful in preventing the excessive light from this sample to undesirably affect imaging of an adjacent sample.

Also associated with the moveable stage 58 is a position sensor 142. The position sensor 142 communicates with the computer 28 and provides a read-out which may be used in position control of the stage 58. In this case, the position sensor 142 includes a string or thin string 144 having one end attached to the moveable stage 58 while the other end is attached to a take-up reel in the position sensor 142. Based on the amount of string 144 wound on the reel and the total length of the string 144, the position sensor 142 is able to determine the length of string between the stage 58 and the sensor 142. This length is then converted into the height of the moveable stage 58 relative to partition 52., e.g., by using a look-up table in computer 28 to carry out the conversion. In another embodiment, the position sensor is a laser positioned in the interior cavity 44 to intercept the moveable stage 58 at a starting vertical position. The laser may then be used to calibrate the position of the moveable stage 58 to the starting vertical position.

In many imaging applications, the low intensity light source may be embodied in any of a variety of light-emitting animals containing light-emitting molecules, such as various mammalian subjects containing luciferase expressing cells. Often, thermoregulatory functioning of the animal has been compromised to facilitate analysis or image capture, e.g., many laboratory mice are genetically hairless or the mice are sedated during imaging to minimize any movement that may compromise imaging. Correspondingly, in one embodiment of the present invention, the system 10 includes a temperature control element 132, e.g., a heater or cooler, configured to control the temperature of one of the sample and the interior cavity 44, or both. For example, while imaging mammals or mammalian cells, it is often desirable to maintain the specimens at or near 37 degrees Celsius. In these cases, the imaging system 10 keeps the stage 58 and sample warm by heat provided by the temperature control element 132 at or near 37 degrees Celsius.

In the embodiment shown in Fig. 11, the temperature control element 132 is provided by a heating blanket placed on top of stage 58. In another embodiment, as shown in Figs. 12 and 13A, the temperature-adjusting element 132 is a thermal sheet 160 for controlling the temperature of the sample (such as M2436 1234 24V 35W as provided by Instrument Labs of Los Angeles, CA) that is fixed, e.g. glued. into a cut-

away portion of stage 58. The temperature-adjusting element 132 includes heating element lead 162 which supplies the power for heating element 160 and extends from thermal sheet 160 and is in electrical communication with electronic components 56 housed in drawer 54. Together, the heating element lead 162, electronic components 56 and computer 28 allow temperature and heat control of the temperature-adjusting element 132.

As mentioned before, it is often desirable to sedate a light-emitting animal during imaging to minimize any movement that may affect imaging. In many cases, an anesthetizing gas is supplied to the animal to keep the animal sedated for an extended period of time. In these cases, the imaging system 10 includes a gas delivery system 220 detachably mounted on the moveable stage 58 to deliver a gas to the sample.

In the embodiment shown in Figs. 13B-C, the gas delivery system 220 comprises a gas manifold 222 detachably coupled to the moveable stage 58. The gas manifold 222 includes a plurality of interfaces 224. Each interface 224 is adapted to provide a gas to a sample resting on the moveable stage 58. In a specific embodiment, the gas manifold 222 comprises five interfaces 224 that are each funnel shaped to accommodate different sized samples. The gas manifold 222 may be screwed using bolts 227 or otherwise fixed to the stage 58 in a detachable manner. A valve 225 associated with each interface 224 controls gas flow to the sample through its respective interface. A tube 226 supplies gas to the gas manifold 222 and extends from outside the box 12. To reach a sample, gas supplied from the tube 226 flows through a channel 229 to an interface 224 that accommodates the sample. In one embodiment, the tube 226 includes a distal end that is open to the environment outside the box 12. In this case, the tube 226 is substantially long and extends along a large distance in the cavity interior 44 with numerous turns. The tube 226 also has a large length to cross sectional area and includes non-reflective surfaces that minimize light passage through the tube 226. As a result, the end of the tube 226 inside the cavity interior 44 emits substantially no light within the cavity interior 44. In a specific embodiment, the tube 226 has a diameter of ¼"ID to 3/8" OD inches and a length of about 85 to about 90 inches and is made of black PVC. A second tube 228 may also be included as a gas outlet.

As light retained in any elements inside the box 12 may undesirably affect subsequent luminescent image capture, the manifold 222 comprises a non-reflective surface and a non-light retaining material. In a specific embodiment, the gas manifold 222 is made of glass that does not retain light introduced to the manifold 222 when the door 18 is open. In another embodiment, the gas manifold 222 is autoclavable. The autoclavable gas manifold 222 allows simple sterilization and contaminants to easily be removed from the gas manifold 222. For example, the gas manifold 222 may be autoclaved by subjecting the manifold 222 to high temperatures and pressures, e.g., up to about 60 psi and about 130 degrees Celsius. Together, the tube 226, gas manifold 222, and interfaces 224 gas supply to one or more samples in the cavity interior 44 from a gas source outside the box 12. A knob may also be included on an outside surface of the box 12 to control gas supply through the tube 226.

In accordance with another aspect of the present invention, a light source is provided in the interior cavity 44 for illuminating the sample or specimen in the imaging box 12. The light source may be continuously illuminated or flashed while capturing photographic images of the sample and is turned off for capturing luminescence images. As illustrated in Fig. 14A, the light source comprises a ring of low-wattage lights 128 mounted on the bottom surface of a partition 130 positioned around the camera lens 100. The partition 130 is positioned below the other components housed in the top area of the box and separates the upper portion of the box 12 from the cavity 44. The partition 130 is attached to at least one side of the box 12 and also includes a hole 129 for camera 100 visibility. In another embodiment, as illustrated in Fig. 14B, the light source comprises four pairs of white-light emitting diodes (LEDs) 131, one pair mounted in each of the corners of partition 130 around the camera lens 100. One advantage of using such LEDs is that the spectral emission thereof may be contained to visible light while excluding infrared light. Wires (not shown) may extend from the light low-wattage lights 128 to the electronic components 56 and computer 28 to allow light levels to be controlled externally through the computer 28.

The light source may also include a fiber optic cable. In a specific embodiment, the fiber optic cable has a first end located in the interior cavity 44 and a second end located outside the box 12. The first end may be used to illuminate the sample while the

second end extends outside the box 12 to a light source that provides the light to the optic cable. Within the cavity interior 44, the fiber optic cable may be contained in a flexible "snake-like" housing that maintains a desired position of the first end as provided by a user. This allows the user to flexibly position the first end relatively close to the sample for illumination in a particular photographic capture. A filter may also be used with the fiber optic light source to provide a particular lighting effect. In a specific embodiment, the fiber optic light source is used to make various samples fluoresce and the filter is used to select the wavelength of light to excite the sample. In this case, filters in the filter select device 117 of Fig. 9 allow a camera to only receive selected light that is fluorescing in the sample. When the second end is located outside the box 12, the imaging box 12 includes a hole that allows said fiber optic cable to pass therethrough. When the optic cable is not in use, the hole may be suitably plugged to prevent light from entering the box. In another embodiment, there is a break in the fiber optic cable at the box 12 wall that allows a user to remove either the outside or inside fiber and cap the remaining end off to minimize light leakage to the inside of the box 12.

To provide additional light protection for the interior cavity 44, one or more walls forming the cavity 44 may be light sealed. For example, it may be advantageous to light seal the partition 52 and/or the top enclosure panel 41 (FIG. 11).

Fig. 1D is a cross-sectional view of a conventional seal 5 that may be used for preventing light from entering an enclosure between two adjacent walls 6 and 7 of the enclosure housing. The seal 5 is formed between two opposed surfaces 6a and 7a of the walls 6 and 7 to be joined. Together, the opposed surfaces 6 and 7 form a channel 8 for receipt of an o-ring 9. Only one of the surfaces, surface 6a, is recessed for the channel 8, while the other surface 7a is flat.

Figure 15 shows a light seal 164 in accordance with one embodiment of the present invention. Light seal 164 is employed, for example, in the assembly of upper housing 16, and at the contact between upper housing 16 and top plate 103 (see Fig. 11 for both cases) and is used for preventing light from entering the cavity 44 from the top portion of the box 12. Light seal 164 includes a gasket 166 which is composed of compressible material, such as a rubber o-ring, and two opposing channels 168a and

168b, each located in one of two substantially planar surfaces 170a and 170b. The surface 170a, defining the first channel 168a, and the second surface 170b, defining the second channel 168b, are configured such that the channels are aligned at least partially with each other. The compressible gasket 166 is disposed in the channels 168a and 168b and configured to contact opposing edges of the channels 168a and 168b when the surfaces 170a and 170b are positioned in opposed relationship to one another . In addition, the gasket 166 and channels 168a and 168b may also be configured such that the gasket 166 is positioned at the intersecting edges between the channels 168a and 168b and the corresponding surfaces 170a and 170b.

In accordance with this aspect of the present invention, this light seal 164 is a more effective light barrier than the conventional seal 5 of Fig. 1D. In the conventional seal, if there is a slight gap between the seal 9 and the surface 7a such as an inconsistency in either of the surfaces 6a and 7a along the o-ring 9 length, light can pass between points C and D with virtually no change in direction. In contrast, when there is an imperfection in the sealing of the o-ring 166 against one of the flat surfaces in Fig. 15, entering light would require multiple direction changes to pass between points A and B. Accordingly, the light seal 164 reduces the amount of light entering the cavity 44 due to any defects in the light seal 164 along its length.

Referring now to Fig. 16, there is shown a top perspective view of drawer 54 and electronic components 56 housed therein. As previously noted, these components interface with the computer 28 and are used to control the various motors and other components of the imaging system 10. A 3 V power supply 137 provides electrical power to the various active components in the drawer 54. A motor control board 146 has four motor controllers 148, 150, 152, 154 mounted thereon. The motor controllers 148, 150, 152, 154 are in communication with each of the F-stop motor 106, lens focus motor 114, and filter wheel motor 126 and stage motor 138, respectively, via wires 134. Each motor controller interfaces, via cable 34, with the computer 28 where the motor controllers and motors may be controlled by appropriate software running on the computer and user input. Drawer 54 also houses a data acquisition board (DAB) 156. On the face of the drawer is a knob 155 which is in communication with light source 128 and allows the user to manually to control the light intensity in the interior cavity 44.

Also on the drawer face is a heater controller 158 which is in communication with heating element lead 162 to control and display its temperature.

B. Comparison of the imaging system of the present invention with a conventional light box

Tests were conducted to compare a conventional light box - model number A4178 as manufactured by Hamamatsu Photonic Systems of Bellerica, MA - and an imaging box in accordance with one embodiment of the present invention. Each box was tested under substantially identical conditions. For the Hamamatsu box, a camera was installed on and operably connected with image capturing equipment. A piece of white paper was placed inside the box, approximately 12" away from the front of the camera lens, to serve as a reflective sample. The door of the box was closed, and an image was acquired with the exterior room lights on. The room lights were then turned off, and a second image was acquired. The total amount of light acquired under the two conditions was compared. The camera was then transferred to an imaging box of the present invention, and images were acquired under the same conditions.

The testing showed that approximately 130% to 140% more external light entered the Hamamatsu box with the exterior lights on than with them off. In comparison, the imaging box of the present invention measured only about a 3% increase in the amount of light entering the box with the exterior lights on as compared with the exterior lights off.

II. Operation of the Imaging System of the Present Invention

The present invention may be employed in a wide variety of imaging applications. Generally, the present invention may be applied with any non-invasive methods and compositions for detecting, localizing and tracking light-emitting entities and biological events in a mammalian subject. For example, the imaging system 10 may be implemented with intensified Charge-Coupled Device (CCD) cameras to detect the

localization of light-producing cells (e.g., certain bacteria or tumor cells made bioluminescent by transforming them with luciferase DNA constructs) inside of living animals, such as mice. In such applications, an animal containing the bioluminescent cells is placed inside of the specimen chamber, and within the field of a photodetection device, such as an intensified CCD camera. The camera is then activated to detect the emitted photons. The photon signal may then be used to construct a luminescent image of photon emission. The luminescent image is constructed without using light sources other than the luminescence from the sample itself. This luminescence is recorded as a function of position to produce the luminescence image. The photographic image may also be taken of the same sample to aid in position visualization of the luminescent image. One approach to generating such composite photographic/luminescence images is described in U.S. Patent No. 5,650,135 issued to Contag et al. on July 22, 1997. The entire disclosure of that patent is incorporated herein by reference for all purposes.

Turning now to Fig. 17, a flow chart illustrates a method of capturing photographic and luminescent images using the imaging system 10 in accordance with of the invention. The method begins by placing a sample or specimen to be assayed for light emission on the stage in the imaging box 12 (202). The imaging box 12 and associated image components are then prepared for capturing a photographic image of the sample (204). The preparation may include launching imaging and acquisition software (e.g., "LivingImage", Xenogen Corporation, Alameda, CA) on the computer 28 and initializing the camera 20. Further preparations may include selecting the desired stage 58 position, closing the door 12, activating the photographic capture option in the software, and turning on the lights (e.g., lights 128 or 132) in the box. Preparations may further include focusing the lens 100, selectively positioning an appropriate lens filter 118, setting the f-stop, etc.

The photographic image is then captured (206). In one embodiment, a "live mode" is used during photographic imaging of the sample to observe the sample in real time. The live mode includes a sequence of photographic images taken frequently enough to simulate live video. Upon completion of photographic capture, the photographic image data are transferred to an image processing unit 26 and/or computer

system 28 (208). These may be used to manipulate and store the photographic image data as well as process the data for display on computer monitor 38.

Subsequently, imaging box 12 and associated image components are prepared for luminescence image capture (210). Such preparation may include, for example, selecting luminescent exposure time and binning level using the computer 28, and turning off the lights in the cavity 44. The CCD camera 20 then captures (212) the luminescence image over a set period of time (up to several minutes). The luminescence image data are transferred to the image processing unit 26 and/or computer 28 (214), which may be used to manipulate and store the luminescence image data as well as process it for display on the computer display 38 (step 214). The manipulation may also include overlaying the luminescent image with the photographic image and displaying the two images together as an "overlay" image, with the luminescence data typically shown in pseudocolor to show intensity. This overlay image may then be the basis for user analysis (216). At this point, the user has the components of a digital overlay image (including the luminescence image and the photographic image) stored in the computer 28. The information contained in these image may be analyzed and manipulated as desired.

Based on the foregoing, it should be readily apparent to those skilled in the art that a substantially improved imaging box for imaging low intensity light sources has been disclosed. The improved sealing arrangements and door securing designs of the present invention provide substantially more light-tight enclosures than have been previously available. Various additional improved features of an imaging box have also been disclosed, including a novel automated filter select device, automated camera focusing, f-stop adjustment, automated stage height, internal illumination and sample temperature control. Moreover, it will be apparent to those skilled in the art in light of the foregoing disclosure that further alternatives, modifications and variations are possible. For example, imaging systems in accordance with the present invention may not necessarily include all the improvements and embodiment disclosed herein and may include any one or more of the above described embodiments. In addition, the present invention is suitable for other imaging applications and may be tailored correspondingly. By way of example, the present invention may be adapted for analysis of high detail in-

vivo applications and thus may include zoom tools for the camera 20 and controlling computer 28. Also, the various properties and characteristics of the compressible material 62 are by way of example only and other materials and variations may be suitable. Although various details have been omitted for brevity's sake, obvious design alternatives may be implemented. Therefore, the present examples are to be considered as illustrative and not restrictive, and the invention is not to be limited to the details given herein, but may be modified within the scope of the appended claims.

What is claimed is:

1. An imaging box for capturing an image of a sample, the imaging box comprising:

a body including an interior cavity for receiving the sample, and having a front wall defining an opening into said cavity;

a door having a rear wall, said door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning said rear wall substantially adjacent the body front wall to prevent access through the cavity opening, the door rear wall adapted to cooperate with the body front wall, in the closed condition, to define a capture space extending substantially perimetrically about said cavity opening; and

a compressible material disposed at least partially within the capture space and extending substantially perimetrically about said cavity opening, said compressible material having a transverse cross-sectional dimension defined at least partially by a first side extending perimetrically therearound and at least partially by an independent second side extending perimetrically therearound, when the door is in said closed position, said door rear wall is adapted to compress the compressible material substantially along the entire first side thereof, and the body front wall is adapted to compress the compressible material substantially along the entire second side thereof such that light entering said capture space from the exterior of the body is intercepted by compressible material.

2. The imaging box of claim 1 wherein the body front wall includes a first wall section extending outwardly therefrom, and extending substantially perimetrically around the cavity opening, said first wall section further having a distal edge portion positioned substantially adjacent the door rear wall in the closed position.

3. The imaging box of claim 2 wherein a ledge portion of the compressible material is adapted to extend outside the capture space when the door is in the closed position.

4. The imaging box of claim 3 wherein the distal edge portion of the first wall section compresses the ledge portion of the compressible material in the closed position.

5. The imaging box of claim 2 wherein said compressible material includes a third side, extending between the first side and the second side thereof, said first wall section of the body front wall being adapted to engage the compressible material substantially along the entire third side thereof.

6. The imaging box of claim 5 wherein the rear wall includes a second wall section extending outwardly therefrom toward the front wall, in the closed position, said second wall section further extending substantially perimetrically around the cavity opening at an orientation engaging said compressible material between the first wall section of the body and the second wall section of the door.

7. The imaging box of claim 6 wherein the door further includes a third wall section extending outwardly therefrom toward the front wall, in the closed position, said third wall section being positioned perimetrically outside and generally parallel to said second wall section, said third wall section further having a distal edge portion positioned substantially adjacent the body front wall in the closed position.

8. The imaging box of claim 1 wherein the door rear wall and the body front wall cooperate with the compressible material to direct light entering the capture space to traverse the transverse cross section of the material for interception of the light thereof.

9. The imaging box of claim 1 wherein the compressible material prevents the door rear wall from contacting the front wall when the door is in the closed condition.

10. The imaging box of claim 9 wherein the compressible material is made of a material having a durometer of between about 10 to about 50.

11. The imaging box of claim 1 wherein the first and second side of the compressible material are positioned on opposite sides thereof.

12. An imaging box for capturing an image of a sample, the imaging box comprising:

a body including an interior cavity for receiving the sample, and having a front wall defining an opening into said cavity, the body further including a first wall section extending outwardly from the front wall and substantially perimetrically around the cavity opening; and

a door having a rear wall, and movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning said rear wall substantially adjacent the body front wall to prevent access through the cavity opening, the door further including a second wall section extending outwardly from the door rear wall and substantially perimetrically around the cavity opening, in the closed position, the door second wall section and the body first wall section further adapted to substantially overlap, relative their respective depth, such that a distal edge portion of the first wall section is positioned substantially adjacent the door rear wall, and such that a distal edge portion of the second wall section is positioned substantially adjacent the body front wall, when the door is in the closed condition.

13. The imaging box of claim 12 further including a compressible material disposed at least partially between said first wall section and said second wall section

14. The imaging box of claim 12 wherein said first and said second wall extend perimetrically around said opening.

15. The imaging box of claim 12 wherein the door further includes a third wall section extending from the second wall section, and oriented substantially adjacent to said front wall when the door is in the closed condition.

16. The imaging box of claim 15 wherein third wall section extends in the direction of the body first wall section.

17. The imaging box of claim 15 wherein third wall section extends from the distal edge portion of the second wall section.

18. The imaging box of claim 12 wherein, in said closed condition, the distal edge of the second wall section forms a gap from said front wall in the range of about 1/1000 inches to about 1/2 inches.

19. The imaging box of claim 12 wherein, in said closed condition, the distal edge of the second wall section forms a gap from said front wall in the range of about 1/1000 inches to about 1/8 inches.

20. The imaging box of claim 12 wherein the first wall is substantially perpendicular to said front wall, an the second wall is substantially perpendicular to said rear wall.

21. An imaging box for capturing an image of a sample, the image box comprising:

a body including an interior cavity for receiving the sample, and having a front wall defining an opening into said cavity;

a door having a rear wall, said door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning said rear wall substantially adjacent the body front wall to prevent access through the cavity opening;

one of the body front wall and the door rear wall including a pair of generally parallel channel walls extending outwardly therefrom to form a channel extending substantially around the perimeter of the cavity opening when the door is in the closed condition; and

the other of door rear wall and body front wall having an interengaging wall extending outwardly therefrom and adapted to extend into said channel in a manner such that said interengaging wall and said channel walls substantially overlap, relative their respective depth, when the door is in the closed condition.

22. The imaging box of claim 21 further including a compressible material disposed in the channel.

23. The imaging box of claim 22 wherein said interengaging wall further engages said compressible material such that light entering said channel from the exterior of the body is intercepted by compressible material.

24. The imaging box of claim 22 wherein the compressible material has a thickness of at most one half of the depth of the channel.

25. The imaging box of claim 21 wherein, in said closed condition, a distal edge of the interengaging wall forms a gap from one of the door rear wall and body front wall in the range of about 1/1000 inches to about 1/2 inches.

26. The imaging box of claim 21 wherein the other of door rear wall and body front wall further includes a third channel wall extending generally parallel to the interengaging wall to form a second channel extending substantially around the perimeter of the cavity opening, when the door is in the closed condition, one of the pair of channel walls being adapted to extend into said second channel in a manner such that said third channel wall and said pair of channel walls substantially overlap, relative their respective depth.

27. The imaging box of claim 26 further including a second compressible material disposed in the second channel, and said one of said pair of channel walls further engaging said second compressible material such that light entering said second channel from the exterior of the body is intercepted by second compressible material.

28. The imaging box of claim 26 wherein the light entering the interior cavity from outside the box is required to make at least six right angles before entering the interior cavity.

29. The imaging box of claim 21 wherein the interengaging wall and the channel walls are substantially parallel one another in the closed condition.

30. The imaging box of claim 21 wherein the door further includes a cover wall section extending outwardly therefrom toward the front wall, in the closed position, said

cover wall section being positioned perimetrically outside and generally parallel to said channel walls and said interengaging wall, said cover wall section further having a distal edge portion positioned substantially adjacent the body front wall in the closed position.

31. The imaging box of claim 30 wherein the door further includes an adjacent wall section extending from the cover wall section, and oriented substantially adjacent to said front wall when the door is in the closed condition.

32. The imaging box of claim 31 wherein said adjacent wall section extends in the inward direction toward interengaging wall and the channel walls.

33. An imaging box for capturing an image of a sample, the image box comprising:

a body including an interior cavity for receiving the sample, and having a front wall defining an opening into said cavity;

a door having a rear wall and an exterior face, said door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning said rear wall substantially adjacent the body front wall to prevent access through the cavity opening;

a compressible material disposed on one of the rear wall and the front wall;

a first latch moveable between a first position disengaged with said front wall and a second position engaged with said front wall in which a securing force is applied between the door and the front wall at a first location; and

a second latch moveable between a first position disengaged with said front wall and a second position engaged with said front wall in which a securing force is applied between the door and the front wall at a second location, wherein the securing force provided by the first latch and the securing force provided by the second latch substantially uniformly compress a portion of the compressible material.

34. The imaging box of claim 33 further including a user handle extending to the exterior face of the door.

35. The imaging box of claim 34 wherein the first latch is operably positioned by the user handle.

36. The imaging box of claim 35 wherein the second latch is operably positioned by the user handle.

37. The imaging box of claim 34 wherein the second latch is operably positioned by a second user handle extending to the exterior face of the door.

38. The imaging box of claim 33 further including a third latch moveable between a first position disengaged with said front wall and a second position engaged with said front wall in which a securing force is applied between the door and the front wall at a third location.

39. The imaging box of claim 37 wherein the third latch is operably positioned by a third user handle extending to the exterior face of the door.

40. The imaging box of claim 37 wherein the securing force provided by the third latch and the securing force provided by one of the first and second latch compress a second portion of the compressible material substantially uniformly.

41. The imaging box of claim 33 wherein the first and second latches are on separate edges of the door.

42. The imaging box of claim 33 wherein the securing force provided by the first latch varies with rotation of the user handle.

43. The imaging box of claim 33 wherein the first latch comprises a circular element that applies said securing force between the door and the front wall at the first location.

44. The imaging box of claim 33 wherein the securing force provided by the first latch is applied by a screw driven by a motor.

45. An imaging box for capturing an image of a sample, the image box comprising:

a body including an interior cavity for receiving the sample, and having a front wall defining an opening into said cavity;

a door having a rear wall and an exterior face, said door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning said rear wall substantially adjacent the body front wall to prevent access through the cavity opening;

a compressible material disposed on one of the rear wall and the front wall; and

a first magnetic element attached to one of said rear wall and said front wall, the first magnetic element providing a first securing force between the door and the front wall when the door is in the closed condition.

46. The imaging box of claim 45 further including a second magnetic element attached to one of said rear wall and said front wall, the second magnetic element providing a second securing force between the door and the front wall when the door is in the closed condition.

47. The imaging box of claim 46 wherein the first securing force and the second securing force magnetic substantially uniformly compresses a portion of the compressible material.

48. The imaging box of claim 45 wherein the first magnetic element is a permanent magnet.

49. The imaging box of claim 45 wherein the first magnetic element is an electromagnet.

50. The imaging box of claim 49 wherein the electromagnet is configured to apply said first securing force during image capture of the sample.

51. An imaging system for capturing an image of a sample, the imaging system comprising:

an imaging box having:

a body including an interior cavity for receiving the sample, and having a front wall defining an opening into said cavity;

a door having a rear wall and an exterior face, said door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning said rear wall substantially adjacent the body front wall to prevent access through the cavity opening; and

an optical filter select device adapted to carry a plurality of optical filters, the filter select device capable of selectively positioning one of the plurality of optical filters to intersect light emitted from the sample.

52. The imaging system of claim 51 further including a processor in electrical communication with the optical filter select device, said processor configured to provide output for controlling the position of said optical filter select device.

53. The imaging system of claim 51 wherein the plurality of optical filters includes one of a neutral density filter, a wavelength cutoff filter, and a fluorescent filter.

54. The imaging system of claim 51 wherein the optical filter select device rotates the plurality of optical filters to selectively provide one of the plurality of optical filters to intersect light emitted from the sample.

55. The imaging system of claim 54 wherein the optical filter select device further includes a first wheel adapted to position a first set of optical filters included in the plurality of optical filters, said first wheel being rotatably coupled to the imaging box.

56. The imaging system of claim 55 wherein the filter select device further includes a second wheel adapted to position a second set of optical filters included in the plurality of optical filters, said second wheel being rotatably coupled to the imaging box.

57. The imaging system of claim 51 further including a light source in the interior cavity.

58. The imaging system of claim 57 wherein the light source comprises a fiber optic cable having a first end located in the interior cavity and a second end located outside the box.

59. The imaging system of claim 58 wherein said imaging box includes a hole that allows said fiber optic cable to pass therethrough.

60. The imaging system of claim 57 wherein the light source comprises a ring of lights positioned around said optical filter select device.

61. The imaging system of claim 60 wherein the light source comprises a plurality of light emitting diodes.

62. An imaging system for capturing an image of a sample, the imaging system comprising:

an imaging box having:

a body including an interior cavity for receiving the sample, and having a front wall defining an opening into said cavity;

a door having a rear wall and an exterior face, said door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and

a closed condition, positioning said rear wall substantially adjacent the body front wall to prevent access through the cavity opening; and

a moveable stage in the cavity interior that supports the sample, the moveable stage having a first vertical position and a second vertical position in the interior cavity, wherein the first vertical position and the second vertical position have the substantially same horizontal position in the interior cavity.

63. The imaging system of claim 62 further comprising a temperature control element configured to alter the temperature of one of the sample or the interior cavity.

64. The imaging system of claim 63 wherein the temperature control element is a thermal sheet in thermal communication with said moveable stage.

65. The imaging system of claim 62 further including a gas manifold coupled to said moveable stage, the gas manifold configured to supply a gas to the sample.

66. The imaging system of claim 62 further including a light shield supported by said moveable stage, said light shield preventing light emitting from said sample to translate horizontally to a portion of the moveable stage that does not support said sample.

67. The imaging system of claim 62 further including a laser positioned in said interior cavity to intercept said moveable stage at said first vertical position.

68. The imaging system of claim 62 further including a worm gear coupled to the moveable stage that provides vertical translation of the moveable stage between the first vertical position and the second vertical position.

69. The imaging box of claim 62 further including a partition located in the cavity interior, the partition forming a compartment within the body separate from the interior cavity.

70. The imaging box of claim 69 wherein the compartment includes an opening formed in the body and the compartment is adapted to slideably receive a drawer through the opening.

71. The imaging box of claim 70 wherein the drawer houses electronics equipment used to control components within the imaging box.

72. An imaging system for capturing an image of a sample, the imaging system comprising:

an imaging box having:

a body including an interior cavity for receiving the sample, and having a front wall defining an opening into said cavity;

a door having a rear wall and an exterior face, said door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning said rear wall substantially adjacent the body front wall to prevent access through the cavity opening;

a stage in the cavity interior configured to support the sample;

an gas manifold in the cavity interior and detachably coupled to said stage, said manifold including a first interface adapted to provide a gas to said sample; and

a tube configured to transport said gas from outside said imaging box to said gas manifold.

73. The imaging system of claim 72 wherein said gas manifold comprises a non-reflective surface.

74. The imaging system of claim 72 wherein said gas manifold comprises a second interface adapted to provide said gas to said sample.

75. The imaging system of claim 72 further including a valve that controls gas flow to said sample through said first interface.

76. The imaging system of claim 72 wherein said tube has a first end that opens outside of said imaging box and a second end inside said cavity interior that emits substantially no light within said cavity interior.

77. The imaging system of claim 72 wherein said gas manifold is autoclavable.

78. The imaging system of claim 72 wherein said stage is moveable.

79. An imaging box for capturing an image of a sample, the image box comprising:

a body including an interior cavity for receiving the sample, and having a front wall defining an opening into said cavity;

a door having a rear wall, said door movable between an opened condition, enabling access to the interior cavity through the cavity opening, and a closed condition, positioning said rear wall substantially adjacent the body front wall to prevent access through the cavity opening; and

at least one light tight seal, the seal comprising:

a first surface including a first channel,

a second surface including a second channel, the first surface opposing the second surface such that the first channel and second channel at least partially face each other, and

a compressible gasket disposed in the first and second channel, the gasket configured to contact opposing edges of the first and second channel when the first and second surfaces are in contact.

80. A light-tight seal comprising:

a front wall having a first wall section extending outwardly therefrom;

a rear wall positioned proximate said front wall such that a distal edge portion of said first wall section is substantially adjacent the rear wall, said rear wall including a second wall section extending outwardly toward the front wall, the front wall, the first wall section, the rear wall and the second wall section cooperating to define a capture space therebetween; and

a compressible material disposed at least partially within the capture space and having a transverse cross-sectional dimension defined at least partially by a first side and at least partially by an independent second side, said rear wall being adapted to compress the compressible material substantially along the entire first side thereof, and the front wall being adapted to compress the compressible material substantially along the entire second side thereof such that light entering said capture space is intercepted by compressible material.

81. The light-tight seal of claim 80 wherein the rear wall and the front wall cooperate with the compressible material to direct light traversing the light-tight seal to traverse the transverse cross section of the material for interception of the light thereof.

82. The light-tight seal of claim 80 wherein the front wall includes a first wall section extending outwardly therefrom, said first wall section further having a distal edge portion positioned substantially adjacent the rear wall.

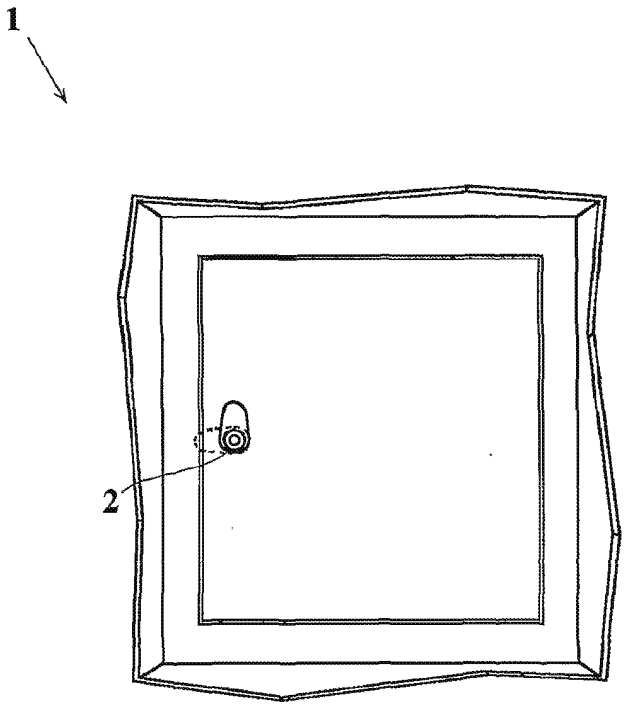


Fig. 1A
(Prior Art)

SPECTRAL_0000409

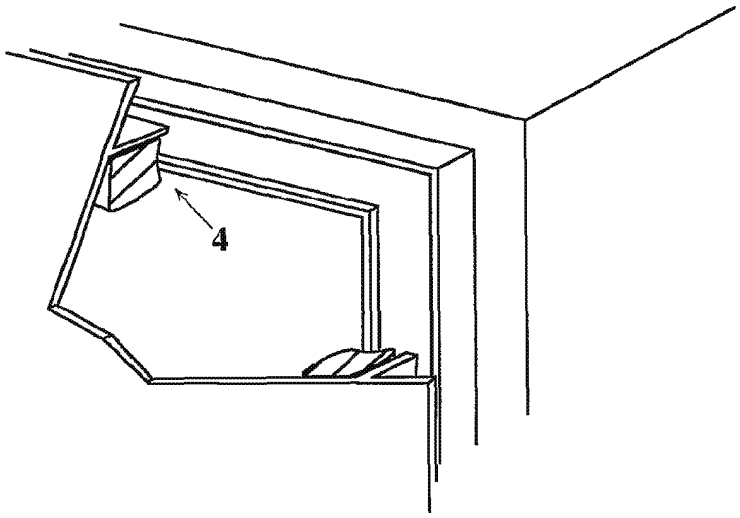


Fig. 1B
(Prior Art)

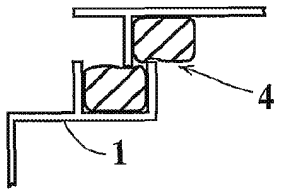


Fig. 1C
(Prior Art)

SPECTRAL_0000410

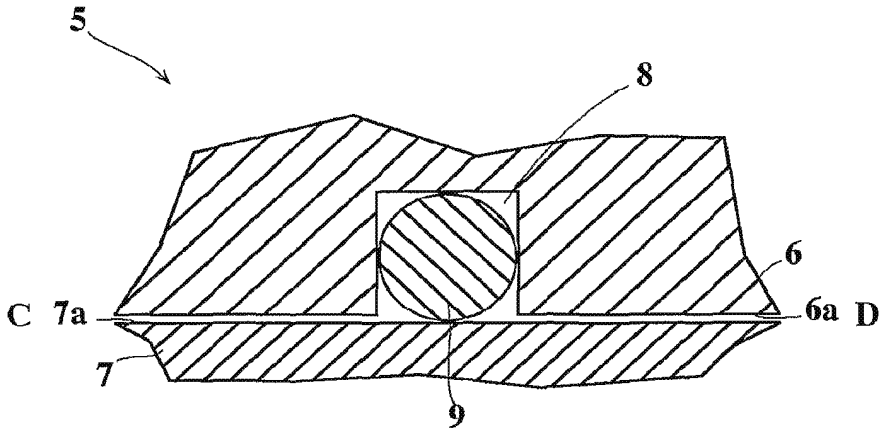


Fig. 1D
(Prior Art)

SPECTRAL_0000411

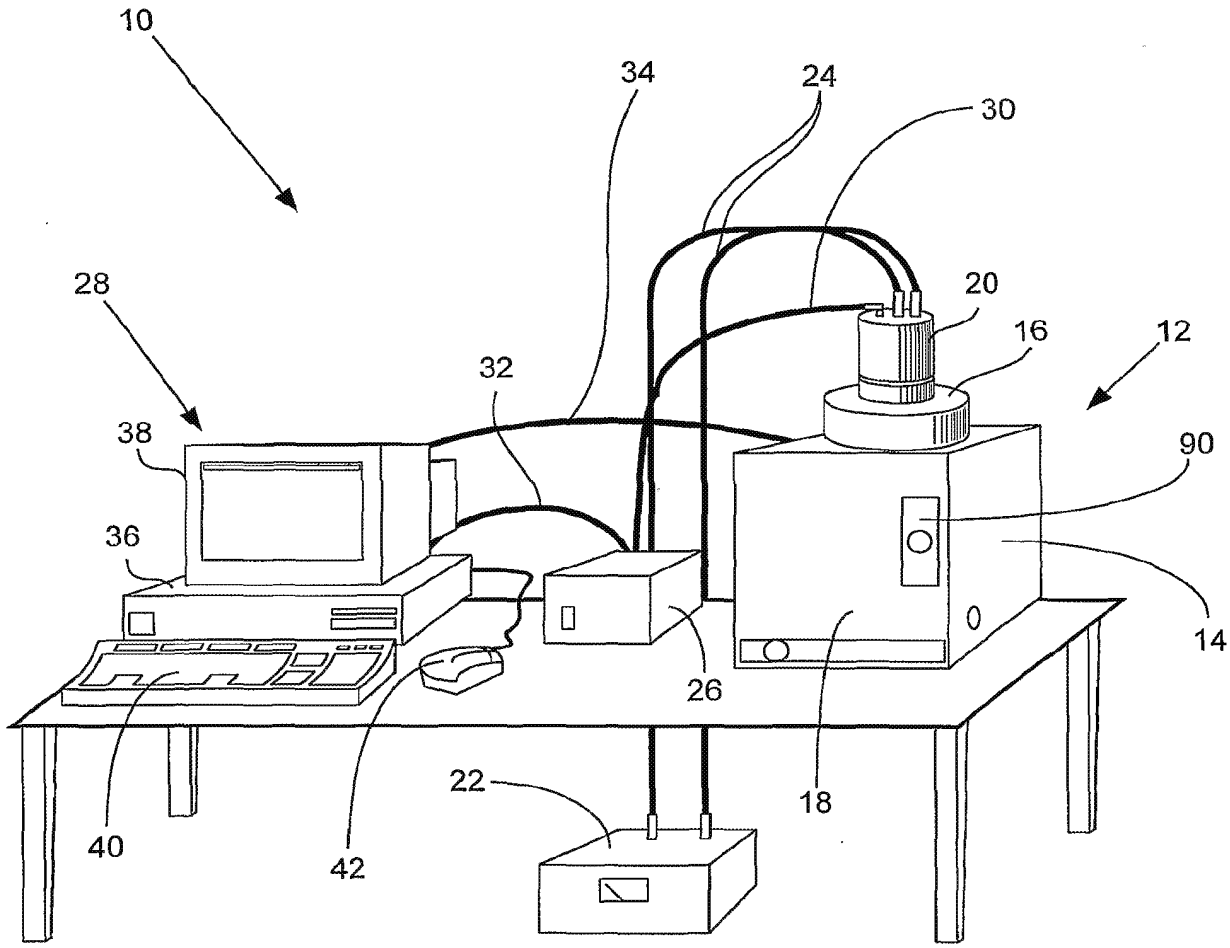


Fig. 2

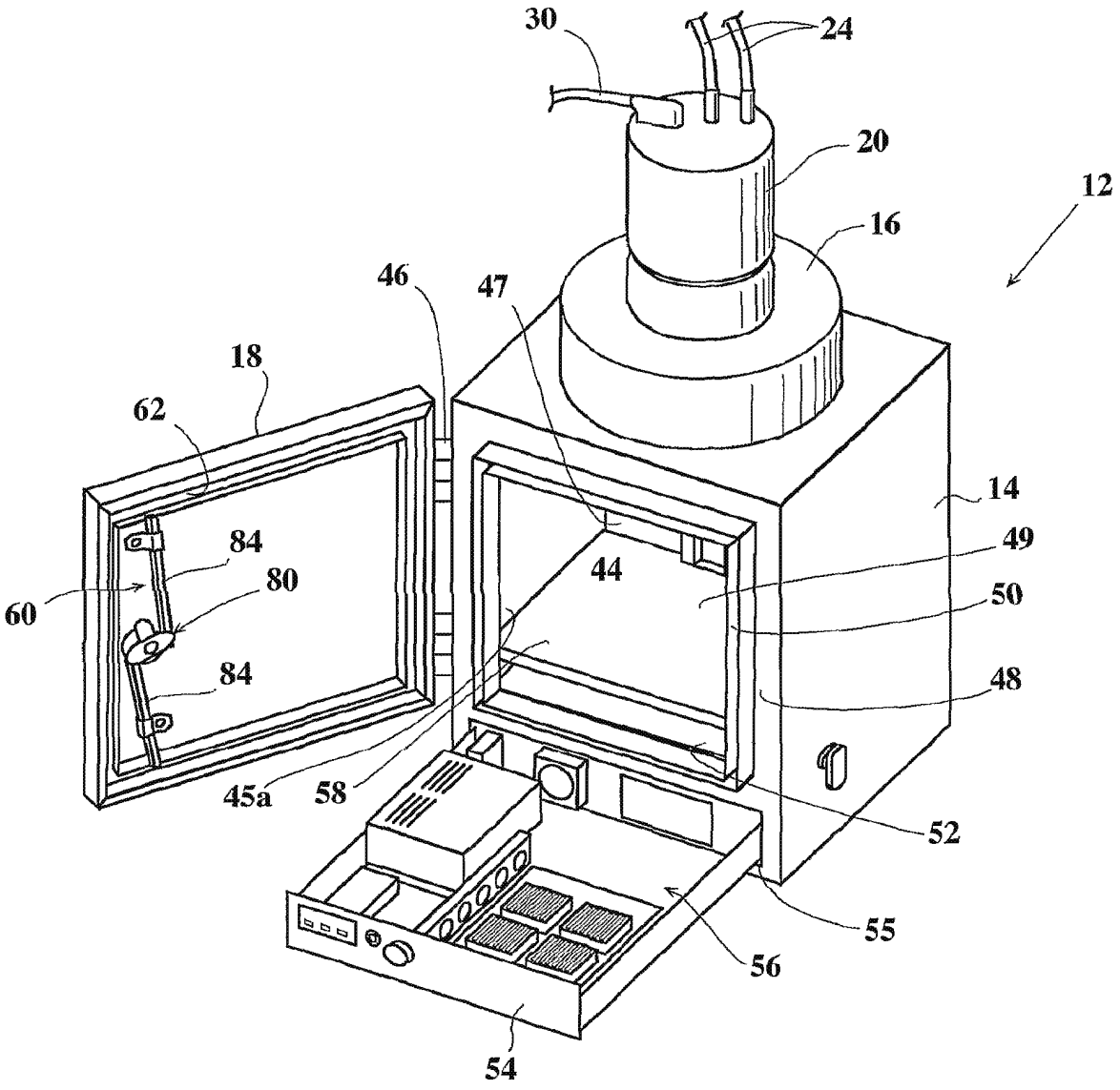


Fig. 3

SPECTRAL 0000413

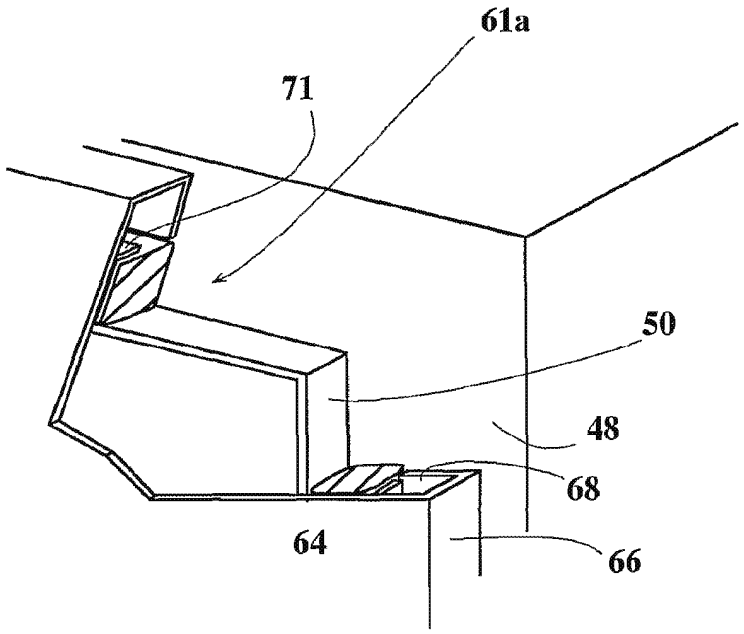


Fig. 4

SPECTRAL_0000414

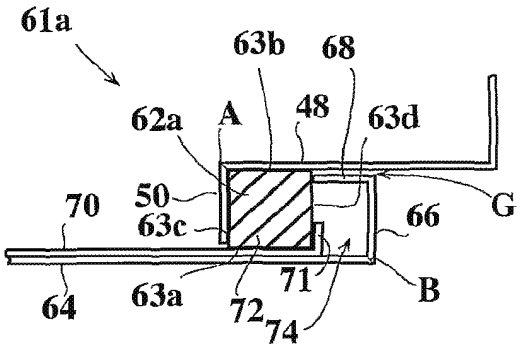


Fig. 5A

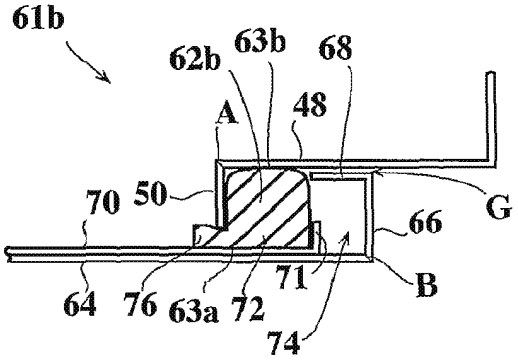


Fig. 5B

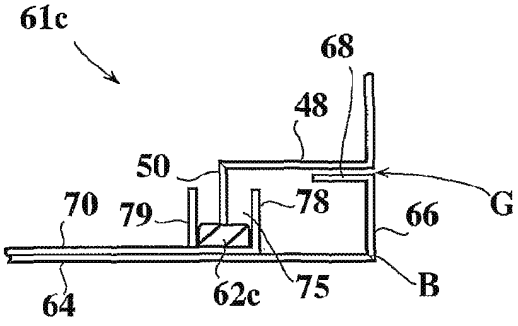


Fig. 5C

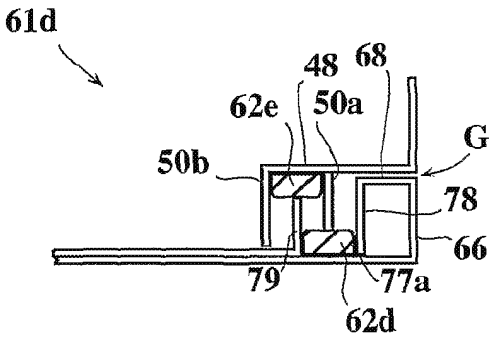


Fig. 5D

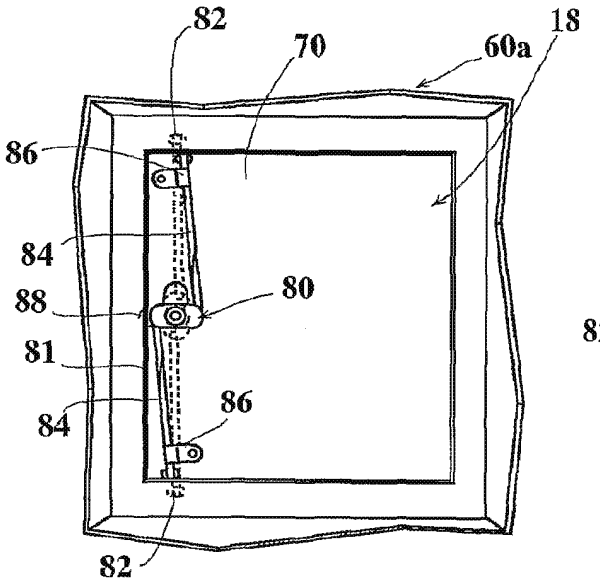


Fig. 6A

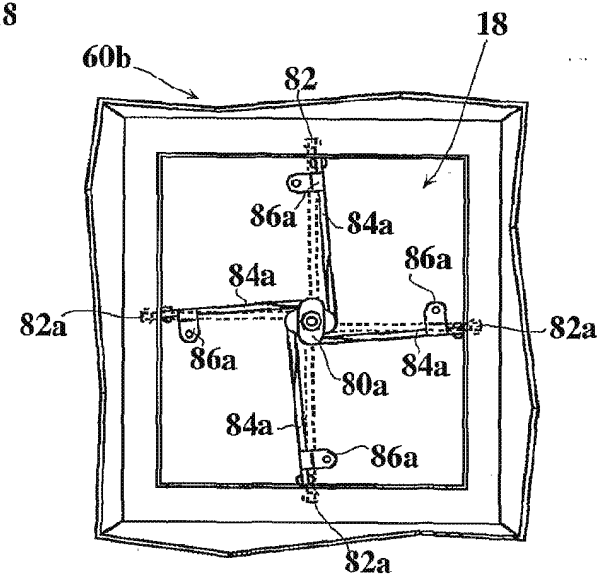


Fig. 6B

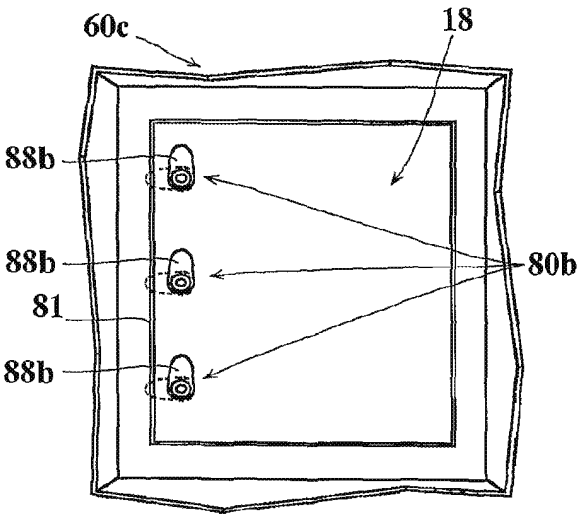


Fig. 6C

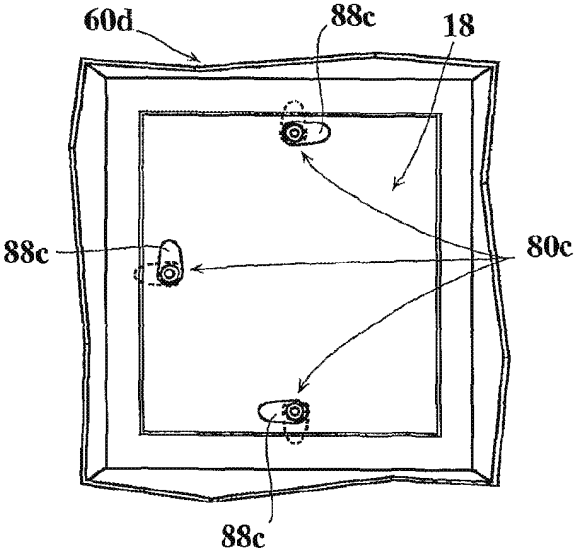


Fig. 6D

SPECTRAL 0000416

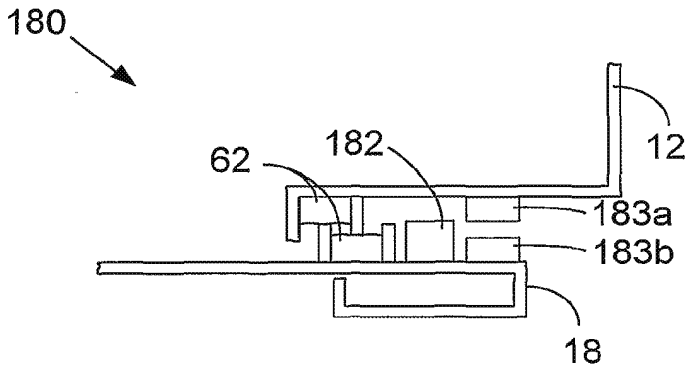


Fig. 6E

SPECTRAL_0000417

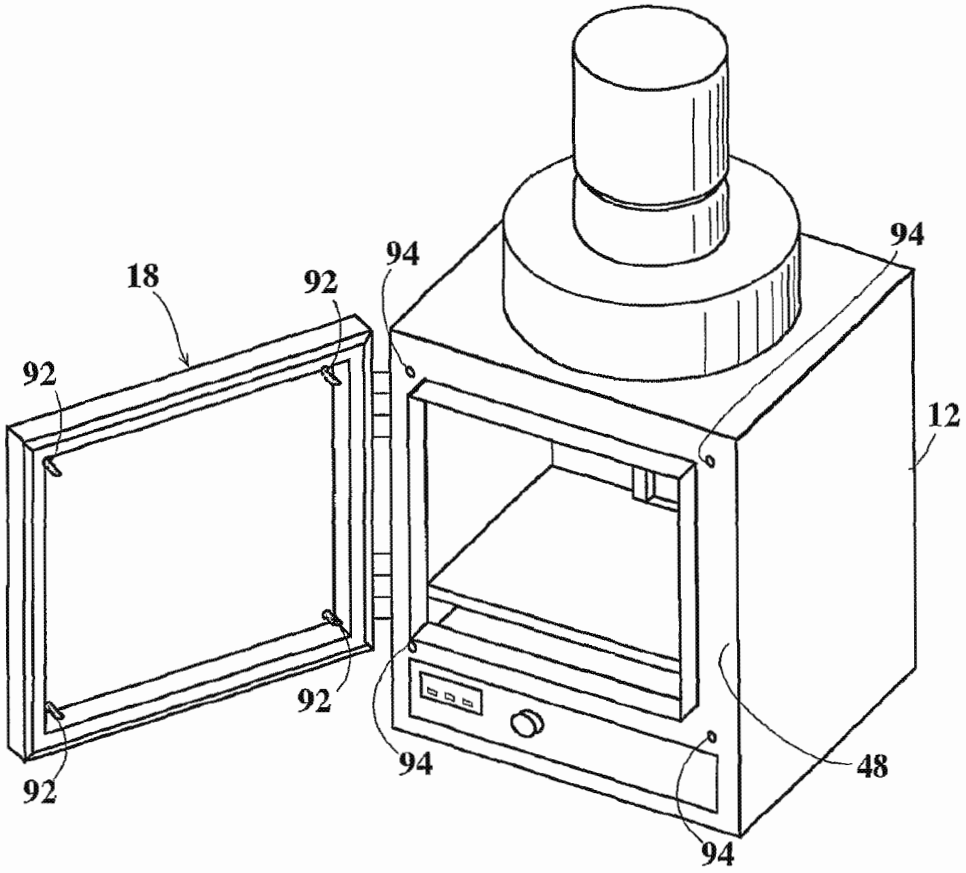


Fig. 7

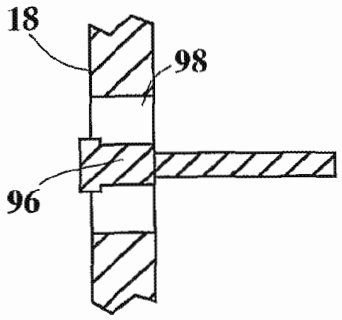


Fig. 8

SPECTRAL_0000418

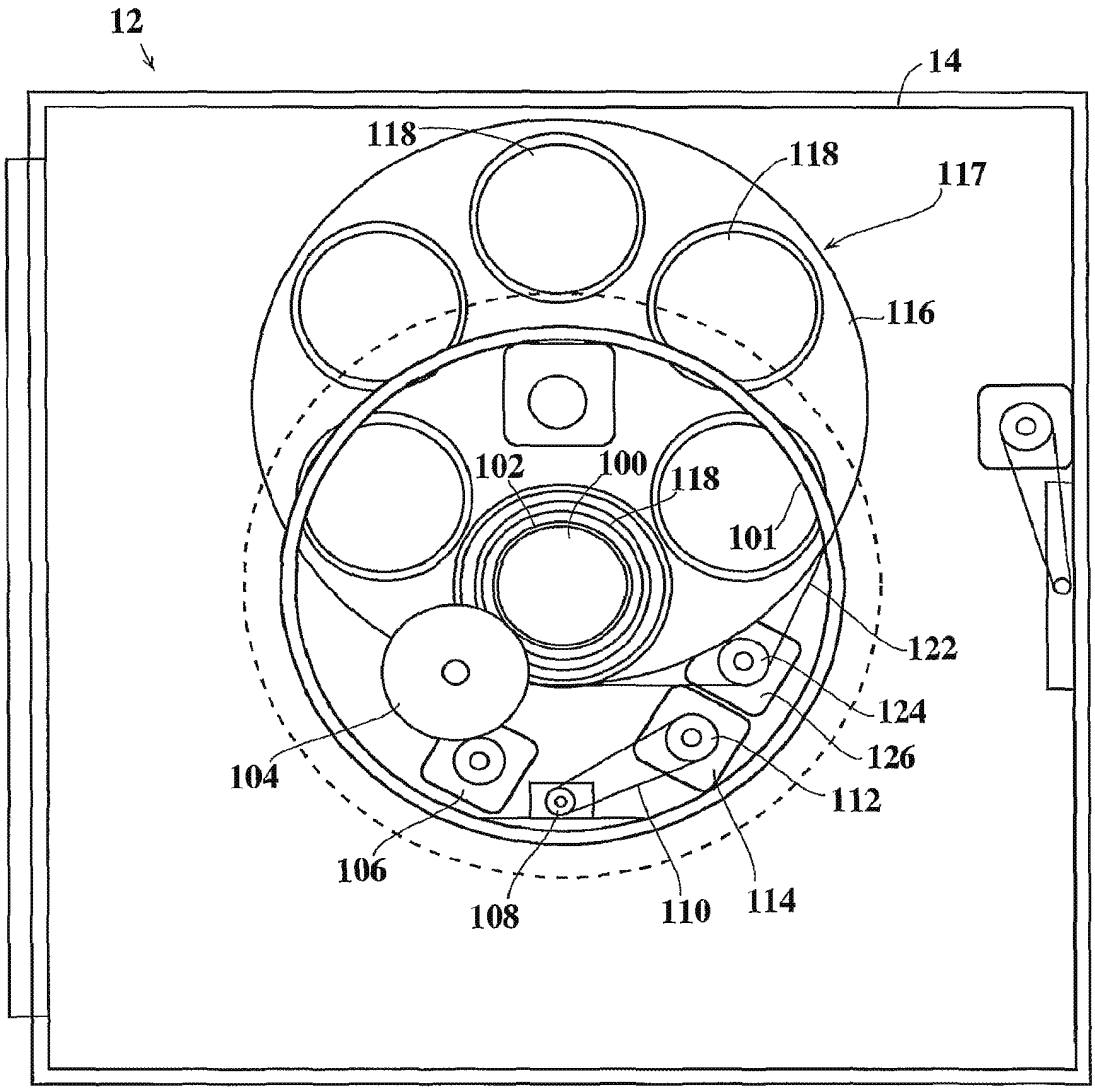


Fig. 9

SPECTRAL_0000419

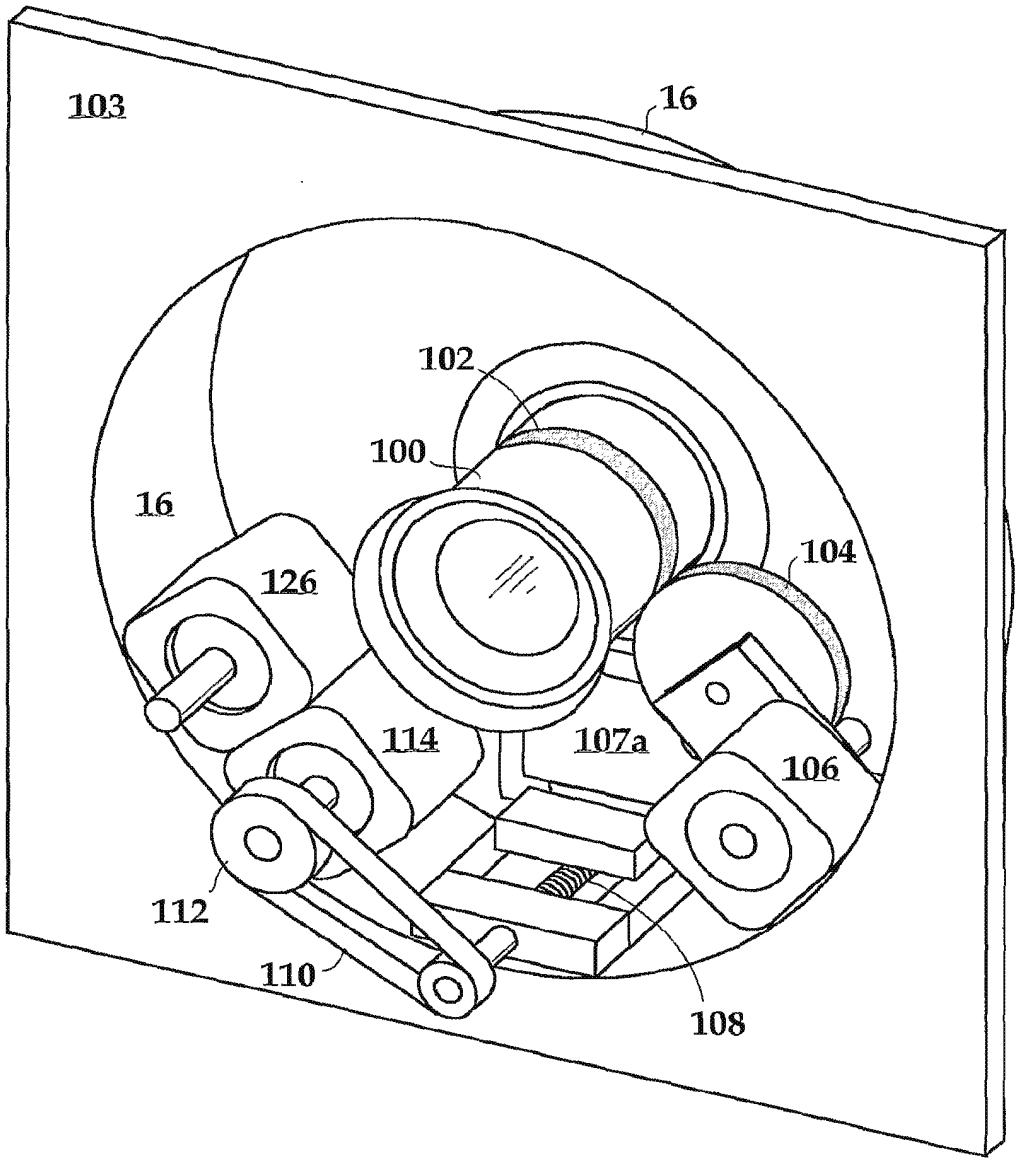


Fig. 10

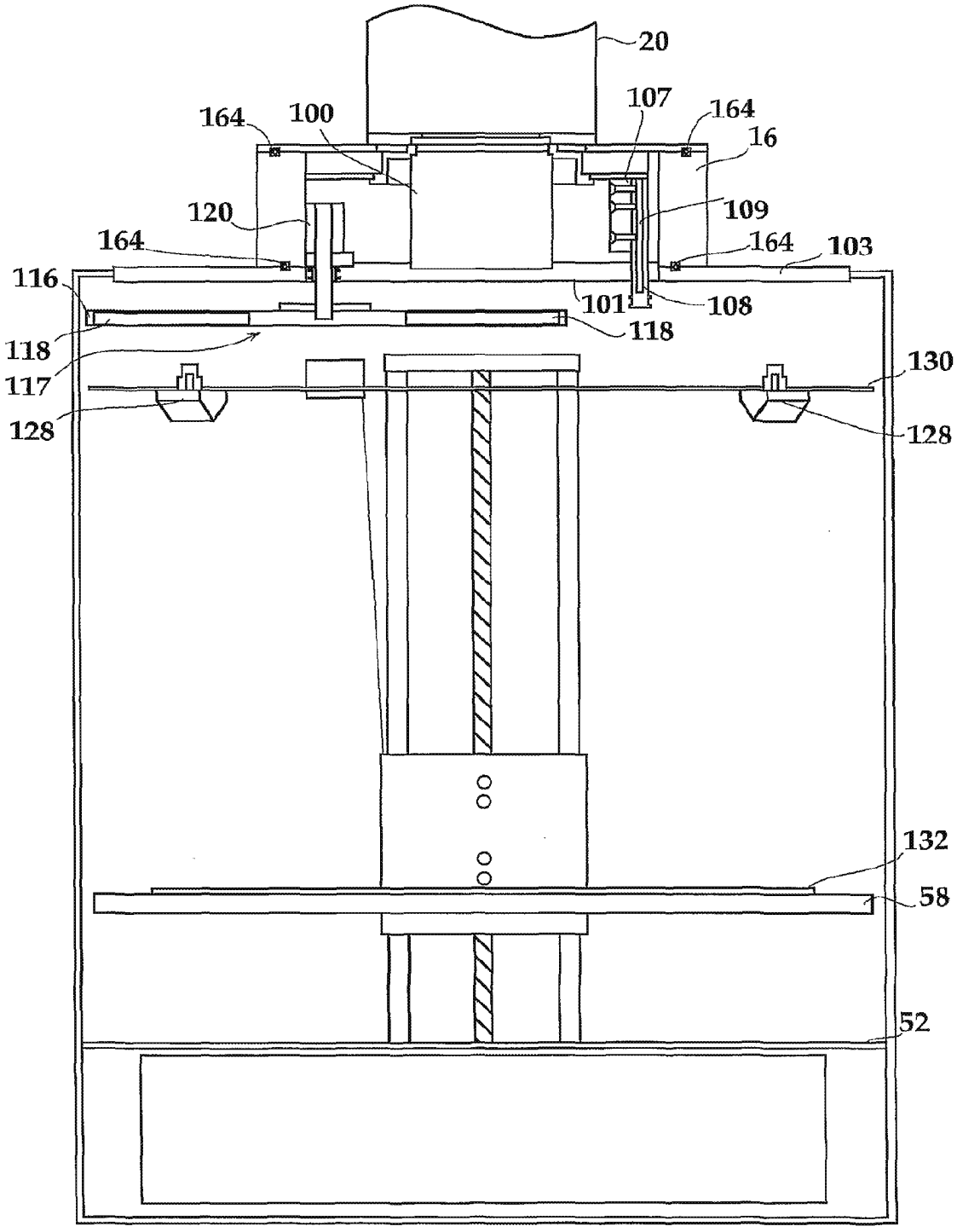

20
164
100
107
164
16
120
109
164
164
103
116
101
108
118
118
117
130
128
128
132
58
52

Fig. 11

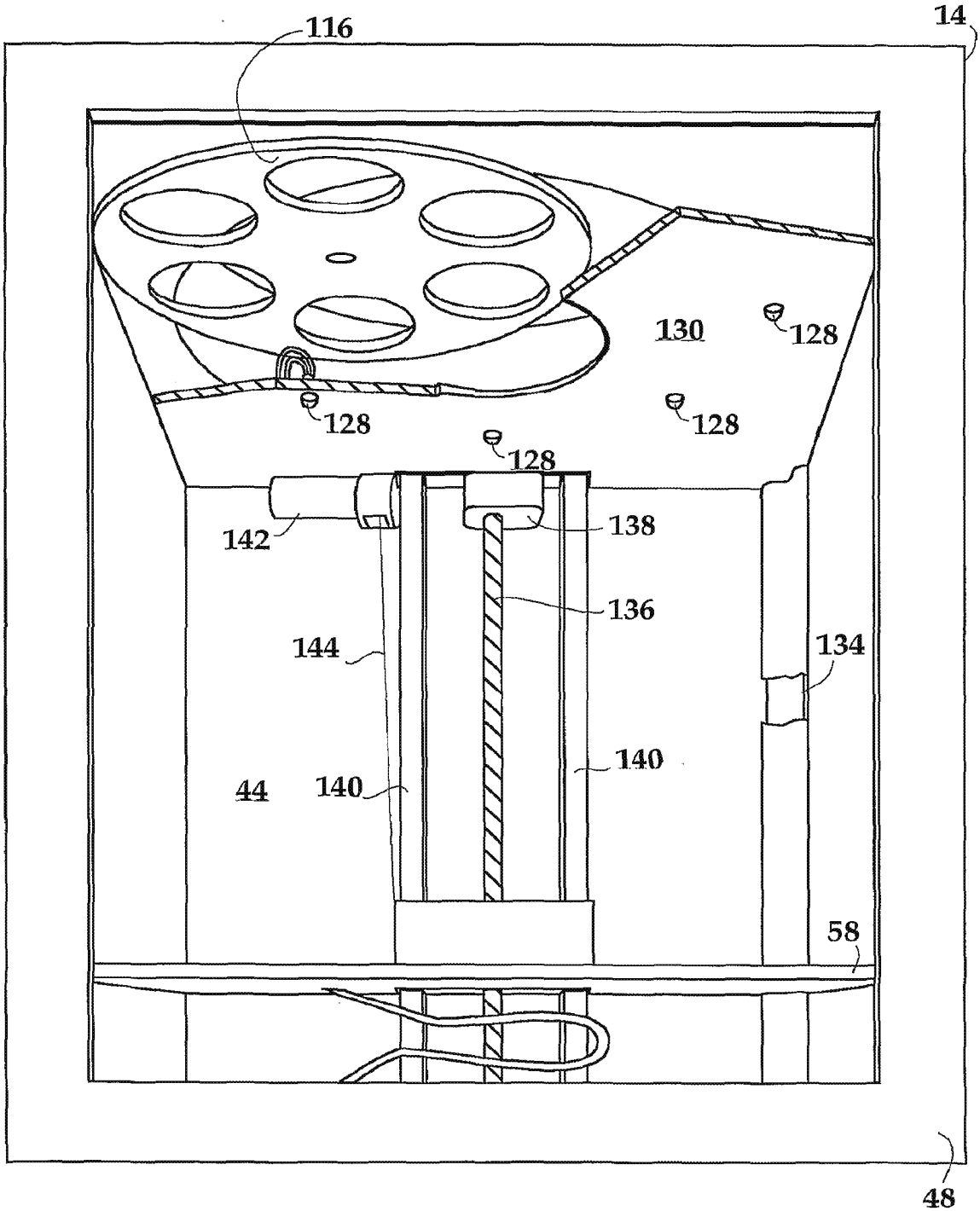


Fig. 12

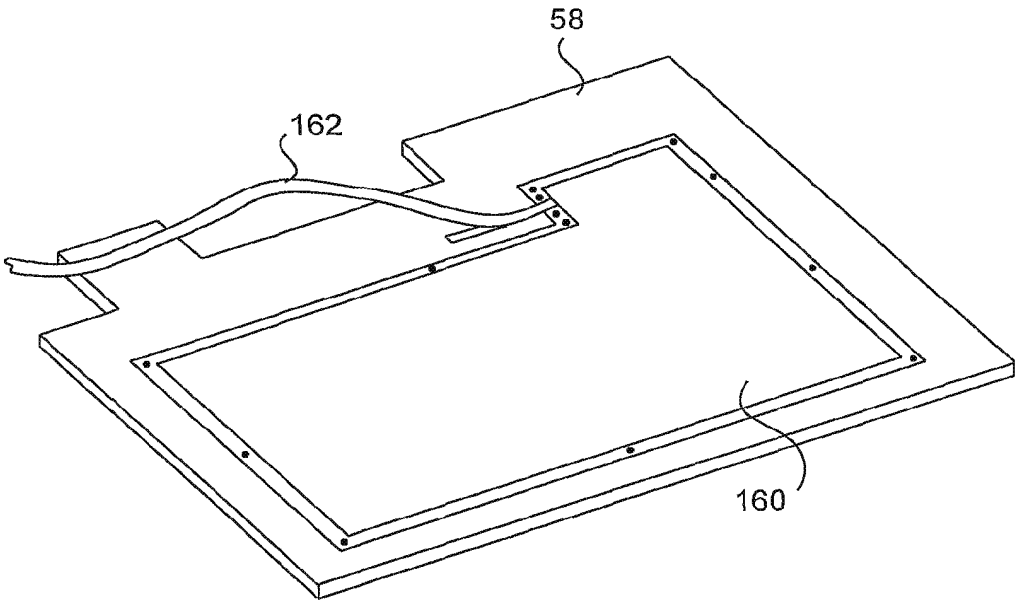


FIG. 13A

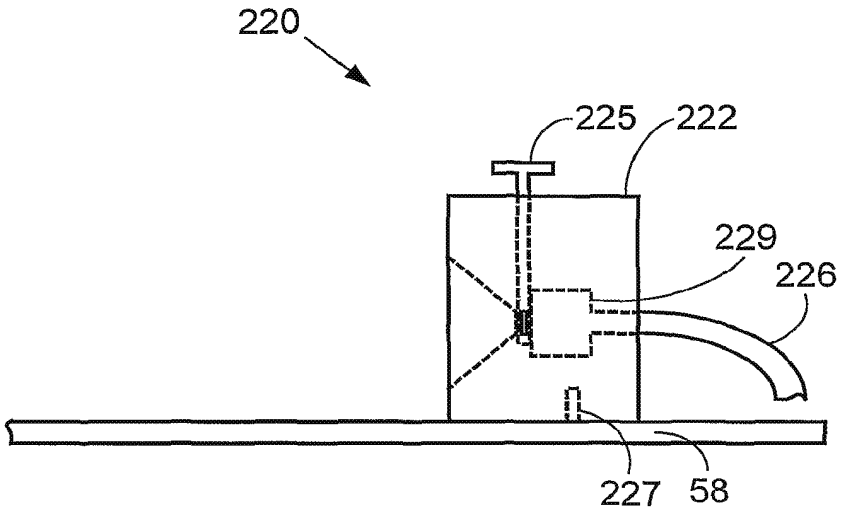


Fig. 13B

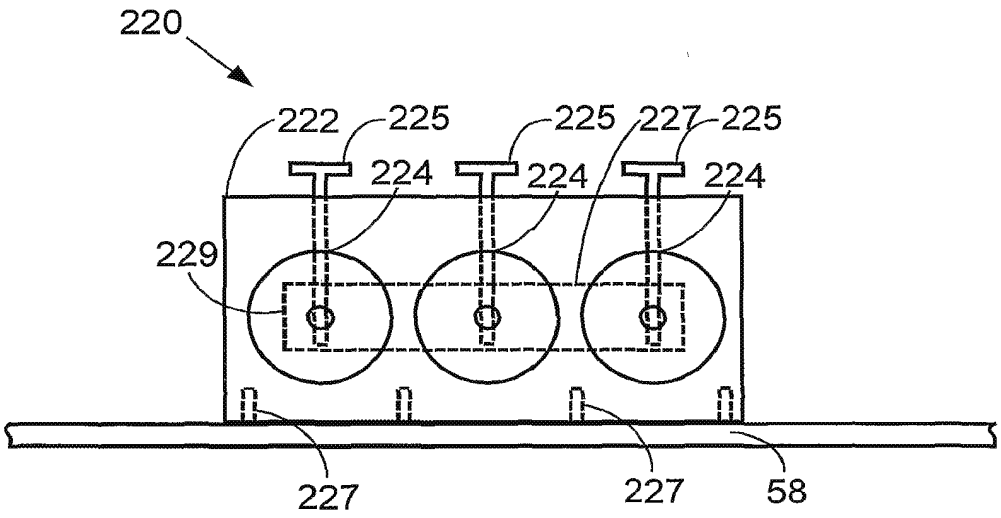


Fig. 13C

SPECTRAL_0000424

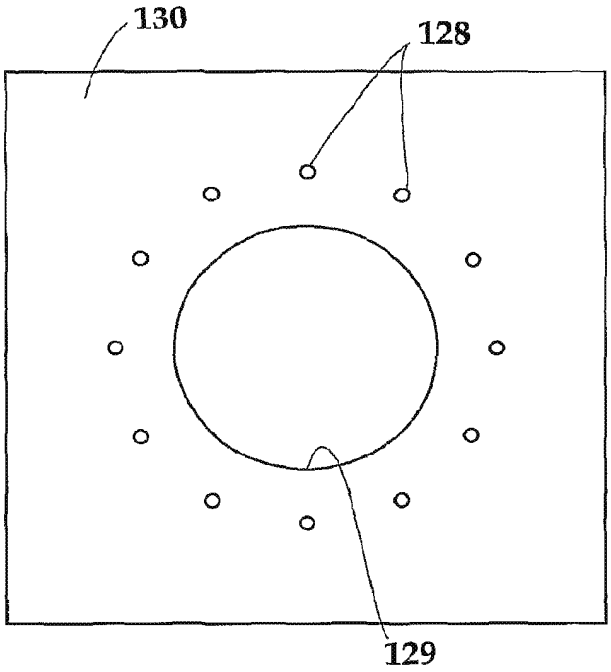


Fig. 14A

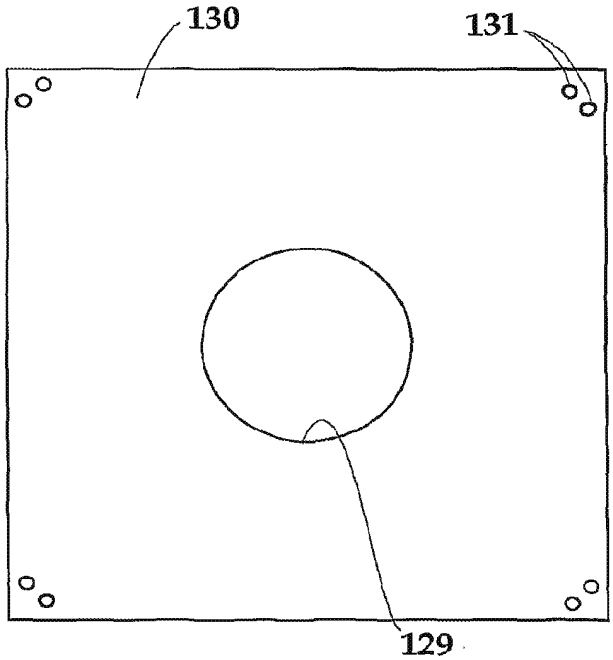


Fig. 14B

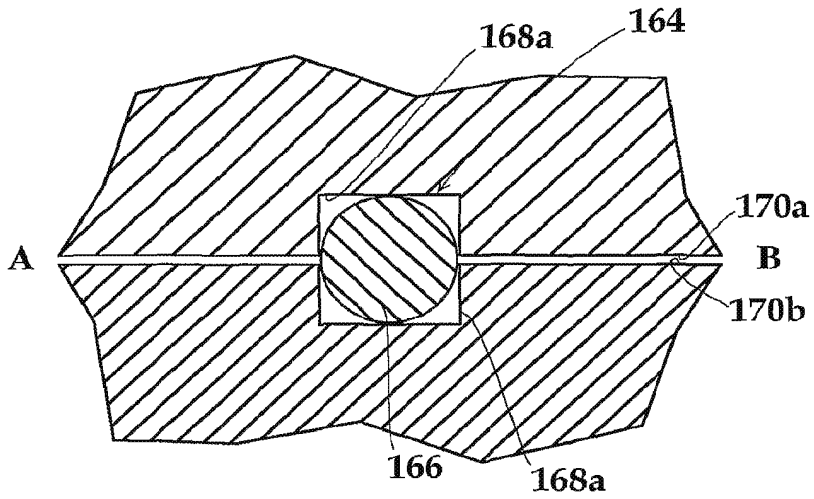


Fig. 15

SPECTRAL_0000426

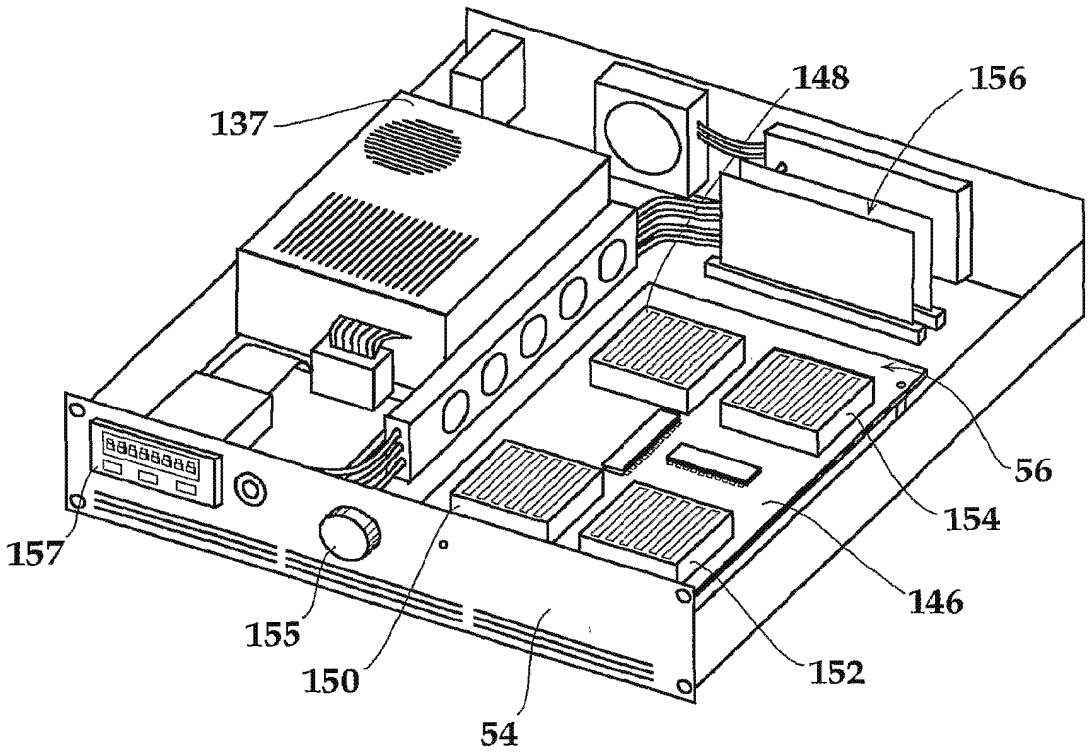


Fig. 16

SPECTRAL_0000427

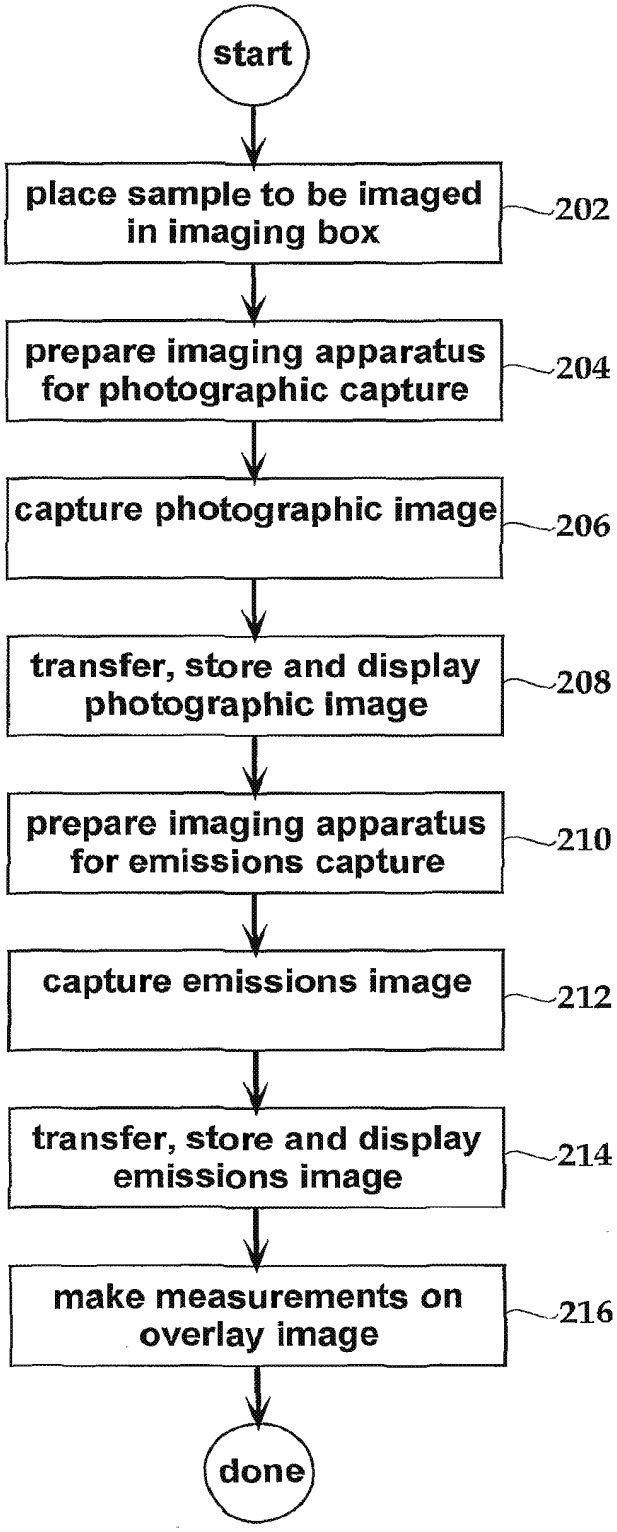

start
place sample to be imaged in imaging box
202
prepare imaging apparatus for photographic capture
204
capture photographic image
206
transfer, store and display photographic image
208
prepare imaging apparatus for emissions capture
210
capture emissions image
212
transfer, store and display emissions image
214
make measurements on overlay image
216
done

Fig. 17

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G06T 7/00 — A1

(11) International Publication Number: **WO 99/08233**

(43) International Publication Date: 18 February 1999 (18.02.99)

(21) International Application Number: PCT/CA98/00762

(22) International Filing Date: 7 August 1998 (07.08.98)

(30) Priority Data:
60/054,892 7 August 1997 (07.08.97) US

(71) Applicant *(for all designated States except US)*: IMAGING RESEARCH INC. [CA/CA]; Brock University, 500 Glenridge Avenue, St. Catharines, Ontario L2S 3A1 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: RAMM, Peter [CA/CA]; 290 Riverview Boulevard, St. Catharines, Ontario L2T 3N4 (CA). SUN, Gang [CA/CA]; 146 Riverview Boulevard, St. Catharines, Ontario L2T 3M5 (CA).

(74) Agent: ROBIC; 55 Saint-Jacques Street, Montreal, Quebec H2Y 3X2 (CA).

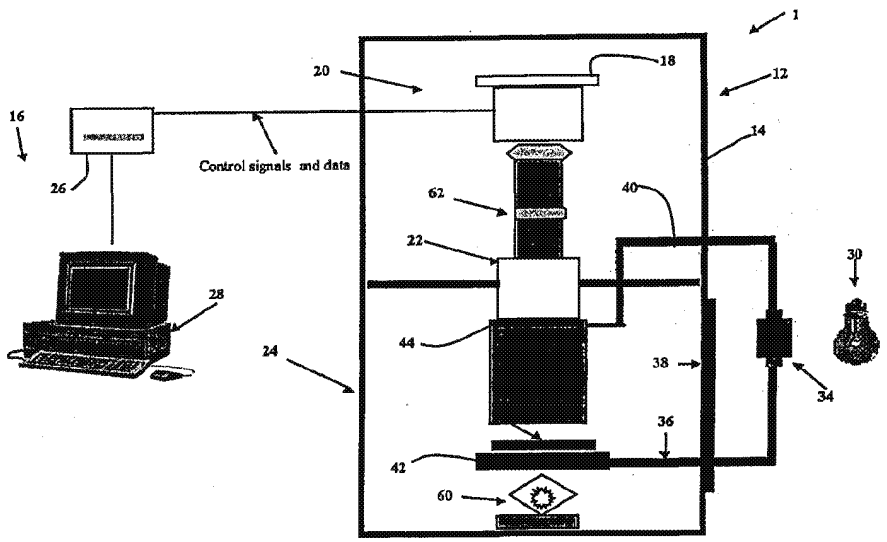
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: A DIGITAL IMAGING SYSTEM FOR ASSAYS IN WELL PLATES, GELS AND BLOTS



(57) Abstract

A method and apparatus are disclosed for use in an area digital imaging system for assays to extract targets on a specimen containing an array of targets that may not be arranged in perfect regularity. A matrix is defined of nominal target locations including a probe template of predefined, two–dimensional size and shape at each of a plurality of fixed, predefined grid points on the specimen, and a determination is made of the most probable location of the probe template corresponding to a specific target by sensing both pixel intensity and the spatial distribution of pixel intensities in an image of the specimen at a plurality of locations in the vicinity of a nominal target location.

SPECTRAL_0000429

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

SPECTRAL_0000430

A DIGITAL IMAGING SYSTEM FOR ASSAYS IN WELL PLATES, GELS AND BLOTS

Field of the Invention

The present invention relates to a system for creating digital images of fluorescent, luminescent, or bright field specimens. The system is flexible, both in its mode of illumination and in that specimens may be arrayed in matrices (e.g. well plates) or randomly arranged (e.g. chemiluminescent colonies, gel media).

Background of the Invention

The present invention is primarily an assay imaging system. An assay may be defined as a measurement of physical properties (chemical, biochemical, physiological or other) within a specimen. Assays are used, typically, in the areas of molecular biology, genomics, and pharmaceutics.

The standard assay specimen container is a plastic or glass plate containing 96 small chambers, termed *wells*. Detection instruments and robotic plate handling technologies have evolved to make efficient use of the 96 well plate, and to provide as high a throughput as possible with this plate format. Current screening technologies can allow a large screening laboratory using 96 well plates to process some thousands of compounds per day.

Recently, there has been a massive growth in the number of compounds available for testing. In part, this is due to an increased exploitation of biodiversity resources to

generate natural compounds. In greater part, the proliferation of new compounds is a result of a new chemical technology, termed combinatorial chemistry. In combinatorial chemistry, large numbers of related compounds are synthesized (using permutations of chemical building blocks), and must then be tested for medicinal value.

With new discoveries in natural compounds, and with the advent of combinatorial chemistry, pharmaceutical companies and biotechnology companies are generating extensive "libraries" of untested compounds. These libraries can easily contain millions of compounds. Standard measurement technologies cannot cope with the volume, and new technologies are needed which will increase the rate at which compounds can undergo initial testing (screening) for medicinal value. To show a major advantage over standard technologies, new technologies should allow throughput to exceed 100,000 compounds/instrument per day. Imaging technologies have the promise to increase throughput to the required levels. They have the additional advantage of flexibility, in that an imaging system can be applied to assays in wells and other formats, and to assays which are static or which change over time.

Most assays are designed so that changes in the absorbance, transmission, or emission of light reflect reactions within the specimen. Therefore, most assay measurement instruments detect alterations in luminance as their operating principle. For detection instruments, bioluminescence or chemiluminescence provides the simplest type of assay, in that there is no need to apply illumination. Absorbance assays involve transilluminating the specimen, usually with monochromatic light. The reaction of interest affects the extent to which the light is absorbed by a fluid, and this absorbance may be measured.

Fluorescence is emitted when a fluorophore interacts with an incident photon (excitation). Absorption of the photon

SPECTRAL_0000432

causes an electron in the fluorophore to rise from its ground state to a higher energy level. Then, the electron reverts to its original level, releasing a photon (fluorescence emission) whose wavelength depends upon the amount of energy that is released during reversion. A given fluorophore may emit at single or multiple wavelengths (creating an emission spectrum), as electrons drop from various orbitals to their ground states. The emission spectrum is constant for each species of fluorophore.

Fluorescence assays require the application of an intense monochromatic illumination beam, termed "excitation". Fluorescence assays are used for the following types of applications:

1. An detector tuned to a specific emission spectrum can be used to localize a fluorophore. For example, wells which contain cells expressing a fluorescently tagged protein can be discriminated from wells which do not.

2. By measuring the intensity of fluorescence, an detector system can estimate the concentration of a fluorescent molecule.

3. Changes in the fluorophore molecule (such as binding of fura-2 to Ca++) will lead to alterations in the emission spectrum. A detector can be used to measure these spectral changes, as an indication of changes in the environment of the fluorophore.

Wells

Each well contains a discrete condition of the experiment, and alterations in light emission are measured to determine whether that condition yields favorable properties. "Well plate" assays are higher in throughput and lower in cost than similar assays in discrete containers.

Reactions within the wells may be of many kinds. In chemistry assays, molecules of different compounds (e.g. a drug candidate and a receptor molecule) are placed into the same

well, and the interaction between those compounds is observed. In cell-based assays, each well contains a population of living cells, and effects of compounds on these cells are observed.

Most assays are conducted by making a single measurement from each well. However, it is also possible to record changes over time, by measuring each well repeatedly. The use of repeated observations could be termed a "dynamic" assay.

Standard well plates contain 96 or 384 wells in an area of about 8 x 12 cm. The trend is towards miniaturization of the wells. Prototypes containing 864 wells or more are under evaluation at many sites. The goal is to develop plates with high density arrays of small "microwells" (e.g. thousands/plate) with small fill volumes. That is, miniaturized wells might contain 1 ul of fluid instead of the 100 ul or more used in a typical 96 or 384 well assay. Miniaturized assay formats promise to achieve dramatic cost reductions and to simplify disposal procedures, while allowing many more assays to be conducted.

Hybridization arrays and genetic assays

Low throughput methods of genetic analysis use various electrophoretic procedures. Methods for increasing throughput and decreasing costs of genomic assays include arraying DNA clones (cDNAs) or synthetic oligonucleotides onto flat support membranes or slips of treated glass. The arrays of cDNAs or oligonucleotides (termed *high density grids*) are then hybridized to samples of genomic material to quantify levels of gene expression, or to localize relevant sequences. In the past, most hybridization assays have been conducted using isotopic label and storage phosphor imaging systems for detection. However, nonisotopic methods (particularly fluorescence) are under investigation in many laboratories. Nonisotopic high density grids provide the potential for very high throughput at low cost, and various detection technologies

are being developed for these specimens.

Free format assays

Assays which occur within a regularly spaced array (wells, cDNAs within a grid) can be referred to as fixed format assays. Specimens that are irregularly distributed can be termed free format assays. Examples of free format assays include electrophoregrams, bacterial colonies in culture, and various combinatorial assays in which bead-bound compounds are distributed over a tissue culture. The common factor in these free format assays is that areas of altered luminance can occur at any spatial location.

Instruments designed for fixed format assays (fluorescence plate readers, liquid scintillation counters, etc.) only read from defined locations in the specimen. They are not useful with specimens in which effects lie at locations that are not predefined. In contrast, imaging systems are able to detect and quantify reactions at any position within an image, and there is an extensive history of image analysis being applied to free format assays.

Summary of types of assays

Pharmaceutical companies are faced with unprecedented numbers of new compounds, which must be screened for medicinal value. Specimens are screened using luminescence, absorbance, or fluorescence technologies. Specimen formats include standard and miniaturized well plates, high density arrays of hybridization assays, and free format assays. The present invention is designed to be useful for all of these technologies and specimen formats.

Area Imaging Systems

An area imaging system places the entire specimen onto a detector plane at one time. There is no need to move PMTs or to scan a laser, because the camera images the entire

SPECTRAL 0000435

specimen onto many small detector elements (usually CCDs), in parallel. The parallel acquisition phase is followed by a reading out of the entire image from the detector. Readout is a serial process, but is relatively fast, with rates ranging from thousands to millions of pixels/second.

Area imaging systems offer some very attractive potential advantages:

1. Because the entire specimen is imaged at once, the detection process can be very quick.

2. It is relatively easy to acquire a timed series of images for dynamic assays.

3. Given an appropriate illumination system, any excitation wavelength can be applied.

4. Luminescence reactions (bioluminescence, chemiluminescence) can be imaged.

5. Free or fixed format specimens can be imaged.

In many applications (e.g. film autoradiography), area imaging has a history, instrument developers have experience, and the problems are well understood. In contrast, assay imaging presents new and significant technical challenges. There is no established practice for imaging assays, and very few instrument developers have practical experience with both area imaging and assay technologies.

Luminescence Area Imaging

We will define luminescence as light emitted from a specimen, without excitation by external light. Most luminescence reactions are quite dim, and this can make extreme demands upon detection. The standard area imaging strategy is to use scientific grade CCD cameras, which achieve relatively high levels of sensitivity. Intensified CCD cameras have also been used.

Typical prior art applies area imaging to luminescent assays on flat membranes. Descriptions of well imaging are flawed in that no correction for parallax error is described.

Telecentric lenses have not been used, because prior art telecentric lenses have not been able to collect enough light.

Prior art also fails to disclose the use of software that would allow automated analysis of targets which do not lie in a perfectly regular array, or software which models variations in measurement efficiency from well to well.

The novel features of the present invention (efficient light collection combined with freedom from parallax, target detection software, calibration software which minimizes variations in measurement efficiency) allow it to be used with luminescent specimens that lie beyond the capabilities of prior art luminescence imaging systems.

Fluorescence Area Imaging

Area imaging is in routine use for fluorescence microscopy, where epifluorescence is the universal method and is easily applied. On a microscope, epifluorescence is implemented via an arrangement incorporating a dichroic mirror and interference filters. Prior art in fluorescence microscopy is irrelevant to the present invention, which is specifically designed for macro specimens.

Macro-level, area fluorescence imaging is routinely applied with gels and blots. Although some specialized research systems have been reported, prior art is dominated by low cost commercial systems for routine gel/blot fluorescence. Typically, this type of imaging has been compromised by sub-optimal delivery of excitation illumination. The most common form of area illumination uses gas discharge illuminators (e.g. UV light boxes). The lamps are coated with a filter that limits emission to the peaks emitted by the excited gas within the lamp. Thus, most prior art systems offer a limited number of excitation wavelengths.

Many prior art systems, as those using transilluminators, pass excitation directly through the specimen and into the collection optics. For the highest

SPECTRAL_0000437

sensitivity, however, it is important that excitation not be delivered directly into the detection optics. With direct delivery, both the excitation and the emission wavelength are detected, with the excitation at much higher levels than the emission. The excitation wavelength can be filtered, selectively, but enough excitation remains to degrade sensitivity.

To avoid the direct detection of excitation, some prior art systems deliver excitation from above, from the side, via dark field, or using refraction (e.g. evanescent wave) into the specimen. In these cases, the detection optics view fluorescence emission from the specimen, with minimal detection of excitation. However, these techniques have severe limitations. Side-mounted fiber optic illuminators deliver uneven light, and can only be used with flat specimens. When they are used with wells, light enters the specimen at an angle and fails to penetrate deeper regions. Similarly, refractive or dark field illuminators do not penetrate into deep wells, cannot be used with opaque specimens, and tend to provide rather dim levels of illumination.

We can summarize the characteristics of prior art systems as follows:

1. *Most are limited to the wavelengths emitted by gas discharge lamps.* Typically some combination of UVA, UVB, UVC, and/or white light lamps is provided. Other wavelengths cannot be obtained, and this is a sever disadvantage.

2. *Wavelengths cannot be altered during an assay.* If the illumination must be changed during the assay (e.g. as for calcium measurement with fura-2), the devices cannot be adapted.

3. *Insensitive to small alterations in fluorescence.* Transillumination comes from directly below the specimen into the detector optics. Therefore, even very good filters fail to remove all of the incident illumination, and this creates a high background of nonspecific illumination.

SPECTRAL_0000438

Small alterations in fluorescence (typical of many assays) are lost within the nonspecific background.

4. *Inefficient cameras and lenses.* A very few systems use high-performance cameras. Even these few systems use standard CCTV or photographic lenses, and can only be applied to bright specimens.

5. *Parallax error precludes accurate well imaging.* As wide-aperture, telecentric lenses have not been available, these systems exhibit parallax error when imaging wells.

In one example of a prior art fluorescence system, a fiber optic is coupled to the specimen on its input, and to a CCD camera on its output. This use of a fiber optic lens in the input to a camera yields freedom from parallax error and efficient light collection. The CCD camera can be run in photon counting mode to obtain single photon detection capability. In achieving parallax-free images and high sensitivity to light, the fiber-coupled camera is like the present invention. However, fiber coupling to the specimen has the following major disadvantages:

1. A fiber-coupled system is limited to specimens that are transilluminated, because there is no place to insert an epi-illumination mechanism. Therefore, the fiber-coupled system would not be as sensitive to subtle variations in fluoresence as is the present invention (epi-illumination).

2. Because fiber optics have a very shallow depth of field (typically about 1 μm), the fiber-coupled system must view the specimen through a thin, clear film of glass, plastic, or other material. Not all assays can be conducted in plates having thin, clear bottoms. If assays must be conducted in opaque plates, the fiber-coupled system becomes inefficient, because it can not be brought close enough to the fluid within the wells to achieve focus.

3. Because of its limited depth of field, a fiber-coupled optic exhibits compromised performance with deep specimens. For example, fluid in well plates is typically

SPECTRAL_0000439

more than 1 mm deep. Only a small part of the fluid is in focus with a fiber-coupled optic. This leads to inefficient light collection from those parts of the fluid that do not lie in critical focus.

4. The fiber coupled system could not be used for fluorescence polarization studies, because of the effects (birefringence, etc.) of the fiber on polarized light.

5. Well plates are 8 x 12 cm. Image forming fiber optics of this size are very difficult and expensive to construct, and must be brought to a much smaller CCD by tapering the fiber bundle into a minified configuration. Minification results in major losses in transmission efficiency, which might drop from 70% to 10%. To avoid both the high cost of a large fiber optic, and the loss of transmission efficiency resulting from minification, the specimen would have to be acquired as a number of subimages. In this case, a translation table moves the specimen or camera, and a straight or slightly minified fiber bundle of reasonable size is used. Following a scan, the subimages would be reassembled to show the entire specimen. This multiple acquisition is slow, affects accuracy and precludes use of the device with assays which change over time.

Other examples of prior art fluorescence systems include use of a cooled CCD camera, with an illuminator that is mounted laterally, or above the specimen. In some cases, an excitation filter wheel can be inserted prior to a fiber optic light delivery system, to achieve some of the functions of the present invention (selection of wavelengths). These prior art systems all have the following major disadvantages:

1. A side or top-mounted illuminator results in very uneven illumination with almost any specimen, and is completely unsuitable for use with wells.

2. Systems lack a telecentric lens, so parallax would preclude imaging of assays in wells.

3. Target detection software is lacking.

SPECTRAL 0000440

There is another prior art fluorescence system (Fluorescence Imaging Plate Reader - FLIPR, Molecular Devices) which uses laser illumination applied at an angle to the wells in a 96 well plate. A pinhole mask is placed over the wells, so that the angled light only reaches a small part of the specimen fluid at the bottom of each well. Data are detected using a cooled CCD camera. FLIPR is inflexible, in that it runs only in 96 well format, uses specific laser lines, does not offer epi-illumination, cannot illuminate the entire fluid contents of deep wells, and is not applicable to luminescence imaging, free format imaging, or higher density well formulations.

Science Applications International Corporation (SAIC, San Diego, CA) was developing the RTFluorimeter, a high throughput fluorescence screening system for kinetic assays in 96 well format. The RTFluorimeter includes a robot for well filling, a high power arc lamp, a filter wheel for excitation wavelength selection, and a CCD array detector. The system illuminated using angled fiber optics placed at the sides of the specimen, and did not include epi-illumination. Performance data and specific imaging design information have not been forthcoming, and this system has been discontinued.

There is extensive prior art in the use of imaging to detect fluorescence assays incorporated within microfabricated devices (often termed "genosensors"). Some genosensors use scanning imagers (e.g. the Molecular Dynamics or Hewlett Packard genechip readers), and detect light with a photomultiplier. Others use area CCDs to detect alterations at assay sites fabricated directly onto the CCD, or onto a coverslip that can be placed on the CCD. It can be envisaged that many other types of genosensors will appear in the near future.

Advantages of the Present Invention for Area Fluorescence Imaging

SPECTRAL_0000441

Macro Fluorescence

Novel features of the present invention minimize the disadvantages of prior art macro fluorescence systems. These features include the following:

1. The device can accept light from almost any source. Therefore, illumination wavelengths may be selected without regard to the peak(s) of a gas discharge lamp or laser.

2. Using a computer-controlled filter wheel or other device, illumination may be altered during an assay,

3. Small alterations in fluorescence emission can be detected, because fluorescence illumination comes via epi-illumination. Direct excitation illumination does not enter the optics, and the nonspecific background is as low as possible.

4. The camera and lens form a highly optimized system for use with dim specimens.

5. The telecentric lens removes parallax error so well plate assays are accurate.

6. The optics can be adapted to many forms of fluorescence, including time resolved and fluorescence polarization assays.

7. The entire fluorescence illumination and detection system is integrated within a single mechanical assembly. This assembly is easy to integrate with automated specimen handling equipment.

For fluorescence, a critical advantage of the present invention is its epi-illuminating lens. It is flexible in application, allows simultaneous imaging of the entire specimen, illuminates, collects data without high levels of background or parallax error, is easily integrated with specimen handling automation, and is usable with dim specimens.

In one particular, adaptability to opaque media, the lens of the present invention minimizes the disadvantages of a

SPECTRAL_0000442

fiber-coupled optic. The lens focuses some distance away from the specimen, uses epi-illumination, and has large depth of field. These features allow the focusing of fluids lying within opaque media and, more generally, focusing of fluids of typical depth (e.g. 1 mm). Another advantage of the present invention is that, as there is no fiber optic lens to act as a light scramble, fluorescence polarization assays may be performed. Finally, the lens of the present invention images the entire well plate in a single acquisition. This avoids the need for translation tables, and makes both accurate calibration and dynamic assays much easier to perform.

Genomic Assays

In one particular, the present invention has advantages for genomic assays. Genomic assays are in flux, and many labeling methods (fluorescence, luminescence, absorbance, isotopic) and formats are likely to appear. Unlike the scanning imagers currently used for these genomic assays, the present invention allows almost any specimen format (including wells), any fluorescence wavelength, and is capable of luminescence detection. Luminescence methods can offer the highest levels of sensitivity in some genomic assays.

The flexibility of the present invention is unique, and makes it suitable as a single tool for laboratories performing genomic assay prototyping. In assay prototyping, is can be difficult to predict the specific wavelengths and specimen formats that will be used. The present invention allows a laboratory to construct an assay using almost any labeling and detection paradigm. There is no need to select from the small number of labeling and detection methods supported by a specific laser scanner.

The present invention could also be used for mass screening (using high-volume fabrication), when scanning laser excitation is inappropriate, or when luminescent detection is

SPECTRAL_0000443

required. In sum, it is a purpose of the invention to provide a more flexible alternative to prior art laser scanning of genomic assays.

Summary of Area Fluorescence Imaging

The present invention includes features that are novel, both singly and in combination. These are:

1. macro epi-illumination;
2. ability of a single instrument to conduct both accurate (parallax free, calibrated) fluorescent and non-fluorescent assays;
3. freedom from parallax;
4. easily adapted to fluorescence polarization, time resolved fluorescence, and other methods;
5. easy integration of fluorescence illumination with specimen handling and dispensing automation;
6. software includes calibration methods that minimize errors inherent to area imaging;
7. software finds and quantifies targets.

A system in accordance with the present invention includes a CCD detector, lens, illumination system, imaging system, and software. Together, these components are configured as a system that can be used with fixed or free formats, with wells or flat specimens, and with any form of illumination (fluorescence, luminescence, transmission). The system exhibits measurement performance similar to that of standard, non-imaging measurement instruments, while having the additional advantages of an imaging system.

Major Components of a System in Accordance with the Invention

a) Sensitive CCD Detector

Area imaging systems use CCD arrays to form images. Factors which influence the ability of CCD arrays to detect small numbers of incoming photons include quantum efficiency, readout noise, dark noise, and dynamic range. Preferably, the

SPECTRAL_0000444

present invention uses a CCD array with high quantum efficiency, low readout and dark noise, and broad dynamic range.

b) A Lens Which Combines Telecentricity and Large Aperture

The lens of the present invention is unique in that it combines telecentricity with a large aperture. This combination allows accurate reading of dim assays within wells.

c) True Epifluorescence Optics

An epifluorescence optic both illuminates and collects through the same lens. Epifluorescence optics must include a source of excitation, and a barrier filter which removes excitation from the detected signal and leaves only the fluorescence emitted from the specimen. The present invention incorporates both a source of excitation and a barrier filter within its lens.

The epifluorescence optics are beneficial in yielding lower backgrounds, broader dynamic range, and more linear fluorescence response than dorsal, lateral, refractive, or transilluminators. The ability to deliver large area, monochromatic epi-illumination is a unique capability of the present invention.

SPECTRAL_0000445

d) Optional Components for Ultra-Low Light Imaging

Although any area detection technology may be used, the preferred detector is a thinned, back-illuminated and large CCD, which is capable of imaging even low levels of fluorescence and luminescence. For the most extreme low light conditions, as with the scintillation proximity assay, the present invention allows use of a camera cooled to very low temperatures (e.g. the CCD runs at -70 degrees C). Cooling may be by liquid nitrogen, forced probe mechanism, multistage thermoelectric, or any other means. As an alternative, the present invention allows substitution of a fiber optic lens for the epi-illuminating lens. This use of a fiber optic lens sacrifices flexibility and fluorescence capability, in return for higher sensitivity with luminescent assays.

The present invention also allows use of a light amplifier, inserted between the lens and the CCD camera. In one preferred configuration, this light amplifier is an image intensifier.

e) Flexible Illumination Source for Fluorescence Excitation

Application of even illumination of selectable wavelength over an 8 x 12 cm area is a feature of the present invention. Illumination is delivered via a standard fiber optic coupling. This allows the use of almost any illumination source (halogen, arc lamp, laser, etc.). In a preferred configuration incorporating a halogen or arc lamp, any wavelength of monochromatic illumination may be selected by precision filters (usually standard interference filters). These are readily available in the small sizes used. An optical coupler/computer-controlled filter wheel is preferred for selection of excitation wavelengths. The output of this device may be taken to a specially designed fiber optic plate for transillumination, to a fiber optic ring, line, or panel light for dorsal illumination, or (preferably) to a fiber

SPECTRAL_0000446

optic illumination assembly within the lens, for epi-illumination.

f) Software That Integrates Control, Detection, and Analysis

Area imaging is inherently more complex than either nonimaging detection, or the use of scanning imagers. In particular, nonimaging counting systems have a relatively easy task. They only need to control the scanning process, control internal calibration, and create a small array of data points representing each well. The sequence of steps might be as follows:

1. Calibrate detector against internal standard.
2. Illuminate one well.
3. Position a PMT over the illuminated well.
4. Read well.
5. Transfer data to spreadsheet.
6. Illuminate next well and repeat.

An area imaging system has a much more difficult task. Imaging a well plate might include the following requirements:

1. Provide adequate illumination over the entire plate.
2. Control a high performance camera.
3. Store geometric and density correction factors.
4. Image specimen.
5. Correct geometric and density variation.
6. If necessary, calibrate image to standards within the specimen.
7. Locate each well and quantify intensity, with respect to a calibration function.
8. Transfer data to spreadsheet.

These tasks can only be performed if the imaging system is equipped with software that performs functions 2-8, above. The present invention incorporates such software, including

SPECTRAL_0000447

novel software for locating specimens, and for calibrating the imaging system to minimize error.

g) Optional Components for Time Resolved and Polarization Fluorescence

A standard way to minimize background is to take advantage of the long-lived fluorescence properties of specific fluors. With a long-lived fluor, the illumination source can be strobed very rapidly, so that excitation is applied for a brief period, and is then blocked. Fluorescence tends to fade gradually (e.g. over a period of 10 msec after blocking of excitation), so measurement can be made during the excitation blocking period. This measurement after blocking of the excitation illumination should be free of reflections, and of direct contamination by the excitation wavelength. This delayed measurement is known as time-resolved fluorescence (TRF).

The major advantage of TRF is that it results in much lower levels of background fluorescence. The major disadvantages of TRF are that it requires specialized hardware for the rapid cycling of excitation and blocking, and that a limited number of special fluors (typically using Europium, Lanthanide, or Terbium chelates) are suitable for the procedure.

Typically, the present invention is applied to time resolved fluorescence by using both a shutter and a strobe light source. The strobe provides brief and precisely gated periods of illumination. The shutter protects the CCD from exposure while the illumination is on.

Various types of shutter are possible:

An image intensifier is mounted between the epi-illuminating lens and the CCD detector. The intensifier serves to amplify the light coming from the fluor and, by gating, the intensifier serves as a shutter. When the intensifier is gated on, light is allowed to reach the CCD.

SPECTRAL_0000448

When the intensifier is gated off, light is prevented from reaching the CCD. Gating can be very quick, so that the intensifier on period coincides with the optimal period of delayed fluorescence from the specimen.

A chopper wheel, liquid crystal, leaf, or other shutter may be mounted within the optical path, after the specimen but prior to the detector. For example, the shutter could be located at the barrier filter position within the lens. The shutter is time-locked to the excitation strobe, so as to achieve the required delay between the on-off cycle of the strobe, and the exposure period of the CCD.

Although the preferred illumination source is a strobe lamp, other forms of illumination (e.g. pulsed laser, pulsed arc lamp, continuous arc lamp) may be envisaged by those skilled in the art. If the illumination cannot be gated precisely, a second shutter mechanism is required to provide a precise on-off cycle.

A critical advantage of the present invention for TRF is that illumination is applied via the telecentric, epi-illuminating lens. This lens provides a convenient means by which fluorescence excitation may be applied, telecentric collection free of parallax, and efficient optics that collect as much as possible of the dim light emitted in delayed fluorescence paradigms.

The lens of the present invention may also be configured for fluorescence polarization studies, by adding a ring polarizer on the output of the internal ring light, and an analyzer into the lens barrel.

Features of the Analysis Software

Finding of Targets

An image contains both targets and background. Often, there is some ambiguity about which parts of the image constitute the target component. Extensive prior art deals with the automated extraction of target data from images. The

SPECTRAL_0000449

extraction process is known as "segmentation". Typically, segmentation algorithms use density, color, texture, or other image characteristics to define a valid target. Any pixel that meets the target definition criteria is designated as a target pixel.

The present invention performs segmentation of the usual kinds, using any combination of image characteristics, in addition to novel forms of segmentation. The usual type of segmentation is preferable for free format specimens. Typically, targets are defined by their luminance value, and are then detected automatically at any points within the image. For imaging of wells, hybridization arrays, and other specimens in a regularly spaced (fixed) format, the present invention uses a novel form of segmentation which allows very large numbers of targets (e.g. 60,000) to be analyzed, with full automation.

The novel segmentation procedure uses a "fixed probe" strategy, in which an array of probes of constant size is placed onto the sample. For this purpose, an analogy is made to liquid scintillation counting. In liquid scintillation counting of isotopically labeled specimens it is common to cut out (using a circular punch) pieces of membrane of fixed size. These pieces are placed into vials of scintillation fluid for counting. As a result of the count, the total amount of radioactivity in each sample piece becomes known. Similarly, with the present software, an array of fixed probes is applied to the image, at intervals specified by the grid definition.

Positioning software is required, because the array of computer-generated probes is unlikely to accord exactly with the true locations of all the targets in the specimen. Variation in target position arises because spotting robots are not perfect, plates may vary slightly in position, and so forth. Therefore, prior to quantification, the image is processed and parsed to rationalize the location of each

SPECTRAL_0000450

target within the array. Essentially, the algorithm looks for any targets that may be recognized on the basis of distinct densities. It fits these recognizable targets into the most likely locations within the grid, using a fuzzy logic algorithm. Then, it fits the less distinct dots into their most likely locations, using the higher-density dots as anchor points for the alignment.

To the user, the procedure appears automatic. Once the fixed probe array has been defined on the specimen, the computer performs automatic alignment of the array elements to the most appropriate positions in the matrix. Any elements that remain misaligned may be manually edited.

Statistical Segmentation

Luminance data from the aligned matrix are output to software, which creates a distribution of data from every fixed probe. Typically, a mean and standard deviation are calculated for this distribution, and targets may be further defined as "hits" using a variance criterion for the distribution. For example, we might define targets that lie more than four standard deviation units from the mean of the distribution as hits. This use of variance-based hit definition is termed "statistical segmentation".

Statistical segmentation requires that every sample in the matrix be analyzed, even if it is not detectable by standard segmentation methods. Therefore, when used for well plate or other grid assays, statistical segmentation requires the use of aligned, fixed probes as described above.

The Measurement Efficiency Calibration

The present invention implements a novel means by which measurement efficiency may be corrected. This allows correction of variations in collection efficiency of the lens or camera. In practice, the system views a set of reference standards which span all or part of the range of intensities

SPECTRAL_0000451

observed in actual specimens. Typically, these standards will be well plates of the same kind, and containing a substance responding to the same wavelengths as are to be used for the assay in question. Preferably, three (or more) plates are made to contain low, medium, and high concentrations of light emitting substance. The use of multiple reference concentrations allows nonlinear variations in measurement efficiency to be accommodated.

Once the reference plates have been viewed, the system can derive discrete models for measurement efficiency at each well. That is, the reference plates allow us to create, at each spatial location in the image, a known relation between the intensity of light observed by the system, and the concentration of light emitting substance that is actually present. The system uses these data to model and correct subsequent measurements obtained from each well. The result is a low level of error variation between wells. Typically, the present invention yields coefficients of variation that are very similar to those obtained from nonimaging counting systems. This use of a correction for well-to-well variation in measurement efficiency is highly advantageous, if an area imaging system is to achieve acceptable accuracy in quantifying wells.

Brief Description of the Drawings

Further objects, features and advantages of the invention will be understood more completely from the following detailed description of a presently preferred, but nonetheless illustrative embodiment, with reference being had to the accompanying drawings, in which:

Figure 1 is a schematic illustration of a system in accordance with a first preferred embodiment (upright) of the invention;

SPECTRAL_0000452

Figure 2 is a detailed illustration of the optical and mechanical components of the lens and the emission filter holder;

Figure 3 illustrates schematically the benefit of the telecentric lens;

Figure 4 is a schematic illustration of how reflections that would be detected on the CCD are avoided;

Figure 5 is a schematic diagram of an imaging system incorporating an image intensifier;

Figure 6 is a schematic representation of the image intensifier;

Figure 7 is a flow chart illustrating the main data analysis process;

Figure 8 is a flow chart showing the process grid definition and alignment;

Figure 9 is a flow chart illustrating the well-by-well calibration process.

Figure 10 illustrates a process for establishing well-by-well calibrations for measured efficacy.

Detailed Description of the Preferred Embodiments

Turning now to the details of the drawings, Fig. 1 is a schematic diagram illustrating a preferred embodiment of an imaging system 1 in accordance with the present invention. System 1 broadly comprises an illumination subsystem 10, an imaging subsystem 12 provided in an housing 14, and a control subsystem 16. The imaging subsystem 12 comprises a CCD camera subsystem 18 housed within a camera chamber 20 of housing 14 and a lens subassembly 22 extending between camera chamber 20 and a specimen chamber 24. In operation, illumination subsystem 10 provides the necessary light energy to be applied to the specimen within chamber 24. Light energy emitted by the specimen is transmitted through lens subsystem 22 to camera 18, where an image is formed and transmitted to the control subsystem 16 for processing. Control subsystem 16 comprises a camera control unit 26, which is a conventional unit matched to the particular camera 18 and a computer 28 which is programmed

SPECTRAL 0000453

to control unit 26 and to receive data from camera 18, in order to achieve unique control and processing in accordance with the present invention.

The light source for the illumination subsystem 10 is preferably an arc lamp 30. Light from lamp 30 is conducted via an optical coupler 34 and filter wheel X mounted on the output of lamp 30. A fiber optic 32 carries illumination from the optical coupler 34 to the ring light 44 within the lens 22. Alternatively, a liquid or fiber light guide (not shown) may be placed on the output of lamp 30, and taken to a simple optical coupler (not shown) and thence to fiber optic 32.

The filter wheel X contains a number of one inch diameter interference filters. The filter wheel position can be rapidly changed under computer direction, giving the ability to conduct procedures which require more than one excitation wavelength.

Fiber optic bundle 32 carries illumination from the optic coupler/filter wheel 34 to the ring light within lens 44. The bundle 32 enters lens 22 through a mounting flange, placed so that bundle 32 does not need to pierce the wall of the light-tight specimen chamber 24.

Two forms of illumination system are described, each fed by a discrete fiber bundle. These are a transilluminating plate (not shown), used for absorbance or colorimetric assays, and a ring light 44 internal to the lens (22) that performs epi-illumination for fluorescence assays.

The internal ring light 44 consists of one or more rings of optical fibers, mounted within and axially aligned with the body of the telecentric lens 22, and behind its front lens element. It is advantageous to have multiple rings, in that one ring may be angled optimally for even delivery of illumination over most of the specimen, while the second ring may be angled optimally to deliver additional illumination to the edges of the specimen.

SPECTRAL_0000454

An input polarizer is positioned in front of the illumination ring 44 to supply the polarized excitation. By putting the polarizer close to the output of the fiber optic, any effects of polarizer fluorescence are minimized. That is, only emitted fluorescence that is reflected in the surface of the front element of the telecentric lens assembly has the potential to reach the CCD detector. The input polarizer is of ring form, with a hole in its center allowing light to travel up the lens barrel without hindrance. A second polarizer functions as an analyzer (not shown), and may be placed at any position within the lens barrel. A preferred location is at the plane of the excitation polarizer.

The specimen well plate is carried within a holder 58 that is mounted to a focusing drive 60. The holder 58 grips the well plate at its edges. The bottom of the holder 58 is empty, so as not to impede viewing of the wells. By adjusting the focus drive 60, the holder 58 moves relative to the lens 22 and the specimen is focused. The lens 22 is telecentric with a wide aperture. The lens contains an emission filter slot 62, which accepts interference filters for fluorescence imaging. It contains an internal fiber optic ring light 44, positioned behind the front lens element. The lens 22 is mounted to the camera chamber by a flange 64 near its middle. The back of the lens projects into the camera chamber 20, providing ready access to the emission filter slot 62 without disturbing the specimen. The front of the lens projects into the specimen chamber 24.

The cooled CCD camera 18 is mounted with the detector placed as close as possible to the lens. Minimal spacing between the final lens element and the CCD is important to an efficient transfer of light from the lens to the CCD.

The entire system 1 is constructed to achieve high transfer efficiency. Three factors dominate the transfer efficiency (photoelectrons generated / photons emitted) of the detector system. These are the light collection efficiency of

the lens, the quantum efficiency of the CCD detector, and the lens transmittance. We can calculate the number of photoelectrons generated as follows:

$$Npe = \tau * \phi_{detector} * c.e. * N_{photons}$$

where τ is lens transmittance, about 85-90% for our lens

where ϕ is quantum efficiency of the CCD detector, typically about 35-40%, up to 80% in our case

c.e is collection efficiency of lens, about 0.3% in our caseIn a typical scientific grade CCD camera system, using the fastest available photographic lens (F/1.2), and with a high quality cooled detector, the CCD will generate 1 photoelectron for about 5,000-10,000 photons generated from a point source in the sample.

The lens of the present invention offers a collection efficiency about that of an F/1.2 photographic lens. The efficiency of the CCD detector is about double that of other CCDs. The result is that the present invention has the theoretical ability to generate one photoelectron for about 2,500-5,000 photons generated from a point source within the sample. This very high transfer efficiency allows detection of specimens that cannot be imaged with prior art systems.

Control Subsystem

The control subsystem 16 comprises control unit 26 and computer 28. The camera control unit is a computer controllable unit provided by the manufacturer of camera 18 to control the camera. Computer 28 is preferably a conventional computer running in the Windows® environment and is programmed to achieve image acquisition and analysis in accordance with the present invention. All software control, imaging, and analysis functions are resident within the computer 28.

SPECTRAL_0000456

Illumination Subsystem

The preferred configuration of the present invention uses a broad-band illumination source, such as a halogen lamp or mercury-xenon arc lamp. The advantage of an arc lamp is that its output can be made into a narrow beam that can be passed through a small and readily available interference filter, before being spread over the entire surface of the specimen. Any wavelength of monochromatic illumination may be selected by precision filters (usually interference filters) mounted after the lamp. Filters are preferred, because variable monochromators or tunable lasers lack sufficient light output when diffused over large areas.

Although the present invention can be configured to apply diffuse transillumination (through the specimen) or other forms of dorsal or lateral illumination, epi-illumination is preferred because it usually results in lower backgrounds, broader dynamic range, and more linear fluorescence response.

Figure 2 shows best the optical components of the telecentric lens 22. The lens has the following characteristics:

Effective focal length	164.436 mm
Numerical Aperture	0.443
Magnification	0.25

The lens has mounted within it a fiber optic ring light 44, which projects excitation through the front lens element onto the specimen (leftward in Fig. 2). The lens is so calculated that the focus plane of the ring light is at B, while the focus plane of the entire lens is in front of that point, at A. Placing the focus of the ring light at a point beyond the specimen minimizes specular reflections from the specimen.

The emission filter slot 62 allows insertion of an interference filter (a barrier filter) that removes excitation illumination and other unwanted light from the incoming rays, leaving only the fluorescence or other relevant signal emitted by the specimen. The barrier filter is coated to minimize

SPECTRAL_0000457

reflections. A critical advantage of the present lens is that the internal beam is collimated at a position appropriate to the insertion of a barrier filter. At this position, rays are nearly parallel. The collimated beam at this point is critical, because interference filters perform best with rays that are incident at 0 degrees. If the incoming illumination is at an angle, the filter exhibits both broadening of its transmission characteristics, and alterations in the wavelengths that it passes.

It is to be appreciated that the lens of the present invention implements a unique form of macro epi-illumination, without need for a dichroic mirror. In epifluorescence microscopy, where small areas are illuminated, a dichroic beam splitter is usually placed behind the objective. A dichroic beam splitter or mirror is a partially reflective surface that reflects one wavelength range, while allowing another wavelength range to pass through.

On a fluorescence microscope, illumination enters the dichroic mirror from the side. The mirror is reflective with respect to the excitation illumination, and is angled to reflect the excitation light down through the objective toward the specimen. Fluorescence emitted by the specimen (shifted up in wavelength from excitation) is collected by the objective, which passes it upwards towards the dichroic mirror. The dichroic mirror is transparent to the emission wavelength, so that the light proceeds through the dichroic to the detector plane. A different dichroic is required for each excitation/emission wavelength. There are major difficulties in applying a dichroic-based epi-illumination system to macro imaging:

1. The dichroic mirror must be at least as large as the objective it must fill. Camera lenses are much larger than microscope objectives, and would need correspondingly large dichroic mirrors. Dichroic mirrors this large are not

SPECTRAL_0000458

readily available, costly, and subject to internal variability.

2. It is critical that the back lens element of a macro lens be mounted as close as possible to the CCD. Any increase in the distance between the rearmost lens and the CCD markedly impairs the working F number and the light-gathering efficiency. Therefore, there is no room for a dichroic to be mounted behind the lens and the dichroic would have to be mounted within the lens itself. Although dichroics have been mounted within microscope objectives, dichroics mounted within a macro lens have not been described. Our own tests with one such arrangement have revealed difficulties in achieving low backgrounds and even illumination.

3. In a normal epi-illumination system, the dichroic reflects excitation through the entire lens. This has two disadvantages.

- Each lens adds its own autofluorescence to the total background. This may not be a major problem with the small lenses and very bright illumination levels found in microscopy. With macro lenses and lower light levels, however, glass autofluorescence becomes a greater component of the total signal seen by the detector.
- Transmission of excitation illumination is highly subject to the optical characteristics of the glasses used in the lens. Very costly (and difficult to work) quartz glass optics are required for UV epi-illumination. These UV-transparent optics can be constructed in the small sizes needed for a microscope objective, but would be astronomically expensive in the large sizes described for the present invention.

4. Dichroic beam splitters absorb light. Typically, they are 80-90% efficient.

The present invention functions without a dichroic mirror. Rather, the illuminator 44 is mounted so that it shines directly at the front lens element X, from behind. This

SPECTRAL_0000459

illuminates the specimen, without any need of a dichroic mirror.

Illuminating from within the lens is possible as a result of the novel features of the present invention. These features include:

a) calculation for avoidance of detection of internal reflections and autofluorescence;

b) calculation for effective use of an emission barrier filter, located within the lens and posterior to the illumination source;

c) design so that only one of the lens elements resides in front of the internal illuminator.

It is a key advantage that the lens of the present invention has only one element X in front of the illumination source 44. This feature has the advantages that internal flare, reflections, and glass autofluorescence are minimized, and that only the front element needs to be transparent to UV. A single UV-transparent lens is costly, but not prohibitively so. A further advantage of having only one lens in front of the illumination system is that generation of a polarized illumination beam is made much less difficult. A polarizer may be placed in front of the illumination source, and has only to pass light through the front lens element before affecting the specimen. In the present invention, the front lens can be specified to have minimal effects upon a polarized light beam. If more lenses resided in front of the illumination system, it would be much more difficult to achieve minimal effects upon polarized light.

The front element of the lens is calculated so as to focus the illumination source at B, beyond the plane of the specimen at A. The defocus of the illumination source at the specimen plane minimizes specular reflections. As many well plates are constructed of polished plastic, and tend to generate specular reflections, this is an important feature.

The lens is highly efficient. A typical collection F/# of the lens is 4.5 (working F/1.15). This implies a collection solid angle of 0.03891 sr, and a collection efficiency of 0.03891/4□ = .3096%. For this typical lens, expected transmission value is 0.85-0.90, giving an overall collection efficiency of 0.263-0.279%. This is about equivalent to the collection efficiency of a photographic lens of F/1.2.

The present telecentric lens has a fixed field of view (12 cm diameter, in this case) but, if larger specimens need to be imaged, a motorized translation table may be mounted within the light-tight chamber. The translation table moves the specimen relative to the lens, under computer control. After each motion, a single "tile" is acquired. When the entire specimen has been imaged, all the tiles are recomposed (by the software) into a single large image, retaining telecentricity, freedom from parallax error, and high resolution over its entire surface.

The lens may also be constructed in various sizes and magnification factors to allow larger or smaller specimens to be imaged onto the full extent of the CCD. When used with small, high density DNA arrays, for example, the lens could be constructed without telecentricity, and at a much higher magnification factor so as to image the array at high resolution in a single acquisition. With this new lens calculation, the key principle of using epi-fluorescence to illuminate the entire specimen would be retained.

Figure 3 shows best the telecentric property of the lens 22. Although an epi-illuminating lens could be constructed according to the present invention and without telecentricity, a telecentric lens collects parallel rays, over the entire area of a well plate. Thus, it does not peer into any wells at an angle and is free of parallax error. In contrast, images of deep, narrow targets, made with standard lenses, exhibit severe parallax. That is, geometric

SPECTRAL_0000461

distortion increases at the edges of the field of view. With a non-telecentric lens, circular targets (such as wells) at the center of the image are seen as true circles. However, the lens peers into lateral targets at an angle. Therefore, these lateral targets are seen as semilunar shapes, and the lens fails to image parts of lateral wells.

Figure 4 shows the path of light rays proceeding from the fiber optic ring light 44. Light from the ring 44 strikes the front lens element X, which is coated to allow most of the light to pass through towards the specimen X. However, a small proportion of the light incident to the front lens is reflected back towards the CCD detector 84. Even though these reflections are much less intense than the illumination source (e.g. <0.2%), they are very intense relative to the weak fluorescence emission of the specimen. That is, fluorescence from the specimen may well be on the order of 0.1% of the excitation intensity. Therefore, reflections within the lens would conflict with fluorescence from the specimen, and would degrade the sensitivity of the imaging system.

In the present invention, reflections from lens surfaces ahead of the illumination source are not detected. In calculating the destination of rays which reflect from the front lens element X, it can be determined that only some of the reflections arising from lens X strike the CCD 84. For example, a ray X is shown emanating from ring light 44 at position A, and striking lens X at position B. The reflection X of this ray X is shown striking a point C that does not lie on CCD 84. However, rays from position A on ring light 44 that struck front lens X at position D would reflect to strike CCD 84.

The lens of the present invention contains a mechanism that blocks excitation rays that would strike front lens X at locations which create bothersome reflections. Light rays emitted from ring light 44 are emitted at an angle

(5 degrees in this case) directed inwards towards the center of lens X. Normally, these rays would strike the entire surface of lens X, generating reflections that would be highly evident in the image formed at CCD X, and which would degrade the sensitivity of the system. These undesirable rays are blocked by a lip X, placed as a ring just inside the ring light 44. In effect, lip X shadows the front lens X from rays which would generate reflections.

The rays which are permitted to pass lip X still generate reflections internal to the lens assembly 22, but these die within the body of the lens assembly 22 or on the body of the camera mount 18. They do not reach the CCD X. As a result of these novel design features, the present lens is able to supply an intense epi-illumination beam without the presence of detectable internal reflections. Rather, the angle of excitation incident to the front lens is so calculated that its reflections strike the inner surface of the camera mount X. Thus, reflections die within the body of the camera or lens mechanism, without reaching the CCD detector X.

Figure 4 shows best the cooled CCD camera. The camera 18 includes a CCD element 84 positioned behind a camera aperture. A particular problem that arises with lenses of the present kind (wide aperture, back element positioned very close to the detector plane) is that the depth of field at the detector plane is very narrow. The lens of the present invention, for example, requires that the CCD be flat with respect to the optical axis of the lens, with a tolerance of 50 microns. Therefore, the present invention implements both checks during manufacture, to ensure that the CCD surface is flat, and a tip-tilt adjustment (not shown) within the camera body, allowing the CCD to be adjusted to flatness.

To reduce dark noise produced by electrons within the CCD, the CCD element 84 is mounted to a heat sink 88, which in turn is thermally coupled to a cooling element and liquid

SPECTRAL_0000463

circulation system for providing enhanced heat dissipation. A lens 90 is positioned over the aperture to focus the image on the CCD element 84. The fast, telecentric lens 22 (Figures 2 and 3) is mounted directly to the camera body by screws, after removing the photographic lens mount. Similarly, the image intensifier 70 (when present) is mounted directly to the camera body.

Preferably, the present invention uses a CCD array with high quantum efficiency, low readout and dark noise, and broad dynamic range. Quantum efficiency (QE) describes the ability of the photodetector to convert incident photons into electron hole pairs in the CCD. Consumer-grade CCDs typically exhibit QE of about 12-15%. Standard, scientific grade cooled CCD cameras exhibit QE of about 40%. A very limited number of thinned, back-illuminated CCDs can achieve QE of as high as 80% at peak detection wavelengths.

Readout noise originates in the output preamplifier of the CCD, which measures the small changes in voltage produced each time the charge content of one or more CCD elements is transferred to it. Readout noise is directly related to the readout rate, and is decreased by use of slow readout.

Dark noise is produced by thermally generated charges in the CCD, which increase the background level. A constant dark noise level can be subtracted from the image, but dark noise also has a random noise component (a variation in level) which cannot be subtracted. This random component adds to the total random noise level of the detector, and has a direct effect upon sensitivity. Dark noise is decreased by cooling the CCD.

The size of the CCD element (typically about 2.25 cm^2) is related to its ability to store photoelectrons (known as the well capacity) and, hence, its dynamic range. The larger each CCD element (pixel) in the array, the larger the full well capacity and dynamic range of that pixel. A broad

SPECTRAL_0000464

dynamic range is desirable, in that it allows the detector to be used for longer exposure times, without saturation. This enhances the detection of very small signals. A further advantage of large pixels is that signal to noise performance is improved, relative to that of smaller pixels. Even so, most area imaging systems use CCDs with small pixels, both because the cost is lower and because resolution is dependent upon pixel size. Large pixels sacrifice resolution to gain signal. Small pixels sacrifice sensitivity and dynamic range to gain resolution. In sum, CCD cameras have tended to trade off light sensitivity against resolution.

To achieve high sensitivity, good resolution, and broad dynamic range, CCD cameras used in astronomy often incorporate a large CCD, containing a large number of large pixels.. The present invention (preferably) incorporates an unusually large CCD array (e.g. 6.25 cm^2), containing many (preferably >1 million) large pixels. In addition, the CCD is selected for the highest possible quantum efficiency (preferably about 70-80%). As a result, the detector of the present invention is characterized by very high sensitivity, broad dynamic range (true 16 bit), *and* high resolution. This synergistic combination of features is novel in its application to assay imaging.

To take full advantage of the CCD characteristics, the preferred support electronics include a high-precision digitizer, with minimal readout noise. The camera provides a 16-bit digital signal output via digitization circuitry mounted within the camera control unit, and an interface card mounted within the computer. Data from the CCD are digitized by the camera control unit are digitized at rates appropriate to the sensitivity required (e.g. 200,000 pixels/second), and transferred directly to the computer memory.

Following the integration period, the CCD camera accepts a trigger pulse from the computer to initiate closure of the electromechanical shutter. With the shutter closed, the

image is transferred from the CCD to the internal frame buffer of the computer.

Although this camera could be used without cooling the CCD element, extended periods of integration are achieved by using a CCD camera with an integral cooling element. The effectiveness of integration is limited by the degree of cooling. With a non-refrigerated liquid cooling device, sensor temperatures of about -50°C (below ambient) can be achieved. At this temperature, dark noise accumulates at a typical rate of about 7-10 electrons/second. This type of cooling has the advantage of low cost and easy implementation.

It is to be appreciated, however, that longer periods of integration are possible if refrigerated liquid or cryogenic cooling are employed.

Figure 5 shows the optional intensifier for extreme low light and time-resolved modes of imaging. The intensifier 70 is mounted between the telecentric lens 22 and the CCD camera 18. Intensification, as for example, is disclosed in U.S. Patent No. 5,204,533 to Simonet, involves the coupling of an image intensifier to a CCD camera. The image intensifier typically includes a photocathode, a phosphor screen, and a microchannel plate (MCP) connected between the photocathode and phosphor screen. Very high light amplification factors (e.g. up to about 90,000) are available with this type of device. The intensifier also provides shuttering capability for time-resolved and photon counting applications. With the intensifier inserted into the optical chain, the present invention becomes an image intensified CCD (ICCD) camera. In all other respects the system is essentially identical to that of Fig. 1 for extreme low light imaging.

Figure 6 shows best the intensifier 70 as being of the GEN 4 type, and including a photosensitive cathode 72, a microchannel plate (MCP) 74, a phosphor screen 76, and a vacuum sealed body or enclosure 78. The epi-illuminating lens 22 (Figures 2,3) is placed in front of this assembly 70. At its

SPECTRAL_0000466

output, the lens is focused on an input window of the cathode 72 so as to transfer the specimen image thereto. The photosensitive cathode 72 is selected to emit electrons in proportion to the intensity of light falling upon it. The MCP 74 is positioned within the vacuum sealed body 78, between the cathode 72, and the phosphor screen 76 and coupled to the cathode 72 at each end. The MCP 74 is provided with an array of small diameter MCP channels, each of which is coated with gallium arsenide. The electrons emitted from the cathode 72 are accelerated along the MCP channels to the phosphor screen 76. As the electrons from the cathode are accelerated along the small diameter channels, they strike the coated channel walls to produce additional electrons. As the multiplied electrons leave the MCP channels, they strike the phosphor screen 76 and produce an intensified image of the specimen on an output window. This image is coupled to the CCD 84 element in the camera by a lens 80.

It has been found that the use of low noise image intensifiers (e.g. GEN 4) is advantageous over other types of intensifiers. It is to be appreciated, however, that many types of intensifiers (e.g. those with photocathode cooling) may be used with the present system. Similarly devices with high intrinsic gain (such as electron bombarded back-illuminated CCD sensors) could be used in place of image intensifiers.

The integrating camera is configured so that the highly amplified image generated on the output window is carried by a fiber optic onto the CCD element. To image low light specimens, the CCD element integrates for a period. During the integration period, photons from the output window incident to the CCD element are stored as negative charges (the signal) in numerous discrete regions of the CCD element. The amount of charge in each discrete region of the CCD element is accumulated as follows:

Signal = Incident light x Quantum efficiency x Integration time

The greater the relative intensity of the incident light coming from the intensifier, the greater the signal stored in the corresponding region of the CCD element.

With the system shown in Figures 5&6, only the CCD sensor is cooled. This is sufficient for most purposes. It is to be appreciated however, that the intensifier photocathode could also be cooled, thereby improving the signal to noise ratio of the intensifier. Similarly, the entire photosensitive apparatus (intensifier + CCD) can be cooled. However, cooling the entire photosensitive apparatus has the disadvantage that the efficiency of the phosphor on the fibre optic output window is decreased.

The CCD camera of the present invention uses an asynchronous reset which takes an external drive signal from the computer. As the CCD camera incorporates mechanisms that provide very low lag, short integration periods (e.g. 1/100 second) can be used. If desired, these integration periods can be locked to a gated power supply in the image intensifier, with the result that the camera can be read out at very short intervals. Using the gating and fast readout feature, and with the intensifier run at highest gain or with a multistage intensifier, the present invention can thereby be operated as a conventional photon counting camera. Thus, the present system can advantageously be used for both direct imaging of faint specimens, or as a photon counting camera by changing its mode of operation from integration to gating.

Figure 7 is a flow chart illustrating the main data analysis process performed by computer 28 in controlling the system 1 and acquiring data therefrom. After initiation of the process, a specimen is acquired for analysis at block 302 as described more fully below. In creating this image, the specimen is illuminated and acquired in such a way as to

SPECTRAL_0000468

demonstrate maximally the effect of interest. This image is referred to as the specific image. It is to be appreciated that in the specific image, and in any other images acquired by system 1, there are many routine operations for removing errors (e.g. offset and bias in the CCD) in the digital imaging system. These routine options will not be shown here.

A grid consists of an array of probe templates. The "probe template" is the nominal definition of a single target (e.g. in terms of shape and area) corresponding to one dot on a membrane, one well in a plate or similar target. Typically the probe template is a circular area, and there is one probe template for each target in the specimen. A matching grid for a specimen image has all its probe templates matched to the targets on the specimen image (whether they are visible or not). This matching grid is also created at block 302, through a process described more fully below.

The system may simply analyze the specific image. Optionally, at block 304, an image of the specimen is acquired under conditions that show best the exact well locations (typically illuminated with white light), and/or conditions that show best filling in the discrete wells, while being minimally affected by the reaction of interest. These images, which are used to improve the quality of data obtained from a specific image, are termed "second sight" images.

A white light image might be used to create a grid that matches well to the specimen. Basically, the location of each well must be established so that data can be extracted from it. Small errors superposition errors between the well and sampling aperture could prove serious. The above-described alignment procedure could be used if there are reasonably good intensities from the specimen. If not, the plate itself is used as a reference, by capturing an image of the plate under dim light. This image shows where all the wells are, and this positioning information can be used to guide data extraction.

SPECTRAL_0000469

Another form of second sight image is used to correct for variation in filling. That is, sample dispensing systems do not place the same amount of sample into each well. In some cases the dispensing error leads to large and irrelevant variations in observed intensities. To remove this error, a second fluor is dispensed. The intensity of the first fluor is related to the reaction of interest. The intensity of the second fluor is not related to the reaction of interest. Rather, its intensity should be the same from well to well. Variations in second fluor intensity can be used to correct out variations in dispensing.

At block 306, the second sight image is used to find the best matching grid, remove nonspecific background fluorescence, or to check filling consistency.

At block 308, an optional process is applied to correct for variations in measurement efficiency. This process, well-by-well calibration, can be used to correct measurements from uneven illumination and/or the collection efficiencies. Well-by-well calibration can also be used to measure directly the target substance concentration. The well-by-well calibration establishment is illustrated in Fig. 10. A second sight image, if available, can provide a confidence factor for each target.

At block 310, the system aligns the sampling grid to the specimen, measures all the targets in the specimen, and makes the data available.

At block 312, the measurements taken from each discrete target in the specimen array are decoded to different conditions. For example, a discrete target may be capable of assuming any of n conditions, and the process of block 312 could decode the sample at each probe to one of those conditions. The actual process is performed using a simple intensity criterion (e.g. target X is brighter than 100 and is placed in category 1. At the user's discretion, decoding of targets to conditions applies the process of statistical segmentation. In accordance

SPECTRAL_0000470

with this process at block 312, the actual levels at every target within the specimen array are measured. A mean and standard deviation are determined for the set of samples, and this results in a working statistical distribution. Each discrete target could then be decoded to a category, by using its position within the distribution of all targets. Typically, this decoding is based upon the target lying a calculated number of standard deviations from the mean. For example, a target that lies more than 5 standard deviation units from the mean might be decoded to category 1.

At block 314, a process is performed to generate a report of the array data, based upon the process performed at block 312. It is contemplated that this may be any form of report writing software which provides the operator a substantial amount of flexibility in preparing reports of a desired format. Once the reports are generated, the data analysis process ends.

Figure 8 is a flow chart illustrating the process performed for image acquisition and in aligning sampling grids to target arrays.

After initiation of the process, a specific image is acquired. Known processes exist for acquiring bias images of a specimen. Such bias images take into account all significant distortions and errors introduced by the system itself when an image is taken. At blocks 320, 322 and 324 the bias images for the specific, nonspecific and second sight images are acquired. These bias images are acquired by utilizing one of the known methods. Once the bias images are acquired, they are stored and used from then on, unless the system environment changes as a result of a light bulb replacement, etc.

At block 502, the specimen specific image is acquired and the bias image is removed at block 508.

At block 504, the specimen nonspecific image is acquired. This image determines the contribution of non-specimen components, such as the support substrate, to the

SPECTRAL_0000471

image. This step is indicated as optional, since it would only be performed in the event that the specimen had to be illuminated in order to acquire the specimen image, in which event some light would also be reflected from non-specimen elements. On the other hand, if the specimen were the source of the light for the image (as in chemiluminescence), the non-specific image would not be acquired. The nonspecific bias image is removed at block 510.

At the block 506, the specimen second sight image is acquired and the bias images are removed at block 512. This yields the second sight image referred to in block 304.

If the specimen nonspecific image is available, it is removed from the specimen specific image at block 514. This yields the specific image referred to in block 302.

At the block 516, if the second sight image is not available, the specific image is used to create the matching grid. If the second sight image is available and has been acquired for grid alignment, the second sight image will be chosen. Otherwise, the selection can be done based on some known criteria, or the selection can be simply done by examining the images.

At the block 518, the system can automatically select one of a plurality of predefined default grids (330) utilizing known criteria, or a user can manually create a new default grid. The "default grid" represents a grid containing an array of probe templates with uniform row and column spacing.

At block 520 a grid alignment process, discussed more fully below, is performed to align all the probe templates to the corresponding targets. The probe templates can be aligned to their corresponding targets by directly aligning each template to its targets if the most of the targets contain detectable signals or a second sight image is used. Otherwise, the operator can define an array of "anchor points", which will aid the system in locating the probe template positions. It

SPECTRAL_0000472

will be appreciated that, in some instances, it may be desirable to align the array using both methods.

At the end of this process, a specimen specific image, a matching grid and a second sight image (optional) have been generated.

The target or anchor alignment processes are essentially the same. In the case of anchor alignment, once the anchors are aligned, the system uses a known regression method to align the rest of probe templates to the aligned anchor points.

Figure 9 is a flow chart illustrating the process performed in block 520 of Fig. 8.

After initiation of the process, image background and noise are estimated at block 602. At block 604, a determination is made whether a group alignment of the grid to the array of targets is necessary. This could be done either visually by an operator or by the system. The purpose of this test is to determine whether the grid is aligned to the targets overall. If done by the system, it would be performed by a conventional procedure for testing alignment of two regular patterns or shapes. If it is determined that adequate alignment of the group exists, control is transferred to block 608.

At block 606, a group alignment is performed. The purpose of this operation is to align the probe template grid roughly with the respective targets. The alignment may be done on the basis of the whole grid or part of the grid selected by the operator. This alignment could be done by the process discussed below with respect to block 608 for maximizing CS, except that CS is maximized over the entire grid.

At block 608, a step-wise process is performed within the area of each individual probe template to locate that point which yields a maximum confidence score (CS), within the probe template as determined by equation (1):

SPECTRAL_0000473

$$CS(x0,y0) = (1-a)\,N_f \int_{S(x0,y0)} D(x,y)\,W(x-x0,y-y0)\,dxdy + a\frac{\int_{S)x0,y0)} D[(x,y)-\bar{D}]\,[W(x-x0,y-y0)-\bar{W}]\,dxdy}{\sqrt{\int_{S(x0,y0)} [D(x,y)-\bar{D}]^2 dxdy \int_{S(0,0)} [W(x,y)-\bar{W}]^2 dxdy}} \quad (1)$$

where:

a	is a weighting (shape) factor in the range of [0, 1];
N_f	is a normalization function, converting a value in the range of (-∞, +∞) to a new value in the range of [0, 1];
(x0,y0)	is the center point of a probe template;
S(x0,y0)	is the probe template area at (x0,y0);
D(x,y)	is the density value (e.g. brightness) at (x,y); and
W(x,y)	is a weighting function (e.g. a two-dimensional Gaussian function with its maximum value at (0,0)).
$\bar{D}$	is the average value of density within S(xo,yo).
$\bar{W}$	is the average value of the weight function.

The first term in equation (1) represents a score for the pixel density values within a discrete target, whereas the second term represents a score for density profile matching. The density profile will match its expected shape closely as noise decreases, so the second term is very sensitive to the presence of noise and other artifacts within the specimen contained in the probe template.

The first and second terms of equation (1) are added in weighted fashion based on the weighting factor, a, which is a shape weighting factor. Preferably, the weighting factor is set by the system on the basis of estimated noise parameters. Alternatively, the weighting factor may have a default value and

can be adjusted downward by the operator in the event that the process of Fig. 9 does not yield good results.

Equation (1) yields an "A location" for each probe template, which is that location that provides the maximum value in equation (1). The probe template location prior to block 608 will be referred to as the "G location."

At block 610, a comparison is performed between the A location and G location, in order to arrive at the final location of the center of each probe template. In effect the weighting factors are utilized to determine the position of the probe center along a path between the A and G locations, with weighting determining how close the point is to the A location. Preferably, this done in a step-wise fashion by moving from location G to the location having the highest value for equation (1), only if that value exceeds a predetermined threshold confidence criterion, such as exceeding the value of equation (1) at the G location by a predetermined amount or proportion. As the threshold criterion is increased, movement from location G becomes less likely.

Figure 10 illustrates the process of block 308 for establishing well-by-well calibrations for measurement efficiency. At block 702, the system acquires a set of reference plates to be used for the calibration. The processes of imaging reference plates, and of aligning sampling grids to the reference plates are as shown in figures 7-9.

Each well in a reference plate is filled with the same or a known concentration of a substance. Each plate in the set of reference plates has a different concentration of the substance. Typically, the substance will have fluorescence or luminance characteristics similar to those of the substance used to label the reaction of interest.

At block 704, numerical data are obtained for the gray level value of each well, in each reference plate. As a result of block 704, computer 28 has a set of data pairs (gray level value and concentration value for each reference plate)

SPECTRAL_0000475

for each well. For example, well #1 yields a gray level value of 100 in reference plate 1 with concentration 10, of 200 in reference plate 2 with concentration 20, etc.

At block 706, the calibration for each well is created by fitting a regression, interpolation or other function to the data pairs associated with that well. As a result, each and every well position is calibrated to the relation between observed gray level value, and concentration in a well plate. This calibration may then be used in classifying and reporting data as shown in blocks 312-312 of figure 7.

Although the detailed description describes and illustrates preferred embodiments of the present invention, the invention is not so limited. Modifications and variations will be apparent to persons skilled in this art which do not depart from the scope and spirit of the invention as defined by the appended claims.

SPECTRAL_0000476

WE CLAIM:

1. A method for use in a digital imaging system for assays to extract targets on a specimen containing an array of targets that may not be arranged in perfect regularity, said method comprising the steps of:

defining a matrix of nominal target locations including a probe template of predefined, two-dimensional size and shape at each of a plurality of fixed, predefined grid points on the specimen; and

determining the most probable location of the probe template corresponding to a specific target by sensing both pixel intensity and the spatial distribution of pixel intensities in an image of the specimen at a plurality of locations in the vicinity of a nominal target location.

2. The method of claim 1, further comprising the step of, using a confidence value indicative of reliability of the most probable location as a weighting factor, shifting the location of the probe template from the nominal target location towards the most probable location for the selected target.

3. The method of claim 2, wherein said determining step is performed iteratively for each target.

4. The method of claim 2, wherein said determining step determines the confidence score, CS(x0,y0), at said plurality of locations, defined by the following formula:

$$CS(x0,y0) = (1-a)\,N_f \int_{S(x0,y0)} D(x,y)\,W(x-x0,y-y0)\,dxdy + a\,\frac{\int_{S)x0,y0)} D[(x,y)-\overline{D}]\,[W(x-x0,y-y0)-\overline{W}]\,dxdy}{\sqrt{\int_{S(x0,y0)} [D(x,y)-\overline{D}]^2 dxdy \int_{S(0,0)} [W(x,y)-\overline{W}]^2 dxdy}}$$

SPECTRAL_0000477

where:

a	is a weighting factor in the range of [0, 1];
N_f	is a normalization function, converting a value in the range of (-∞, +∞) to a new value in the range of [0, 1];
(x0,y0)	is the center point of a probe template;
S(x0,y0)	is the probe template area at (x0,y0);
D(x,y)	is the density value (e.g. brightness) at (x,y); and
W(x,y)	is a weighting function (e.g. a two-dimensional Gaussian function with its maximum value at (0,0)).
$\bar{D}$	is the average value of density within S(x0,y0).
$\bar{W}$	is the average value of weight function.

the most probable location being the one which maximizes the formula.

5. The method of claim 4, wherein the image used in said deriving step is produced by generating a primary image of the specimen showing to best advantage the effect of interest, generating a secondary image which shows minimally the effect of interest but which shows at least one of a second effect and the location of wells.

6. The method of claim 1, wherein said determining step determines the confidence score, CS(x0,y0), at said plurality of locations, defined by the following formula:

$$CS(x0,y0) = (1-a)\,N_f \int_{S(x0,y0)} D(x,y)\,W(x-x0,y-y0)\,dxdy + a\,\frac{\int_{S)x0,y0)} D[(x,y)-\bar{D}]\,[W(x-x0,y-y0)-\bar{W}]\,dxdy}{\sqrt{\int_{S(x0,y0)} [D(x,y)-\bar{D}]^2 dxdy \int_{S(0,0)} [W(x,y)-\bar{W}]^2 dxdy}}$$

where:

a	is a weighting factor in the range of [0, 1];
N_f	is a normalization function, converting a value in the range of (-∞, +∞) to a new value in the range of [0, 1];
(x0,y0)	is the center point of a probe template;
S(x0,y0)	is the probe template area at (x0,y0);
D(x,y)	is the density value (e.g. brightness) at (x,y); and
W(x,y)	is a weighting function (e.g. a two-dimensional Gaussian function with its maximum value at (0,0)).
$\bar{D}$	is the average value of density within S(x0,y0).
$\bar{W}$	is the average value of weight function.

the most probable location being the one which maximizes the formula.

7. The method of claim 6, wherein the image used in said deriving step is produced by generating a primary image of the specimen showing to best advantage the effect of interest, generating a secondary image which shows minimally the effect of interest and combining the secondary image with the primary image.

8. The method of claim 1, wherein said determining step is performed iteratively for each target.

9. The method of claim 8, wherein the image used in said deriving step is produced by generating a primary image of the specimen showing to best advantage the effect of interest, generating a secondary image which shows minimally the effect of

interest and combining the secondary image with the primary image.

10. The method of any one of claims 1-4, wherein the specimen is provided with predefined anchor points, the matrix being initially oriented relative to the actual target locations in the specimen by placing specific probe templates over one of: the anchor points; and those target points which are clearly definable.

11. The method of claim 1 wherein the confidence value for a target is determined by the detectability of the target.

12. The method of claim 4 or 6 further comprising the step of shifting the location of the probe template from the nominal target location towards the most probable location for the selected target only when the confidence value exceeds a predefined threshold value.

13. In a digital imaging system for assays, an apparatus for extracting targets on a specimen containing an array of targets that may not be arranged in perfect regularity, comprising:

a grid generator providing a matrix of nominal target locations including a probe template of predefined, two-dimensional size and shape at each of a plurality of fixed, predefined grid points on the specimen; and

a probe template locator, jointly responsive to pixel intensity and the spatial distribution of pixel intensities in an image of the specimen at a plurality of locations in the vicinity of a nominal target location, to determine the most probable location of the probe template corresponding to a specific target.

SPECTRAL_0000480

14. The apparatus of claim 13, further comprising a shifter, responsive to a confidence signal related to the reliability of the most probable location, to shift the location of the probe template from the nominal target location towards the most probable location for the selected target.

15. The apparatus of claim 14, wherein said probe template locator comprises a processor determining the integrated density, ID(x0,y0), at each of said plurality of locations, in accordance with the following formula :

$$ID(x0,y0) = aN_f\int_{S(x0,y0)} D(x,y)\,W(x-x0,y-y0)\,dxdy + (1-a)\,\frac{\int_{S)x0,y0)} D[(x,y)-\bar{D}]\,[W(x-x0,y-y0)-\bar{W}]\,dxdy}{\sqrt{\int_{S(x0,y0)}[D(x,y)-\bar{D}]^2 dxdy \int_{S(0,0)}[W(x,y)-\bar{W}]^2 dxdy}}$$

where:

a	is a weighting factor in the range of [0, 1];
N_f	is a normalization function, converting a value in the range of (-∞, +∞) to a new value in the range of [0, 1];
(x0,y0)	is the center point of a probe template;
S(x0,y0)	is the probe template area at (x0,y0);
D(x,y)	is the density value (e.g. brightness) at (x,y); and
W(x,y)	is a weighting function (e.g. a two-dimensional Gaussian function with its maximum value at (0,0)).
$\bar{D}$	is the average value of density within a valid weight function range.
$\bar{W}$	is the average value of weight function.

the processor selecting as the most probable location the one which maximizes the formula.

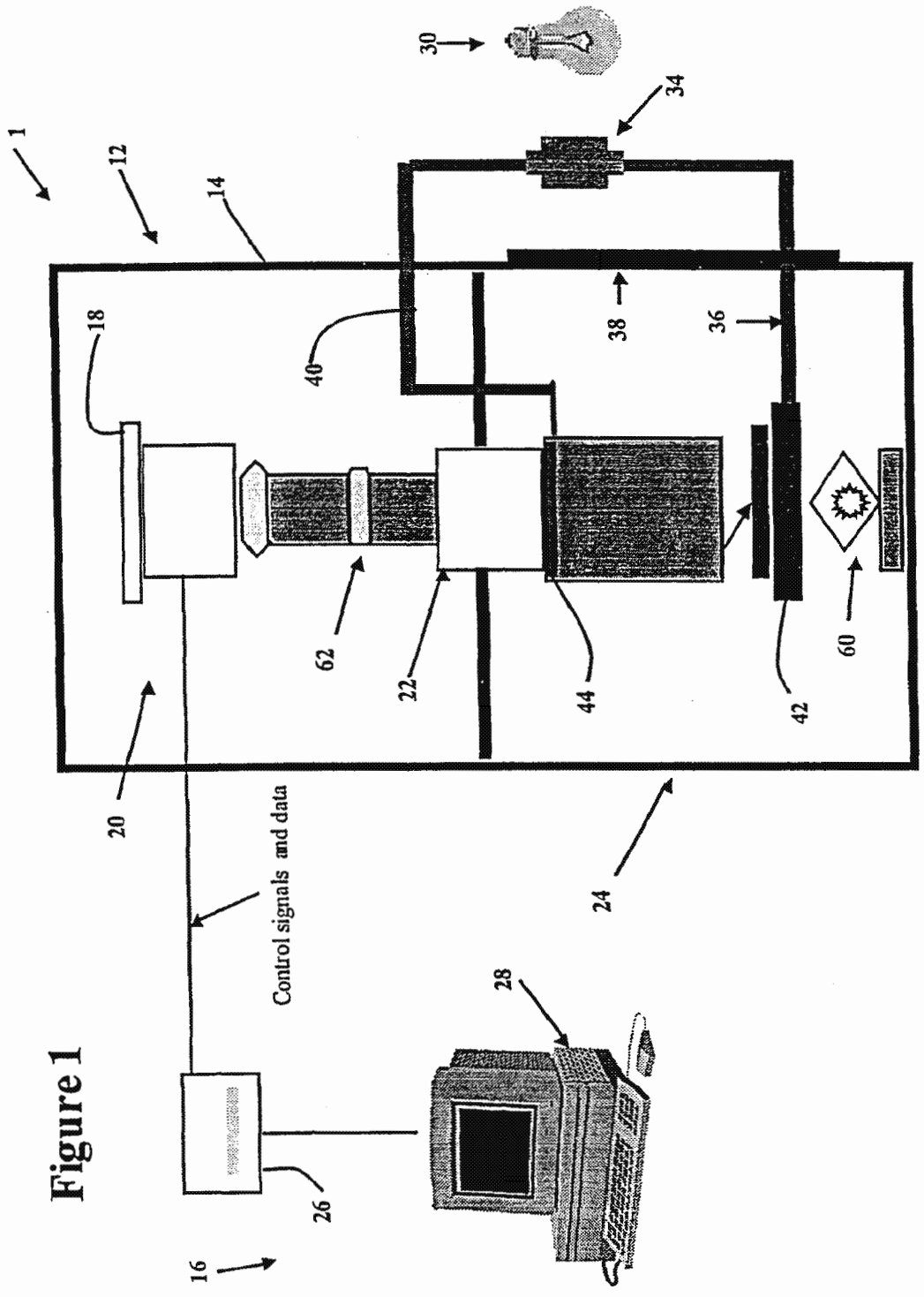


Figure 1

SPECTRAL_0000482

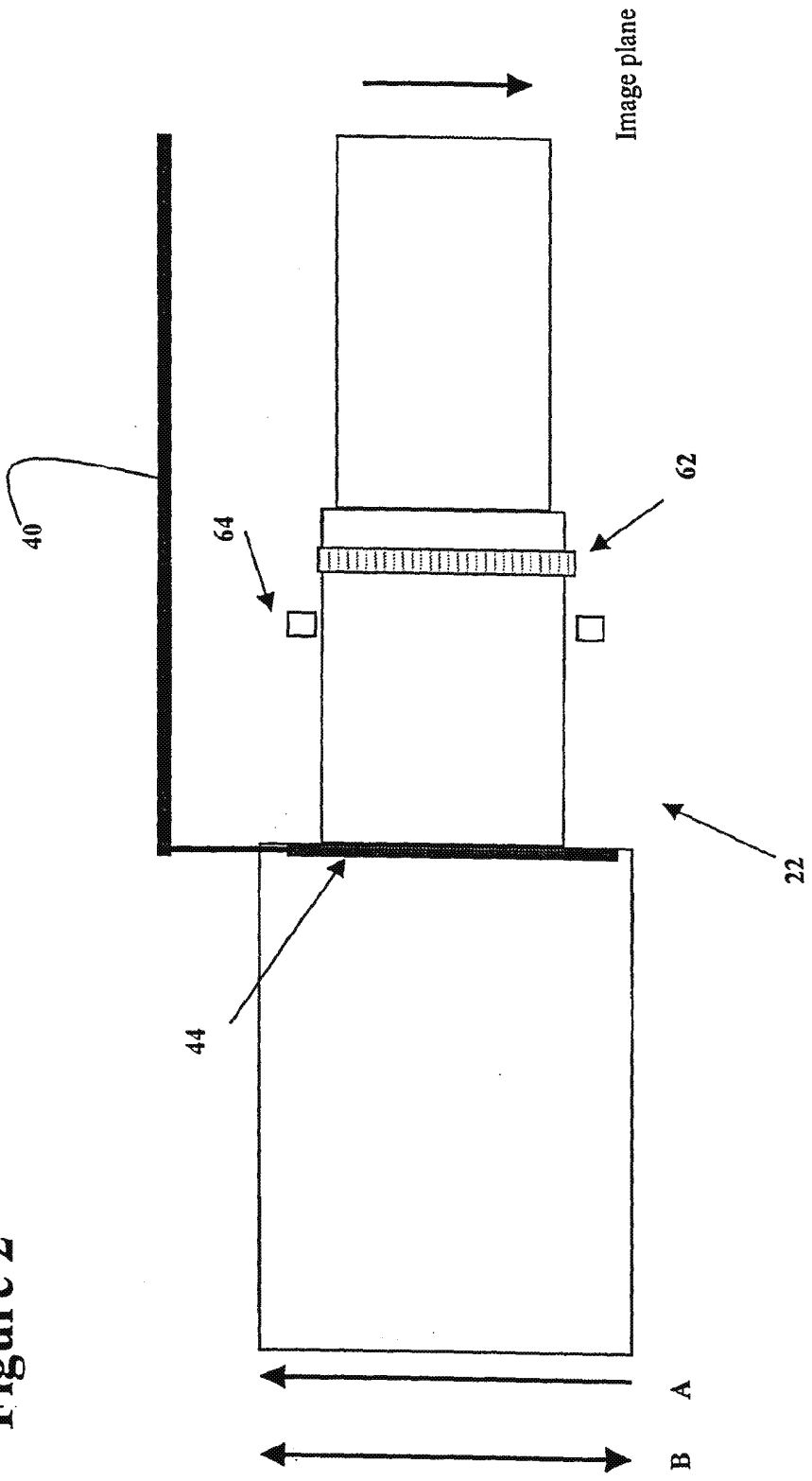
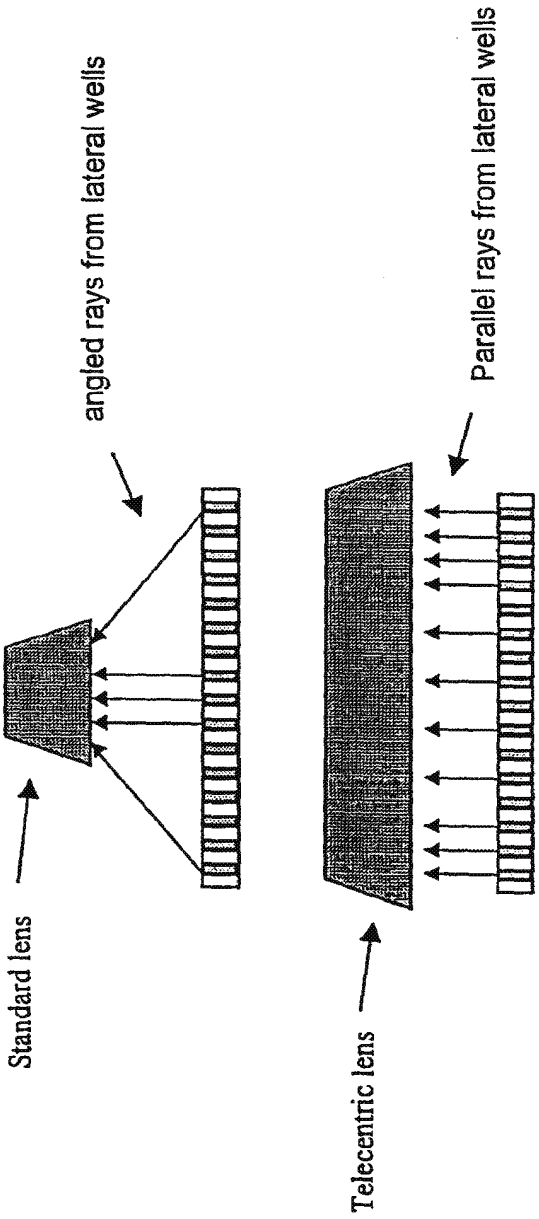


Figure 2

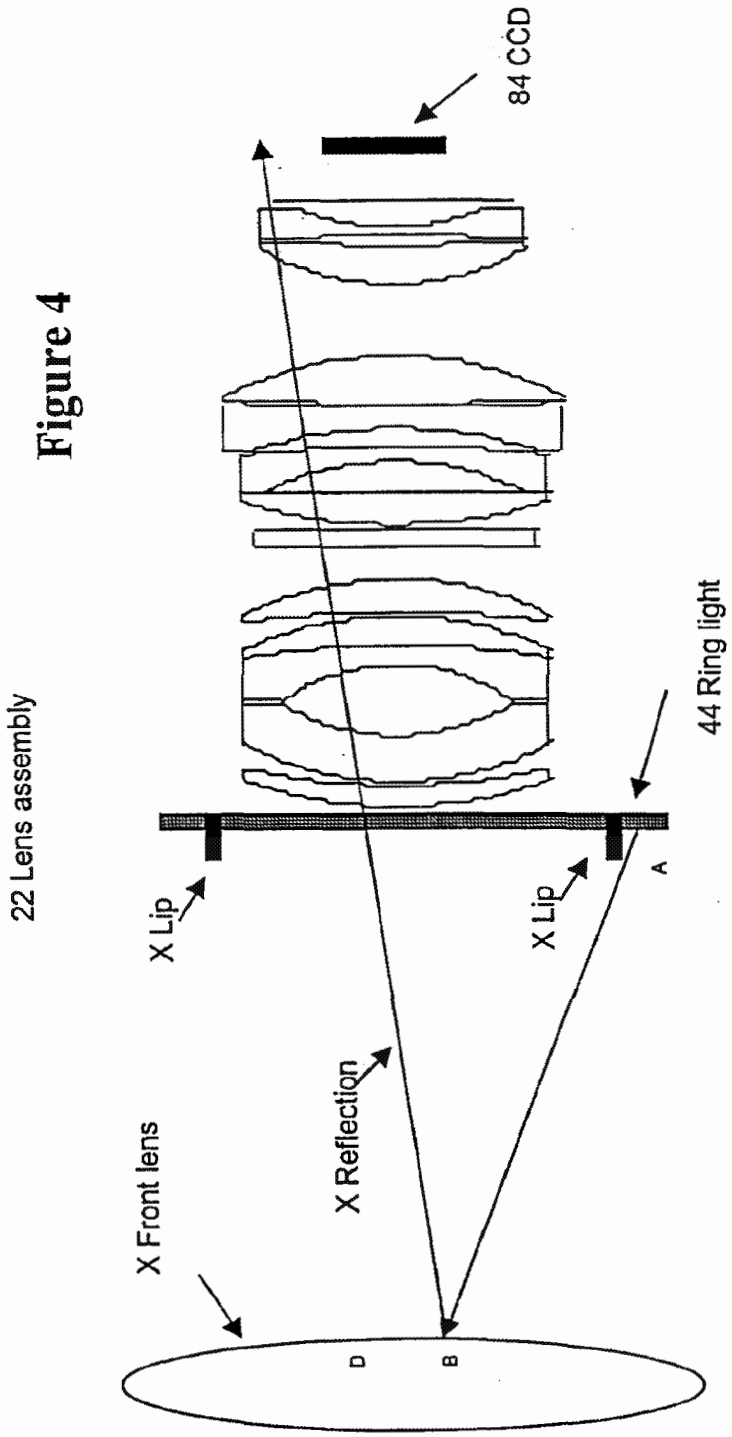
SPECTRAL 0000483

Figure 3



SPECTRAL 0000484

Figure 4



SPECTRAL_0000485

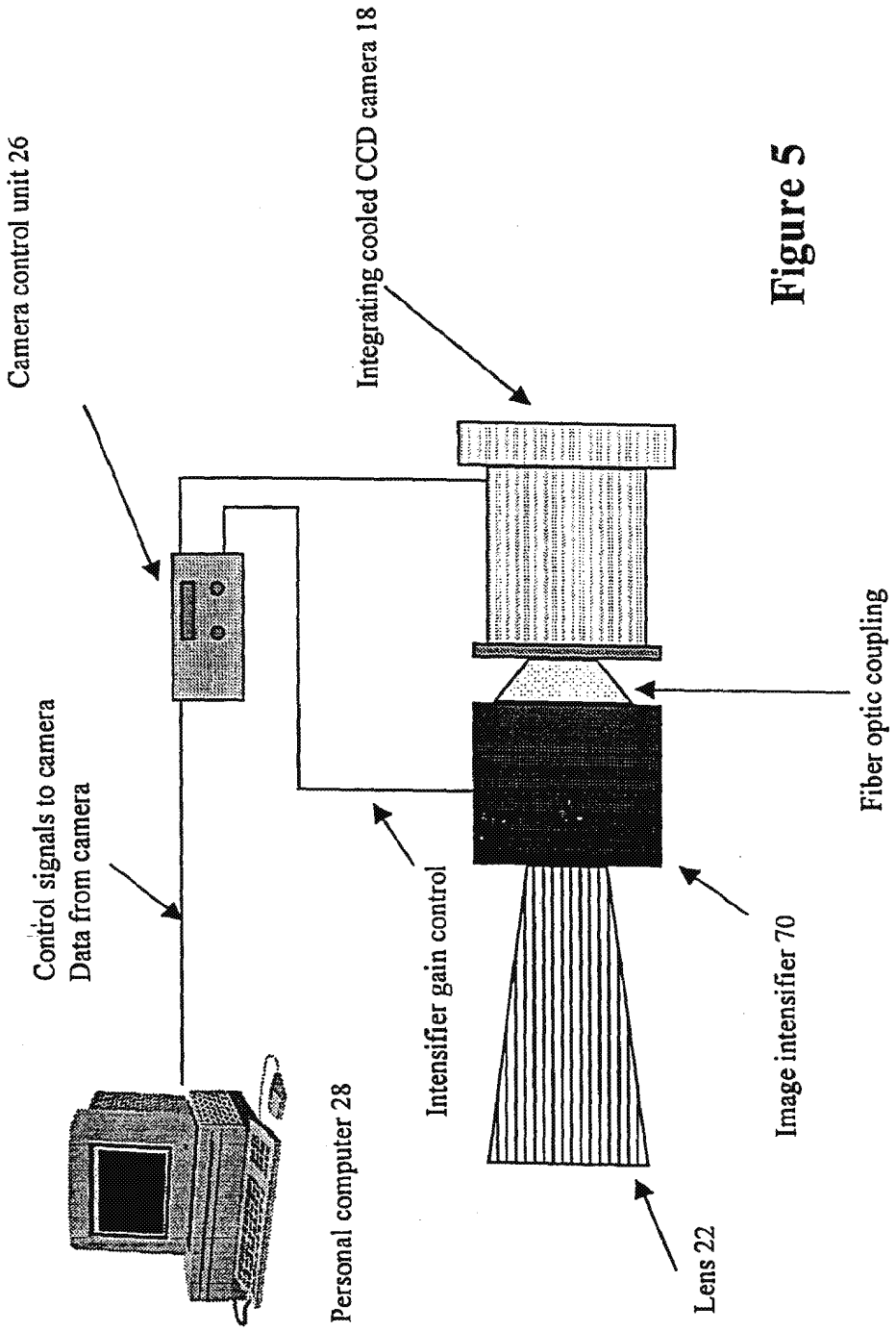

Camera control unit 26
Integrating cooled CCD camera 18
Control signals to camera
Data from camera
Intensifier gain control
Fiber optic coupling
Image intensifier 70
Personal computer 28
Lens 22

Figure 5

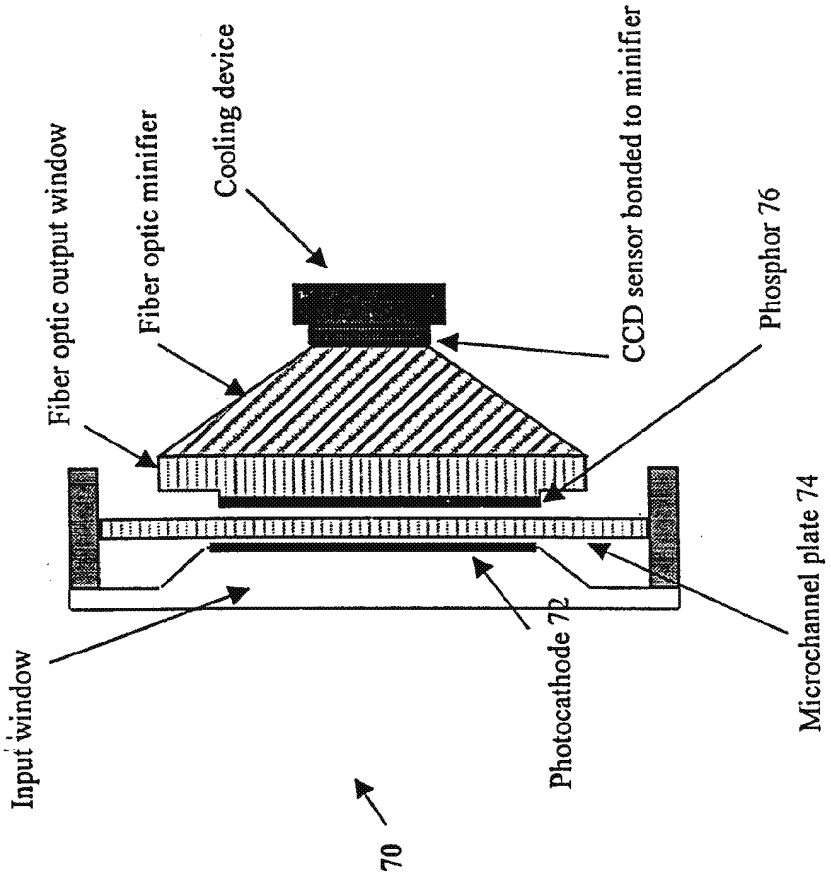

Fiber optic output window
Fiber optic minifier
Cooling device
CCD sensor bonded to minifier
Phosphor 76
Input window
Photocathode 72
Microchannel plate 74
70

Figure 6

Figure 7

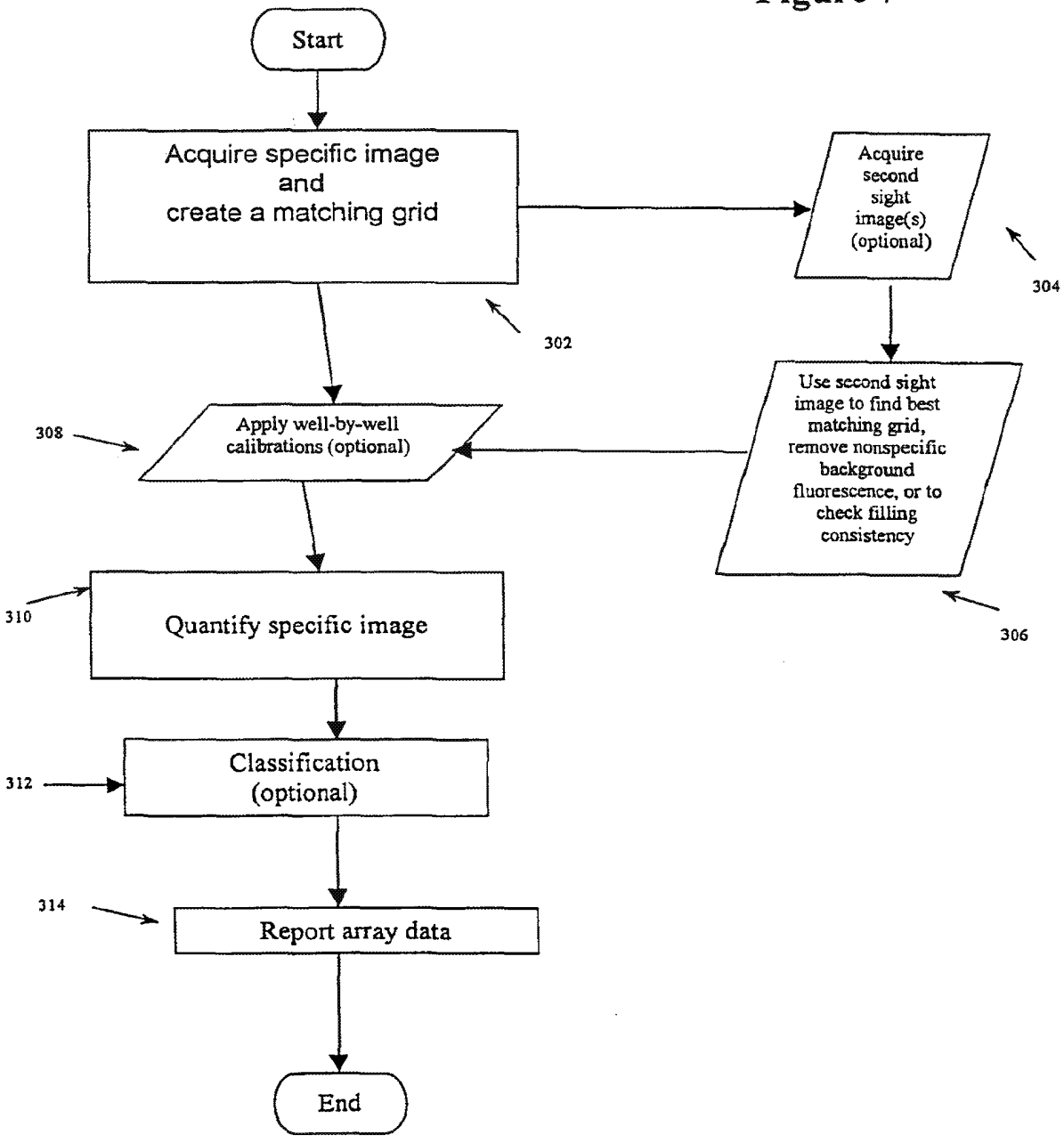
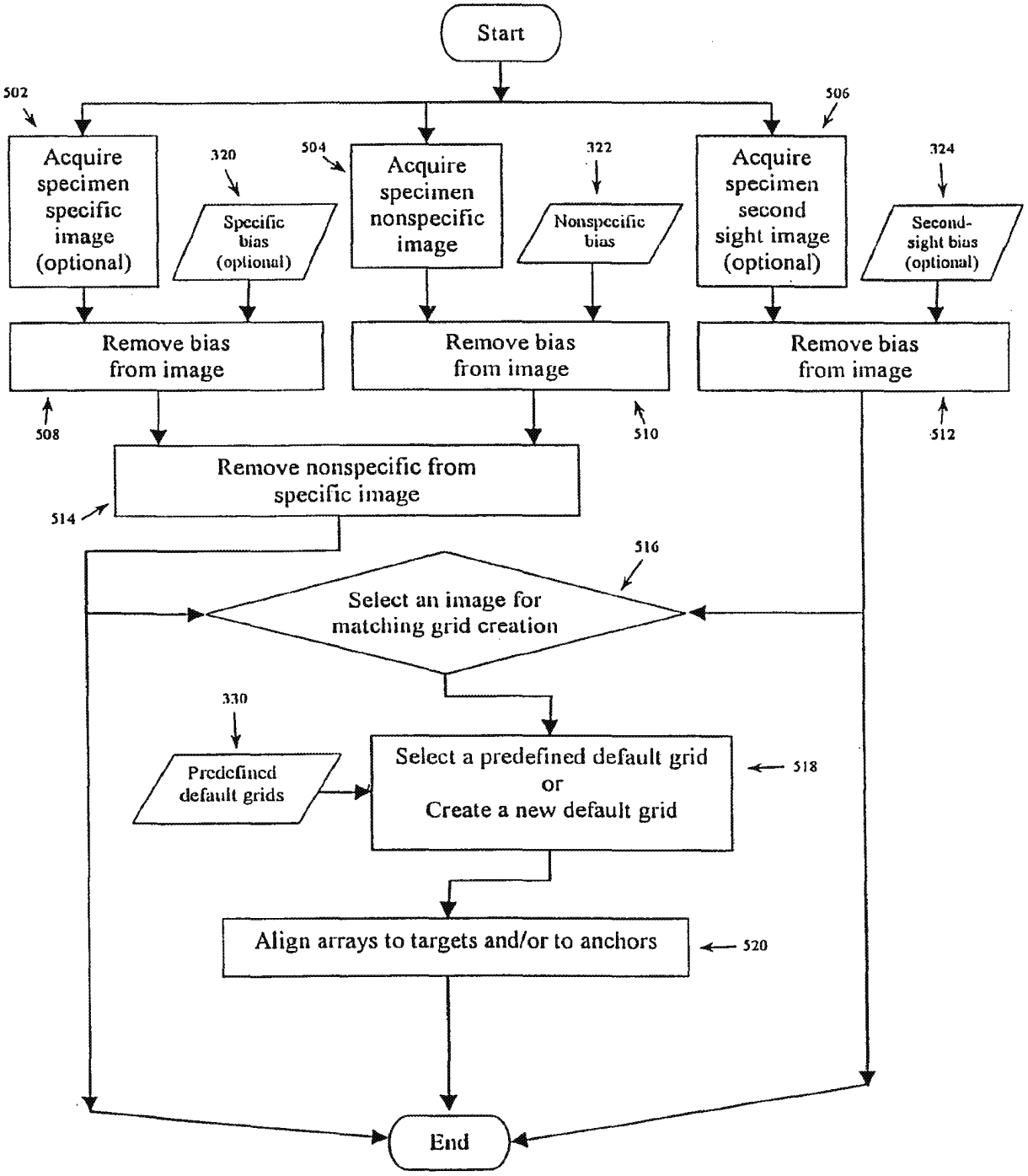

Start
Acquire specific image and create a matching grid
302
Acquire second sight image(s) (optional)
304
Use second sight image to find best matching grid, remove nonspecific background fluorescence, or to check filling consistency
306
308
Apply well-by-well calibrations (optional)
310
Quantify specific image
312
Classification (optional)
314
Report array data
End

Figure 8



SPECTRAL_0000489

Figure 9

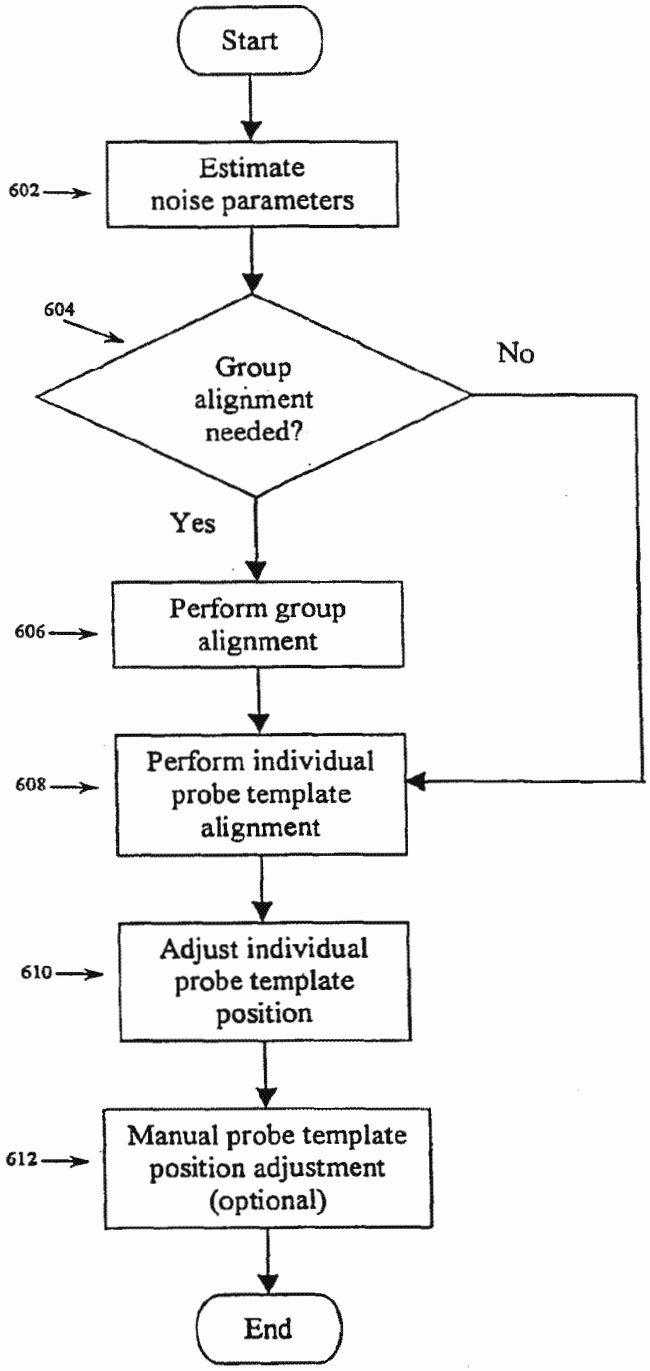
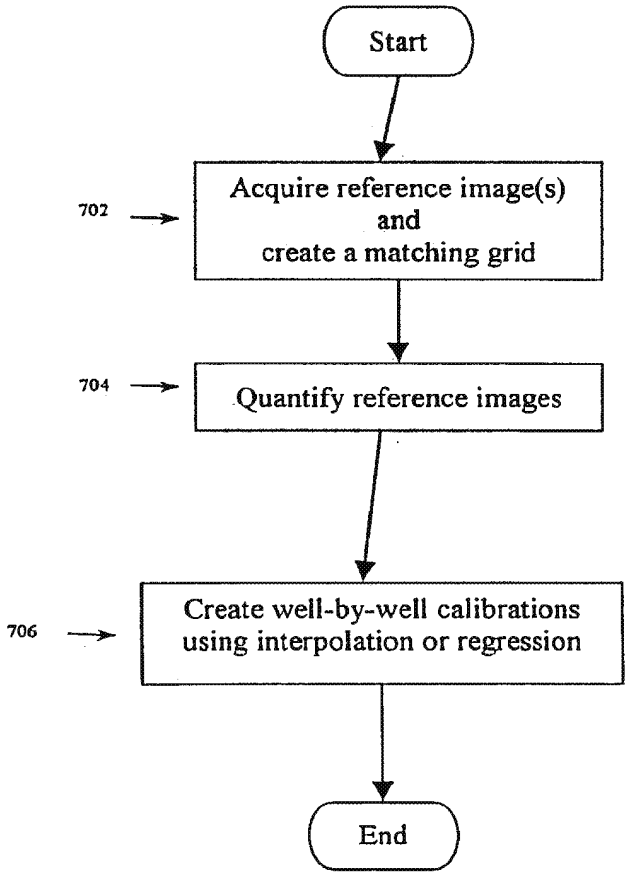

Start
Estimate noise parameters
602
604
Group alignment needed?
No
Yes
Perform group alignment
606
Perform individual probe template alignment
608
Adjust individual probe template position
610
Manual probe template position adjustment (optional)
612
End

Figure 10



SPECTRAL_0000491

INTERNATIONAL SEARCH REPORT

Ir. .ational Application No
PCT/CA 98/00762

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G06T7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G06T

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 98 11508 A (FEY STEPHEN ET AL.) 19 March 1998 see page 6, line 14 - page 7, line 30	1,13
A	WO 92 22879 A (ABBOTT LABORATORIES) 23 December 1992 see page 48, line 28 - page 50, line 29	1,13
A	US 5 592 573 A (CHRISTOPH EISENBARTH ET AL.) 7 January 1997 see column 6, line 9 - line 42	1,13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 December 1998	10/12/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chateau, J-P

1

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

SPECTRAL_0000492

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir. ational Application No
PCT/CA 98/00762

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9811508 A	19-03-1998	AU 4132597 A	02-04-1998
		AU 4569397 A	22-05-1998
		WO 9819271 A	07-05-1998
WO 9222879 A	23-12-1992	AU 2193492 A	12-01-1993
		AU 2250892 A	12-01-1993
		AU 2257592 A	12-01-1993
		AU 2259592 A	12-01-1993
		AU 2266892 A	12-01-1993
		CA 2109944 A	23-12-1992
		EP 0588931 A	30-03-1994
		EP 0588967 A	30-03-1994
		EP 0588968 A	30-03-1994
		EP 0588969 A	30-03-1994
		EP 0588972 A	30-03-1994
		JP 6507495 T	25-08-1994
		JP 6507496 T	25-08-1994
		JP 6507497 T	25-08-1994
		JP 6507498 T	25-08-1994
		JP 6507499 T	25-08-1994
		WO 9222800 A	23-12-1992
		WO 9222801 A	23-12-1992
		WO 9222802 A	23-12-1992
		WO 9222880 A	23-12-1992
		US 5275951 A	04-01-1994
US 5592573 A	07-01-1997	AU 4421593 A	10-02-1994
		CA 2100324 A	07-02-1994
		CN 1083646 A	09-03-1994
		EP 0582548 A	09-02-1994
		JP 6208618 A	26-07-1994

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01J 1/46 — A1

(11) International Publication Number: WO 94/00742

(43) International Publication Date: 6 January 1994 (06.01.94)

(21) International Application Number: PCT/GB93/01356

(22) International Filing Date: 29 June 1993 (29.06.93)

(30) Priority data:
9213737.1 29 June 1992 (29.06.92) GB

(71) Applicant *(for GB only):* HOLMES, Michael, John [GB/GB]; 15 Campion Road, Putney, London SW15 6NN (GB).

(71) Applicant *(for all designated States except US):* NYCOMED PHARMA AS [NO/NO]; Slemdalsveien 37, N-0301 Oslo (NO).

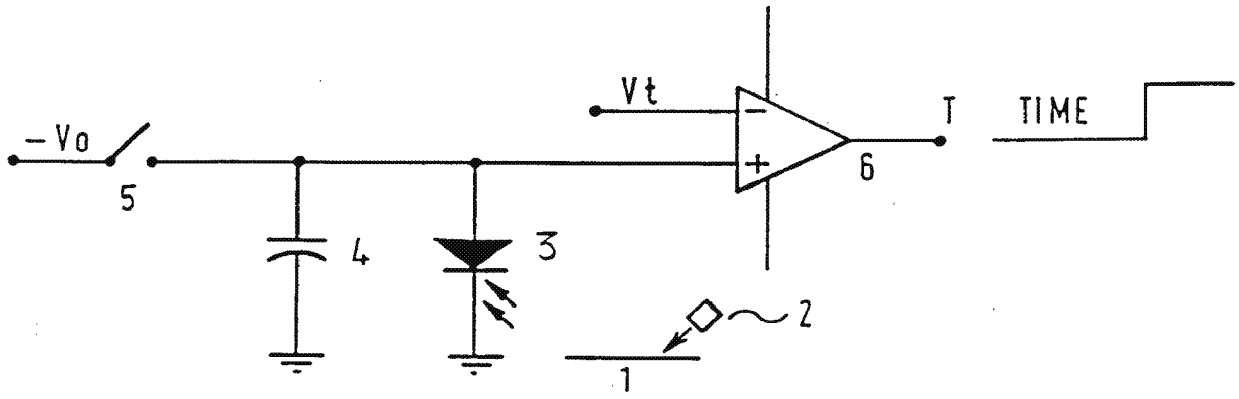
(72) Inventor; and
(75) Inventor/Applicant *(for US only) :* SEIM, Torstein [NO/NO]; Nedre Åsvei 63, N-1300 Sandvika (NO).

(74) Common Representatives: HOLMES, Michael, John et al.; Frank B. Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).

(81) Designated States: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: LIGHT MEASUREMENT



(57) Abstract

There is provided an apparatus for the measurement of reflected light comprising a low intensity light source (2) such as an LED and a light-dependent detection means (3) such as a photodiode, together with capacitative means (4) and means for charging said capacitative means (4) to a predetermined voltage, means for discharging said capacitative means through the light-dependent detector means (3) and means (6) for measuring the rate of discharge of said capacitative means.

SPECTRAL_0000494

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FR**	France	**MR**	Mauritania
AU	Australia	**GA**	Gabon	**MW**	Malawi
BB	Barbados	**GB**	United Kingdom	**NE**	Niger
BE	Belgium	**GN**	Guinea	**NL**	Netherlands
BF	Burkina Faso	**GR**	Greece	**NO**	Norway
BG	Bulgaria	**HU**	Hungary	**NZ**	New Zealand
BJ	Benin	**IE**	Ireland	**PL**	Poland
BR	Brazil	**IT**	Italy	**PT**	Portugal
BY	Belarus	**JP**	Japan	**RO**	Romania
CA	Canada	**KP**	Democratic People's Republic of Korea	**RU**	Russian Federation
CF	Central African Republic			**SD**	Sudan
CG	Congo	**KR**	Republic of Korea	**SE**	Sweden
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovak Republic
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LU**	Luxembourg	**TD**	Chad
CS	Czechoslovakia	**LV**	Latvia	**TG**	Togo
CZ	Czech Republic	**MC**	Monaco	**UA**	Ukraine
DE	Germany	**MG**	Madagascar	**US**	United States of America
DK	Denmark	**ML**	Mali	**UZ**	Uzbekistan
ES	Spain	**MN**	Mongolia	**VN**	Viet Nam
FI	Finland				

SPECTRAL_0000495

Light Measurement

This invention relates to an apparatus and method for carrying out light measurement, and in particular for measuring intensity of reflected light.

In reflectometers and similar apparatus for measuring the intensity of reflected light, an internal light-emitting means is provided in association with light-detection means so that a standard level of illumination is available. In larger reflectometers, relatively powerful light sources may be used, for example lasers, but for some uses, where portability of the device is important, a less powerful light source such as a light emitting diode (LED) is more appropriate.

However, when using LED's and similar low intensity light sources, in relatively simple hand held devices, it is important to use a sensitive means of light-detection which can readily be miniaturised into a compact form.

Many different types of electrical or electronic components are known, a characteristic of which, e.g. the resistance, varies in response to the light incident on the component, or a current is generated due to the illumination. As examples one can cite phototransistors, which are mostly used for detecting a light intensity above or below a certain threshold, and photodiodes which are used to determine a light of varying intensity. A number of circuit configurations are known which permit the light-dependent characteristic of such a component, e.g. a photodiode, to be exploited in order to produce a measurement of light intensity.

Fig. 1 illustrates one such known technique in which the photodiode D1 is connected to the inverting input of a conventional operational amplifier. In this

way the current generated in the photodiode by light incident thereon is converted to an output voltage V_{OUT} which may be used as a measurement of the light intensity. This method is satisfactory up to a point, but it suffers from the disadvantage that the current in the photodiode is low and requires high amplification. The need for high amplification introduces problems such as instability and drift, mainly due to temperature-related effects.

In an alternative approach, used for example in video-cameras (Fig. 2), the photodiode D2 is first charged to an initial voltage. Light incident on the photodiode D2 causes a current to flow that discharges the photodiode and reduces the voltage across it. This voltage is then transferred to the output of a Sample-and-Hold circuit (S/H). This constant output voltage may then be converted to a numerical value by an AD converter and processed in any chosen manner. Fig. 2 represents a simplified version, since a video-camera will have many photodiodes. A disadvantage of this method, however, is that the act of sampling may introduce noise into the signal. S/H circuits and AD converters also increase the cost of the equipment.

Thus there remains the need for a simple, effective and compact circuit arrangement for reliably measuring intensity of reflected light to a high degree of accuracy when the target is illuminated by a low intensity light source such as an LED.

Viewed from one broad aspect the present invention provides apparatus for the measurement of reflected light comprising a low intensity light source and light-dependent detection means together with capacitative means, means for charging said capacitative means to a predetermined voltage, means for discharging said capacitative means through the light-dependent detection means, and means for measuring the rate of discharge of said capacitative means.

With this arrangement, the intensity of the incident light is measured by detecting the rate of discharge of the capacitative means, which rate of discharge depends on the current flowing through the light-dependent detection means, which in turn depends on the intensity of the light thereon. This permits a simple, reliable and accurate determination of the light intensity to be made.

The low-intensity light source is preferably a light emitting diode (LED). These have the advantage of small dimensions, particularly suitable for hand-held reflectometers.

The light emission of LEDs and, indeed, some other light sources is linearly but inversely dependent on temperature over the range 0° to 100°C. While variations in light emission can in part be compensated by a control reading of a surface of standard reflectance, it is advantageous to provide a light source having reduced variability with temperature. It is thus advantageous to connect the LED to voltage correction circuit adapted to compensate for temperature variations. This can be achieved using a voltage regulator to supply a constant voltage to the LED via a resistor. We have found that an LED can be regarded as equivalent to a Zener diode in series with a resistor. The voltage across this Zener diode is constant for constant temperature but decreases with increasing temperature and the light output of the LED will also be decreased. This light output decrease will be partially corrected by keeping the current through the LED constant. However by appropriate setting of the voltage regulator connected to the LED via an appropriate resistor, the light output of the LED can be stabilised.

The discharge rate of the capacitative means may be determined in a number of ways, but it is preferred to provide comparing means whereby the time may be measured until the voltage across the capacitative means has

fallen to a predetermined level. A differential amplifier may serve as such a comparator with the voltage across the capacitative means being one input and a reference voltage being the other input.

The light-dependent element is preferably a photodiode whose conductance varies in response to an incident light, but any component may be employed that has an electrical or electronic characteristic that varies in response to incident light, e.g. a photo-transistor, a photovoltaic element, a photoresistor or an equivalent.

Viewed from another broad aspect the present invention provides a method for measuring reflected light intensity comprising illuminating a target surface with light from a low intensity light source, detecting light reflected from said surface with light-dependent detection means by charging capacitative means to a predetermined voltage, discharging said capacitative means through said light-dependent detection means, and measuring the rate of discharge of said capacitative means.

Preferably, the rate of discharge is determined by measuring the time taken for the voltage across the capacitative means to fall to a second predetermined value.

The present invention has a wide range of possible applications. A particularly preferred application, however, is as a reflectance meter for determining the colour and/or intensity of biomedical test cards which are subject to a colour change on application of a test sample depending on the contents of the test sample.

It should also be understood that although reference is made in this specification to the term "light", it is not intended that the invention be limited to visible light, but rather the invention may also extend to the non-visible parts of the electromagnetic spectrum.

An embodiment of the invention will now be described by way of example and with reference to the accompanying drawings, in which:-

Figs. 1 and 2 are circuit diagrams illustrating prior art arrangements as discussed hereinabove;

Fig. 3 is a circuit diagram showing an embodiment of the light detection system of the invention;

Fig. 4 is a graph showing measurement results of voltage drops across the capacitative means for different light intensities; and

Fig. 5 is a circuit providing stabilised light output from an LED.

Referring to Fig. 3, a target surface 1 is illuminated by an LED 2 and reflected light from the surface 1 falls on a photodiode 3. One side of capacitor 4 is earthed, the other is connectable via switch 5 to a source of a first predetermined voltage -Vo. Closing switch 5 causes capacitor 4 to be charged to voltage -Vo. Connected in parallel with capacitor 4 is the photodiode 3, the photodiode 3 being connected across the capacitor in the non-conducting direction relative to Vo polarity. The capacitor voltage is provided as the non-inverting input to a differential amplifier 6 acting as a comparator. A second predetermined reference voltage Vt is provided to the other, inverting, input of the comparator.

In operation, switch 5 is firstly closed to allow the capacitor 4 to charge to Vo. When constant reflected light is incident on the photodiode 3 to provide a constant value for the conductance and the switch 5 is opened, the capacitor discharges through the photodiode linearly with respect to time, the rate being proportional to the conductance and thus to the incident light intensity, as demonstrated in Fig. 4. The rate of discharge is detected by measuring the time taken for the capacitor voltage to fall to voltage V_t supplied to the other input to the comparator 6, such measurement

being made by any suitable conventional timing technique.

One possible application for the measurement circuit described above is for use in conjunction with a biomedical solid phase immunoassay test card, for example when using light measurement apparatus of the type disclosed in our co-pending application of even date entitled "Light Measurement Apparatus" the contents of which are incorporated herein by reference. Such test cards are designed to indicate the presence of a compound, e.g. a protein, in a liquid, e.g. blood or serum. A card is prepared with one or more test sites which change colour to a varying extent depending on the presence and amount of the particular compound concerned. Measurements of actual protein concentration can be made to a certain extent simply by eye by comparing the test site with a reference colour chart. This is, however, clearly unsatisfactory for accurate readings, and the present invention may be applied to an automated system in which the colour change is detected by measuring the reflectance of the test site.

By measuring the time T for the voltage to be reduced to V_t, the light intensity L is given by

$$L = \frac{1}{CT}$$ C being a constant.

When measuring reflected light from a sample, the reflectance r may easily be calculated by comparing the reflected light from the sample Ls with reflected light from a reference Lr;

$$r = Ls/Lr = Tr/Ts$$

where Tr and Ts are measured time for Lr and Ls respectively.

Possible sources of error in this technique are dark current Ld in the photodiode, and scattered and/or

SPECTRAL_0000501

reflected light Lc from other components, but these possible errors can be taken into account. The measured light Lm will consist of

$$Lm = Ls + Ld + Lc,$$

where Ld can be measured with the light source switched off, and Lc can be measured with the light on but the sample removed.

One of the advantages of the present invention over the prior art is that no additional equipment is required to convert the light intensity to a numerical value. Converting from time T to a number is done by the micro-computer in the apparatus simply by counting clock pulses generated by the processor's crystal oscillator. The high impedance of the capacitor/diode circuit, combined with a high input-resistance amplifier, will reduce temperature drift due to thermic resistance changes in the equivalent-resistance of the diode. However, the diode should have low dark current, compared to the light current used, since the dark current will vary with temperature. If photodiodes with I_d of about $20*10^{-12}$ A, compared to I_r of about 10^{-6} A, the dark current will lead to insignificant errors. Due to the small dimensions of modern LEDs and photodiodes, these can be placed close to the sample as done in the light measurement apparatus described in our co-pending application of even date, and thereby minimising the error due to I_d since I_r will be sufficiently large even when LEDs are used as light sources.

Fig. 5 shows a circuit in which an adjustable voltage regulator U1 is supplied with an input voltage V-IN and capacitors C1 and C2 and resistors R1 and R2 to provide a stabilised output voltage which is passed through a resistor R3 to the LED. As the voltage across the LED decreases with increased temperature, the voltage drop across the resistor R3 changes to produce

an increased current across the LED, thereby countering any decrease in light output due to temperature.

The invention provides advantages of simplicity in contrast to the prior art, enabling accurate measurements with reduced errors from, e.g. temperature changes. These advantages may be obtained without requiring high-intensity light sources.

Claims

1. Apparatus for the measurement of reflected light comprising a low intensity light source (2) and light-dependent detection means (3), together with capacitative means (4) and means for charging said capacitative means (4) to a predetermined voltage, means for discharging said capacitative means through the light-dependent detector means (3) and means (6) for measuring the rate of discharge of said capacitative means.

2. Apparatus as claimed in claim 1 in which the low intensity light source is stabilised.

3. Apparatus as claimed in claim 1 or claim 2 in which the low intensity light source is a light-emitting diode.

4. Apparatus as claimed in claim 3 in which the light-emitting diode is connected to a voltage correction circuit adapted to compensate for temperature dependent light output variations.

5. Apparatus as claimed in any of the preceding claims in which the rate of discharge of the capacitative means is determined by measuring the time for the voltage across the capacitative means to fall to a predetermined voltage.

6. Apparatus as claimed in claim 5 in which a differential amplifier is used to compare the voltage across the capacitative means with a reference voltage.

7. Apparatus as claimed in any of the preceding claims in which the light-dependent detection means is a photodiode.

SPECTRAL_0000504

8. A method for measurement of reflected light intensity comprising illuminating a target surface with light from a low intensity light source and detecting light reflected from said surface with light-dependent detection means by charging capacitative means to a predetermined voltage, discharging said capacitative means through said light-dependent detection means and measuring the rate of discharge of said capacitative means.

9. A method as claimed in claim 8 in which the light source is stabilised to produce light of constant intensity.

10. A method as claimed in claim 8 or claim 9 in which the target surface is a coloured surface on a biomedical test card.

FIG. 1.

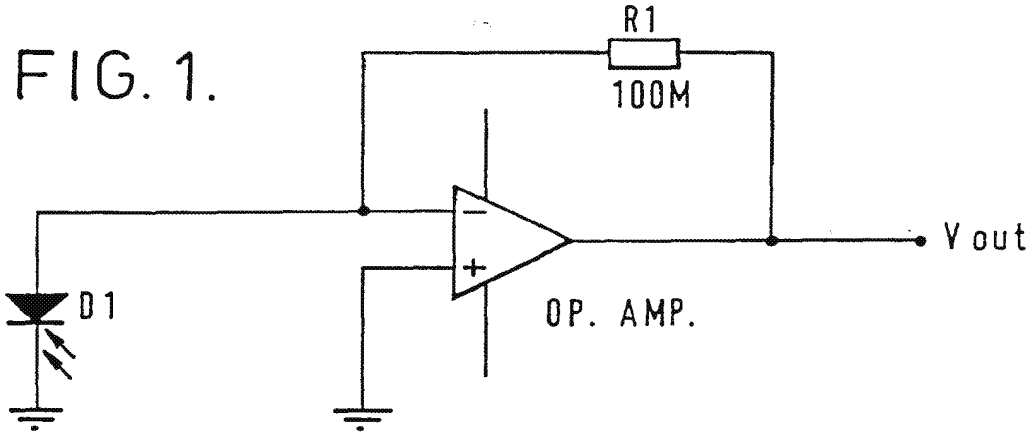


FIG. 2.

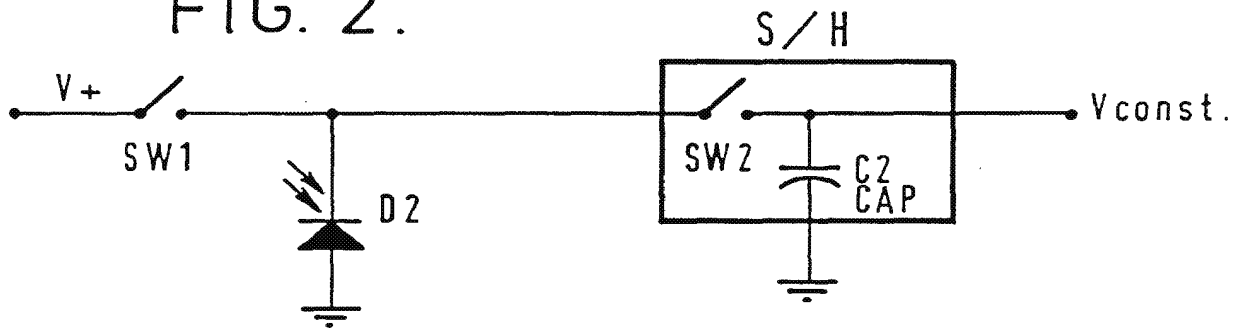
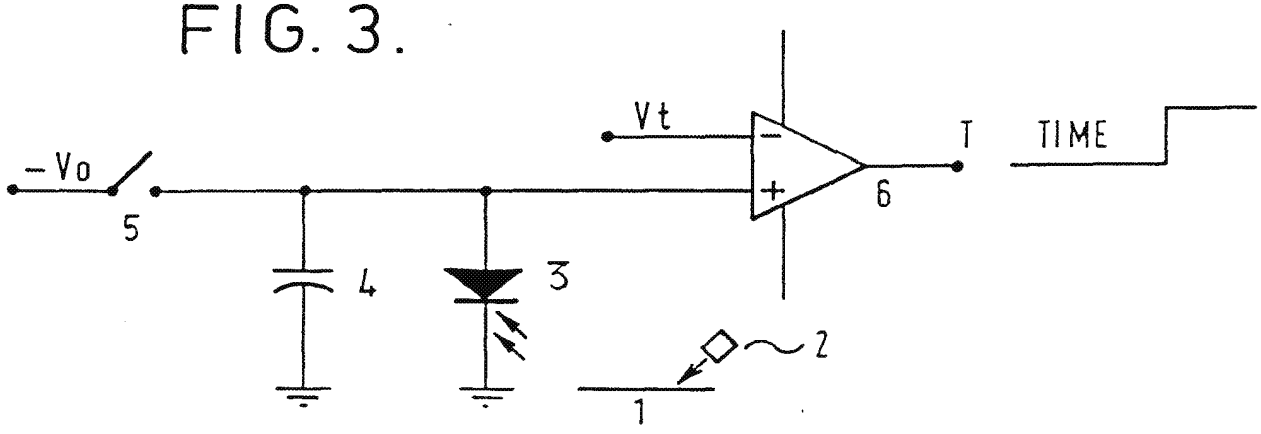


FIG. 3.



SUBSTITUTE SHEET
ISA/EP

SPECTRAL_0000506