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(54) **A SYSTEM COMPRISING A DUAL ILLUMINATION SYSTEM AND AN IMAGING APPARATUS AND METHOD USING SAID SYSTEM**

SYSTEM MIT EINEM DUALEN BELEUCHTUNGSSYSTEM UND EINER ABBILDUNGSVORRICHTUNG SOWIE DAS SYSTEM BENUTZENDES VERFAHREN

SYSTÈME COMPRENANT UN SYSTÈME D'ÉCLAIRAGE ALTERNANT ET UN DISPOSITIF D'IMAGERIE ET PROCÉDÉ UTILISANT LEDIT SYSTÈME

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- **VASILIS NTZIACHRISTOS ET AL: "Planar fluorescence imaging using normalized data", JOURNAL OF BIOMEDICAL OPTICS, vol. 10, no. 6, 20 December 2005 (2005-12-20), pages 064007-1-064007-8, XP055126295, ISSN: 1083-3668, DOI: 10.1117/1.2136148**

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Description

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation-in-part of a U.S. Patent Application entitled "BOTTOM FLUORESCENCE ILLUMINATION ASSEMBLY FOR AN IMAGING APPARATUS" by Nilson et al., filed June 17, 2005, U.S. Application Ser. No. 11/155,078; which in turn is a continuation of a U.S. Patent Application entitled "BOTTOM FLUORESCENCE ILLUMINATION ASSEMBLY FOR AN IMAGING APPARATUS" by Nilson et al., filed February 21, 2003, U.S. Application Ser. No. 10/372,763, which in turn 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. 10/189,886, now issued as U.S. Patent No. 6,894,289, 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 optical imaging systems, and more particularly, relates to fluorescent illumination sources and their associated components to illuminate targeted fluorescent probes in tissue.

BACKGROUND

[0003] One specialized type of imaging involves the capture of low intensity fluorescence from animal subjects such as mice. 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 1 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 fluorescent illumination needs required during image capture. Fluorescent image capture, of course, involves the sample being illuminated with an illumination source, while the minute amounts of light emitted from the "excited" sample are detected using a light detector, e.g., a CCD camera. Depending on the application, there are benefits to both epi-illumination (reflection) and trans-illumination for fluorescence imaging. Epi-illumination provides a faster survey of the entire animal, but is subject to higher levels of autofluorescence. Trans-illumination, on the other hand, provides lower levels of autofluorescence and is useful for performing 3D tomographic reconstructions. Therefore, it is desirable to have both epi- and trans-illumination options on a fluorescence imaging system.

[0007] It is also desirable to determine the 3D location and concentration of fluorescent probes in tissue. Diffuse tomographic reconstruction algorithms are often used for this purpose. To perform 3D diffuse tomographic reconstructions in fluorescence imaging, however, it is necessary to determine a 3D surface topography of the target specimen. The surface topography is used to define the boundary conditions within the diffuse tomography algorithm. One particularly suitable technique to determine the 3D surface topography of a specimen is through the application of structured light imaging. It would be desirable to provide a single imaging system that is capable of both structured light imaging, to obtain 3D surface topography, and fluorescent imaging, to perform 3D diffuse tomographic reconstructions of a specimen. The present invention describes a dual illumination system that accomplishes these objectives.

[0008] US2005201614 describes systems and methods for obtaining a three-dimensional (3D) representation of one or more light sources inside a sample, such as a mammal. Mammalian tissue is a turbid medium, meaning that photons are both absorbed and scattered as they propagate through tissue. In the case where scattering is large compared with absorption, such as red to near-infrared light passing through tissue, the transport of light within the sample is described by diffusion theory. Using imaging data and computer-implemented photon diffusion models, embodiments of the present invention produce a 3D representation of the light sources inside a sample, such as a 3D location, size, and brightness of such light sources.

[0009] WO2003/073079 describes a fluorescence imaging assembly including an imaging apparatus having an enclosure wall defining a view port into a light-tight imaging compartment thereof. A specimen platform is positioned in the imaging compartment having a support surface facing toward the view port. An illumination assembly includes a light source and a frame disposed in the imaging compartment. The illumination assembly is positioned proximate to and substantially peripherally en-

circling the view port. A bundle of fiber optic strands extends into the imaging compartment wherein proximal ends of the strands are in optical communication with the light source and distal ends thereof terminate at the frame to emit a conical directional beam of light onto the specimen platform. The distal ends of the fiber optic strands are sufficiently spaced peripherally about the view port such that the plurality of directional beams collectively illuminate the specimen platform in a substantially uniform manner.

[0010] An article by VASILIS NTZIACHRISTOS ET AL: "Planar fluorescence imaging using normalized data", JOURNAL OF BIOMEDICAL OPTICS, vol. 10, no. 6, 20 December 2005 (2005-12-20), pages 064007-1-064007-8, describes that fluorescence imaging of tissues has gained significant attention due to the emergence of appropriate reporter technologies that enable noninvasive sensing of molecular function *in vivo*. The article mentions epi-illumination (reflectance) imaging and trans-illumination.

DISCLOSURE OF INVENTION

[0011] The invention is defined in the accompanying claims.

[0012] Embodiments of the invention provide a system comprising a dual illumination system and an imaging apparatus. The imaging apparatus defines a light-tight imaging compartment with an interior wall having a view port extending into the imaging compartment. This view port enables data acquisition of a biological specimen contained in the imaging compartment. The dual illumination system includes a first illumination assembly configured to direct structured light imaging onto a first side of the specimen to enable structured light and surface topography measurements thereof. A second illumination assembly then directs excitation light at the specimen wherein diffused fluorescent light emanates from a surface thereof for receipt through the view port to acquire fluorescence data of the specimen.

[0013] Accordingly, a single imaging apparatus is provided that utilizes a dual illumination system capable of both structure light imaging and diffused fluorescent excitation light imaging. Hence, the structured light imaging is applied to determine the 3D surface tomography of the specimen, while the fluorescent excitation light imaging is applied to determine the fluorescence imaging data. Using this collection of data, 3D diffuse fluorescence tomographic reconstructions may be obtained.

[0014] According to the invention the second illumination assembly includes a trans-illumination device and an epi-illumination device. The trans-illumination device is configured to direct light into a first surface of the specimen wherein the diffused light exits a second surface thereof for receipt through the view port. The epi-illumination device is configured direct light onto a third surface of the specimen wherein the diffused light exits the third surface thereof for receipt through the view port.

[0015] More particularly, the trans-illumination device is configured to direct light into a bottom surface of the specimen wherein the diffused light exits a topside surface thereof for receipt through the view port. Further, the epi-illumination device is configured to direct light onto the topside surface of the specimen wherein the diffused light exits the topside surface thereof for receipt through the view port.

[0016] In another configuration, the epi-illumination device includes a first light transmission unit having a proximal end thereof in optical communication with an excitation light source and a distal end thereof terminating proximate the view port. The trans-illumination device includes a second light transmission unit having a respective proximal end thereof in optical communication with the excitation light source and a respective distal end thereof configured to direct the light into the bottom side surface of the specimen.

[0017] The trans-illumination device includes an illumination output device configured to cooperate with the distal end of second light transmission unit to emit a pinpoint beam of the light toward a window portion of a support platform in the compartment. The trans-illumination device further includes a translation mechanism supporting the illumination output device to selectively position the pinpoint beam of light at one of a plurality of positions adjacent the window portion.

[0018] In yet another specific embodiment, the epi-illumination device includes an illumination output end disposed directly into the imaging compartment, and positioned proximate to and peripherally encircling the view port such that the specimen platform is illuminated in a substantially uniform manner. The epi-illumination device includes a frame substantially peripherally encircling the view port, and adapted to support the output end of the illumination device. The epi-illumination device includes a bundle of fiber optic strands extending into the imaging compartment at the output end. The fiber optic strands have respective distal ends thereof terminating at the frame to emit a conical directional beam of light onto the specimen platform. The distal ends of the 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.

[0019] Another particular arrangement provides a first illumination assembly that includes a laser galvanometer device.

[0020] In another specific embodiment, the second illumination assembly includes an optical light switch positioned between an excitation light sources and the proximal ends of the respective first and second light transmission units. This optical light switch is configured to selectively direct the excitation light to one of the epi-illumination device and the trans-illumination device.

[0021] In particular, the light switch includes a housing defining an interior cavity. A proximal end of the first light transmission unit optically communicates with the cavity

along a first light path. Further, a proximal end of a second light transmission unit optically communicates with the cavity along a second light path, while a distal end of a third light transmission unit optically communicates with the cavity along a third light path. The light switch further includes an optical switch element that is selectively movable between a first position and a second position. In the first position, substantially all of a light passing through the third light transmission unit from the distal end thereof enters the proximal end of the first light transmission unit, while in the in the second position, substantially all of a light passing through the third light transmission unit from the distal end thereof enters the proximal end of the second light transmission unit.

[0022] In one specific arrangement, the first light path is in substantially linear, co-axial alignment with the third light path, and the second light path is in non-linear alignment with the third light path. Further, the switch element, in the first position, is positioned out of the optical path of the light transmitted in substantially linear, co-axial alignment substantially along the third light path to substantially along the first light path. In the second position, the switch element is positioned in the optical path of the light transmitted along the third light path and configured to optically redirect the light substantially in the direction the second light path and into the proximal end of the second light transmission unit. In particular, the switch element includes reflective element configured to reflect the excitation light in the direction from the third light path to the second light path, in the second position.

[0023] In yet another specific embodiment, the second light path is oriented substantially perpendicular to the first light path and the third light path. Further, the first light path, the second light path and the third light path are contained in substantially the same plane.

[0024] The switch element includes a cover device configured to cooperate with the proximal end of the second light transmission unit, in the first position, to substantially block the passage of light into the proximal end of the second light transmission unit from the housing cavity. The cover device further includes an aperture extending therethrough. When the switch element is oriented in the second position, the aperture is co-axially aligned with the second light path to enable the passage of the redirected light through the cover device and into the proximal end of the second light transmission unit.

[0025] The invention is also directed to a method for performing 3D diffuse fluorescence tomography reconstruction using the system of the invention. The method includes applying structured light to a surface of the specimen in the imaging compartment to determine the 3D surface topography thereof, and applying fluorescence excitation light to the specimen to acquire fluorescence imaging data thereof. The fluorescence excitation light may be directed toward the specimen in epi-illumination (reflection) mode or trans-illumination mode. The method further includes calculating the 3D diffuse fluorescence tomography reconstruction using the 3D surface topography data where the 3D surface topography obtained from structured light images acquired within the single imaging apparatus.

[0026] An imaging system is described for a specimen including an imaging apparatus defining a light-tight imaging compartment with an interior wall having a view port extending into the imaging compartment. The imaging system includes a support surface disposed in the compartment that is configured to support the specimen thereatop. The support surface further contains a window portion upon which light can pass therethrough. The imaging system further includes a trans-illumination device positioned adjacent the window portion. This trans-illumination device is configured to direct excitation light into a first surface of the specimen wherein diffused light passes therethrough and emanates from a second surface thereof for receipt through the view port to acquire fluorescence data of the specimen.

[0027] The trans-illumination device is positioned on one side of the window portion such that when the first surface of the specimen faces toward an opposite side of the window portion, the second surface of said specimen faces toward the view port.

[0028] The window portion is selectively sized and dimensioned such that the specimen, when supported atop the support surface, can be positioned and seated over the window portion to minimize light leakage around the specimen there between. The trans-illumination device is configured to emit the light in a beam toward the window portion and into the first surface of the specimen.

[0029] The trans-illumination device includes a low profile illumination output device configured to focus the excitation light in a pinpoint beam through the window portion and proximate to the first surface of the specimen. The trans-illumination device further includes a translation mechanism supporting the illumination output device, and is configured to selectively position the pinpoint beam of light at one of a plurality of positions adjacent the window portion.

[0030] Furthermore, a fluorescence illumination system is described for use with an imaging apparatus. The imaging apparatus defines a light-tight imaging compartment with an interior wall having a view port extending into the imaging compartment to enable data acquisition of a specimen contained in the imaging compartment. The illumination system includes a trans-illumination device configured to direct excitation light into a first surface of the specimen wherein diffused light emanates from a second surface thereof for receipt through the view port to acquire fluorescence data of the specimen. The illumination system further includes an epi-illumination device configured to direct excitation light onto a third surface of the specimen wherein the diffused light exits the third surface thereof for receipt through the view port to acquire fluorescence data of the specimen.

[0031] Accordingly, a single imaging apparatus is provided that is capable of both epi-illumination and trans-illumination. The epi-illumination system is applied to de-

termine the surface topography of the specimen, while the transillumination system is applied to excite the fluorescent reporter.

[0032] In one arrangement the trans-illumination device is configured to the direct excitation light into the first surface of the specimen when the first surface faces away from the view port, and wherein the diffused light exits the second surface of the specimen for receipt through the view port when the second surface faces toward the view port. The epi-illumination device is configured to direct the excitation light onto the third surface of the specimen wherein the diffused light exits the third surface thereof for receipt through the view port when the third surface faces toward the view port.

[0033] In another arrangement the illumination system includes a common, remote, excitation light source outputting the excitation light and an optical light switch selectively movable between a first position and a second position. In the first position, the outputted excitation light is directed to one of the epi-illumination device and the trans-illumination device, and in the second position, the outputted excitation light is directed to the other of the trans-illumination device and the epi-illumination device.

[0034] The epi-illumination device, in one arrangement, includes an illumination output end disposed directly into the imaging compartment, and positioned proximate to and peripherally encircling the view port such that the support surface is illuminated in a substantially uniform manner. The epi-illumination device further includes a bundle of fiber optic strands extending into the imaging compartment at the output end. This bundle includes distal ends thereof terminating at the frame to emit a conical directional beam of light onto the support surface. The distal ends of the fiber optic strands are sufficiently spaced peripherally about the view port such that the plurality of directional beams collectively illuminate the support surface in the substantially uniform manner.

[0035] A trans-illumination system is described for use with an imaging apparatus. The imaging apparatus includes a light-tight imaging compartment with an interior wall having a view port extending into the imaging compartment to enable viewing of a specimen supported on a support surface contained in the imaging compartment. The support surface includes a window portion that enables the passage of light there through. The trans-illumination assembly includes an illumination output device having an output end positioned proximate the window portion. The output device is configured to focus a beam of excitation light through the window portion and proximate to a first surface of the specimen. Diffuse light from within the specimen exits a second surface thereof for receipt through the view port. The trans-illumination assembly further includes a translation mechanism supporting the illumination output device. This mechanism is configured to selectively position the output end of the illumination output device at one of a plurality of positions adjacent the window portion such that the light beam impinges the first surface at one of a plurality of positions along the specimen.

[0036] The trans-illumination system includes a control system that is operably coupled to the translation mechanism for precise positioning of the output end relative to the window portion. The translation mechanism further includes an X-control arm and a Y-control arm that cooperate with one another to position the output end along the window portion.

[0037] A low profile, trans-illumination assembly is provided for trans-illumination of a specimen. The assembly includes a housing defining an interior cavity, and a light transmission device having a distal output end optically communicating an excitation light from an excitation source into the cavity generally in a first direction. A lens assembly is included having an input end optically communicating with the cavity. An output end of the lens assembly emits the excitation light there from focused in a substantially pinpoint beam generally in a second direction. The trans-illumination assembly includes an optical element disposed between the light transmission device and the lens assembly. This optical element is configured to direct a substantial portion of the excitation light exiting the transmission unit output end toward the input end of the lens assembly in a manner where the focused pinpoint beam enters a first surface of the specimen and exits as diffused fluorescent from a second surface thereof.

[0038] In one specific arrangement the optical element includes a reflective surface oriented to reflect the excitation light exiting the transmission unit distal end toward the lens assembly input end. Further, the first direction of the transmission unit output end and the second direction of the lens assembly are generally perpendicular to one another. In this configuration, the reflective surface is substantially planar, and oriented at about a 45° angle relative to the transmission unit output end and the lens assembly input end.

[0039] In another arrangement the lens assembly includes a plano-convex lens proximate to the input end thereof, and a bi-convex achromatic lens disposed at the output end spaced-apart from the plano-convex lens.

BRIEF DESCRIPTION OF THE DRAWING

[0040] The system 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.

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 an enlarged, side elevation view, in cross-section, of the filter wheel assembly of the illumination assembly of FIGURE 1.

FIGURE 4 is an enlarged, bottom perspective view of the imaging apparatus of FIGURE 1, and illustrating an alternative illumination assembly.

FIGURE 5 is an enlarged, bottom perspective view of the alternative illumination assembly of FIGURE 4 mounted to the upper interior wall of the imaging apparatus.

FIGURE 6 is an enlarged, bottom perspective view of a light dispersion assembly of the illumination assembly of FIGURE 4.

FIGURE 7 is an enlarged, side elevation view, in cross-section, of the filter wheel assembly of the illumination assembly mounted to the imaging apparatus.

FIGURE 8 is a top perspective view of an alternative bottom illumination assembly providing bottom illumination of the specimen constructed according to the present invention.

FIGURE 9 is an enlarged, top perspective view of the bottom illumination assembly of FIGURE 8 without the specimen thereatop.

FIGURE 10 is a schematic view of a system constructed in accordance with the present invention.

FIGURE 11 is a schematic view of the system of FIGURE 10 with an alternative embodiment trans-illumination device.

FIGURE 12 is a schematic view of the system of FIGURE 10 with another alternative trans-illumination device.

FIGURE 13 is an enlarged, side elevation view, in cross-section, of a low profile illumination output device of the illumination system of FIGURE 10.

FIGURE 14 is a reduced top perspective view of a translation platform movably supporting the low profile illumination output device of the illumination system of FIGURE 10.

FIGURE 15 is a top perspective view of X-arm and Y-arm of the translation platform of FIGURE 14, and illustrating a mounting bracket for mounting the output device to the Y-arm.

FIGURE 16 is an enlarged side elevation view of the low profile illumination output device of FIGURE 13 in an alternative application with a well plate cell.

FIGURE 17 is an enlarged top perspective view, partially broken away, of a switch element for the dual illumination system of FIGURE 10, shown in a first position.

FIGURE 18 is an enlarged top perspective view, partially broken away, of the switch element of FIGURE 17, shown in a second position.

FIGURE 19 is a top perspective view, partially broken away, of the switch element of FIGURE 17, shown in a first position.

FIGURE 20 is a top perspective view, partially broken away, of the switch element of FIGURE 17, and illustrating a lens of a third light transmission unit

FIGURE 21 is a side perspective view, partially broken away, of the switch element of FIGURE 17, shown in a second position.

FIGURE 22 is a front perspective view, partially broken away, of the switch element of FIGURE 17, shown in a first position.

FIGURE 23 is an enlarged, side elevation view, of an imaging apparatus incorporating a scanning laser galvanometer as the first illumination system.

FIGURE 24 is an enlarged, side elevation view, of a structured light source for the scanning laser galvanometer of FIGURE 23.

BEST MODE OF CURRYING OUT THE INVENTION

[0041] 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 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.

[0042] Referring to FIGURES 10-12, a dual illumination system 200 is described for use with the same imaging apparatus 21 (generally of FIGURES 1-9) described in detail below. Similar to those designs, the imaging apparatus at least defines a light-tight imaging compartment 25 with an interior wall 22 having a view port 23 extending therein. This view port 23 enables optical imaging data acquisition of a specimen contained in the imaging compartment. In this embodiment, the illumination system 200 includes a first illumination assembly, generally designated 201, that is configured measure

the surface topography of a specimen, and a second illumination assembly, generally designated 202, that is configured to measure the either general or tomographical fluorescence. Briefly, the first illumination assembly is configured to direct structured light onto a first side of the specimen to enable structured light and surface topography measurements and/or data acquisition thereof. In contrast, the second illumination assembly 202 is configured to direct light at the specimen 145 wherein diffused fluorescent light emanates from a surface thereof, preferably facing the view port 23, for receipt therethrough to acquire fluorescence data of the specimen.

[0043] Accordingly, a single imaging apparatus is provided with a dual illumination system, the first illumination assembly of which applies structured light imaging to determine the 3D surface topography, and the second illumination assembly of which applies fluorescent excitation light to excite the fluorescent reporter. This is advantageous in that the 3D surface topography and fluorescence emission measurements can be performed on a single system. These measurements can then be applied in a fluorescence tomography algorithm to provide 3D localization information for the fluorescent source.

[0044] Briefly, *In vivo* fluorescence tomography refers to the technique of determining the location and brightness of a fluorescent reporter within a living research animal. Since photons in the visible to near-infrared part of the spectrum are strongly scattered in tissue, tomography techniques in this wavelength range utilize diffusion models for photon transport. Typically, a tissue specimen is illuminated with excitation light at several different locations and an image of the fluorescent light emission is acquired for each illumination location. These images are fed into a diffuse tomography code, which then localizes the source from the image information. By moving the illumination source relative to the embedded fluorescent reporter, additional accuracy on the localization of the source is achieved.

[0045] Typically, the best sensitivity is achieved by illuminating the specimen in a trans-illumination geometry. In the present inventive system, the source impinges on the specimen from the bottom (away from CCD camera), and light emission is imaged from the top side (toward CCD camera). The trans-illumination geometry gives improved sensitivity because auto-fluorescence generated by the tissue is reduced compared to an epi-illumination (reflection) geometry.

[0046] A requirement for executing a diffuse tomographic reconstruction algorithm is knowledge of the specimen surface shape, or surface topography. In order to measure surface topography, a structured light technique is utilized. Here a grid of lines is projected onto the animal at an angle of 20-30 degrees to the CCD camera optical axis. An image of the deflection of the lines passing over the specimen can be analyzed to determine the surface topography.

[0047] FIGURES 1-9 will now be described in detail to provide the requisite background imaging assembly foundation that the dual illumination system works in conjunction with. Hence, referring now to FIGURES 1-2 and 4, 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 4) into a light-tight imaging compartment 25 thereof. A specimen platform 26 is positioned in the imaging compartment 25 that 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.

[0048] Briefly, FIGURES 1 and 2 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.

[0049] The illumination assembly 28 includes a frame 32 supporting the illumination device 30 that 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 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.

[0050] 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 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 of the fiber optic strands. To optimize the system for use in fluorescent image capture in accordance with the present invention, the material composition of the fiber optic strands is selected to have low auto-fluorescence properties. 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. The distal ends of each independent strand, terminating at the illumination device, emit a conical directional beam of light which collectively form the substantially uniform

conical beam (illustrated by broken lines 39) onto the specimen platform.

[0051] The direct light is provided by a bulb contained in the housing 45, and is 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.

[0052] 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.

[0053] 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.

[0054] Briefly, as best illustrated in FIGURES 2 and 3, 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 that has low phosphorescence.

[0055] The optical filters are typically interference-type filters that 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 that 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.

[0056] The housing, as viewed in FIGURE 3, 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 that 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.

[0057] 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 that 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 0,00635 m (1/4 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/illumination output device couples into the 0,0127 m (1/2 inch) diameter fiber optic bundle portion 51 in order to mate up with the ring light, which also as a 0,0127 m (1/2 inch) bundle size.

[0058] 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 3 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.

[0059] In another embodiment, 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.

[0060] 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 to about 0.8:1.

[0061] FIGURE 3 further illustrates that each plate member 75 defines a respective central aperture 72 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 against the abutting surface 71 of the collimating lens 67. Further, each aperture 72 has a respective transverse cross-sectional area smaller than that of the collimating lens abutting surface 71. However, each adjacent downstream plate member 75 defines a respective aperture 72 having a diameter incrementally larger than its adjacent upstream plate member 75. Preferably, the area of each successive downstream aperture 72 is about 10% to about 25% larger.

[0062] Referring now to FIGURES 4 5 and 7, another specific arrangement 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.

[0063] 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.

[0064] Referring now to FIGURE 6, 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.

[0065] 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 that is essentially parallel to the specimen platform 26.

[0066] 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.

[0067] 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 that 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 SPECTRALON®, which diffuses the reflected light as it impinges the surface thereof.

[0068] Referring now to FIGURES 8 and 9, in still another embodiment 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.

[0069] 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 specimen 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.

[0070] 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.

[0071] 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.

[0072] 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.

[0073] 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 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.

[0074] Similar to the dispersion assembly 110 of the top illumination assembly above, a reflector device 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 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) 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.

[0075] Referring now to FIGURES 10-12, the dual illumination system 200, upon which this application is primarily based, is provided for use with the same imaging apparatus 21 disclosed in the above-mentioned embodiments. Similar to those designs, the imaging apparatus at least defines a light-tight imaging compartment 25 with an interior wall 22 having a view port 23 extending therein. This view port 23 enables optical imaging data acquisition of a specimen contained in the imaging compartment. In this embodiment, the illumination system 200 includes a first illumination assembly, generally designated 201, that is configured measure the surface topography of a specimen, and a second illumination assembly, generally designated 202, that is configured to measure the either general or tomographical fluorescence. Briefly, the first illumination assembly is configured to direct structured light onto a first side of the specimen to enable structured light and surface topography measurements and/or data acquisition thereof. In contrast, the second illumination assembly 202 is configured to direct light at the specimen 145 wherein diffused fluorescent light emanates from a surface thereof, preferably facing the view port 23, for receipt therethrough to acquire fluorescence data of the specimen.

[0076] Accordingly, a dual illumination system is provided for an imaging apparatus. In the first illumination assembly, structured light is applied for surface topographic imaging of a specimen. By analyzing an image of structured light projected onto the specimen, the surface topography of the specimen positioned atop the specimen platform 26 in the imaging apparatus 21 can be constructed. The second illumination assembly 202 is then applied to fluoresce the specimen, as will be described. However, using the surface topographic data of the specimen determined by the first illumination assembly 201, in one embodiment, the surface topography of the specimen can be determined, relative to the support surface. Collectively, these two illumination assemblies cooperate with one another to provide the information necessary to perform a 3D diffuse fluorescence tomography and thereby localize the source of fluorescence within the specimen.

[0077] The first illumination assembly 201 applies a light source for 3-D imaging of a specimen. In particular, a scanning laser galvanometer is applied for structured light and surface topography determinations of the specimen, although the structured light source may be provided by a light projector (not shown) as well. For example, a projection device may be employed consisting of Ronchi ruling that is illuminated by an LED and optically projected onto the specimen.

[0078] Referring to FIGURE 23, however, scanning la-

ser galvanometer 300 is disposed at the top of imaging chamber 25 and reflects structured light 301 down onto a top surface of the animal. Referring to FIGURE 24, scanning laser galvanometer 300 comprises a laser 303 and a pair of mirrors 302a and 302b, and projects structured light onto the top surface of stage 26. The grid size produced on stage or platform 26 (or an animal resting thereon) will depend on position of stage 26 and control of each mirror according to a desired grid size.

[0079] Laser 303 generates light. Mirrors 302a and 302b each direct light provided by laser 303. The two mirrors cooperate to provide two degree of freedom control for positioning a light beam provided by laser 303. A maximum transmission field 304 defines the spatial range for direction of light by scanning laser galvanometer 90. Actuators 305a and 305b position mirrors 302a and 302b, respectively, and may create any line, shape, grid or pattern of light within field 304. For example, actuators 305 and mirrors 302a and 302b may form a set of parallel lines normal to the head to toe facing of a mouse (for any position of the mouse).

[0080] In general, the light output by a structured light source may include any lines or shapes suitable for generating structured light surface information that is useful in building a surface topography. In one embodiment, a structured light source transmits a grid of lines onto the animal. Spacing between lines in a parallel grid may be adapted to a specific object or image. A parallel grid of lines having line spacing in the range of about 0.2 to about 2 lines per mm is suitable for a mouse. Other line spacings are suitable for use with the present invention. The line spacing may vary based on the object surface texture and object size. Closer line spacing provides higher resolution.

[0081] As mentioned above photographic information may be used offset limitations of structured light at high resolutions, thus enabling even closer spaced structured light lines and more accurate surface representations.

[0082] Such a structured light system is detailed in our U.S. Patent Application S/N 11/127,346, filed May 11, 2005, by Rice et. al. and entitled "3-D IN-VIVO IMAGING AND TOPOGRAPHY USING STRUCTURED LIGHT", which is a continuation application of U.S. Application No. 10/606,976, filed June 25, 2003, by Daniel G. Stearns et al. and entitled, "METHOD AND APPARATUS FOR 3-D IMAGING OF INTERNAL LIGHT SOURCES". These applications also claim priority under 35 U.S.C. §119(e) from U.S. Provisional Applications No. 60/395,357, entitled "Method and Apparatus for 3-D Imaging of Internal Light Sources", by Daniel G. Steams, et al.; U.S. Provisional Application No. 60/396,458, entitled "In Vivo 3D Imaging of Light Emitting Reporters", by Bradley W. Rice, et al.; and U.S. Provisional Application No. 60/396,313, entitled "3D in Vivo Imaging of Light Emitting Reporters", by Bradley W. Rice, et al. These provisional applications were all filed on July 16, 2002.

[0083] The second illumination assembly 202, as mentioned, is applied for fluorescence imaging of the specimen. With the system of the present invention, either epi-illumination or trans-illumination techniques, or both, can be applied to fluoresce the targeted tissue of the specimen. In one specific embodiment, epi-illumination, which is preferably reflection-mode imaging, is provided by an epi-illumination device 203 (i.e., emitting white light or UV such as the above disclosed epi-illumination designs) is applied which involves reflecting an excitation light off the targeted surface of the specimen to generate contrast (absorption) for white light applications, e.g., colorimetric membranes (opaque); or excitation by filtered light (via filter wheel assembly 47) to measure fluorescence emission. By way of example, the above-mentioned epi-illumination device 203 may be applied by the light-ring embodiment 30 shown in FIGURE 2, or by the plurality of light dispersion assemblies 110, shown in FIGURES 4-7, disposed about the view port 23. Briefly, a remote excitation light source is optically coupled to the epi-illumination device 203 (or devices) through a first light transmission unit 205. As has been described, and as will again be described below, the first light transmission unit 205 is preferably provided by a plurality or bundle of fiber optic strands extending into the imaging compartment 25. Proximal ends of the strands are in optical communication with a remote excitation light source, while a distal end of the strands terminates at the epi-illumination device 203.

[0084] In accordance with the present invention, generally fluorescence imaging, or tomographic fluorescence imaging, of the second illumination assembly 202 may also be acquired by a trans-illumination technique, which involves transmitting an excitation light (i.e., UV through infrared light) through the specimen. Similar to the epi-illumination device, a trans-illumination device 206 emits excitation fluorescence energy in the UV range to near infrared range (about 400-900 nm) that causes fluorescence emission.

[0085] The trans-illumination device may be applied using the bottom trans-illumination assembly 140 shown in FIGURES -8 and 9. In this configuration, however, an entire surface section of the specimen that is placed over the window portion 142 is trans-illuminated simultaneously. While this is advantageous to observe large sections of the specimen simultaneously, it does not provide 3D localization (tomographic) data.

[0086] Accordingly, according to the present invention, the trans-illumination device 206 is provided by one or more point sources of light beams that are applied to pinpoint illuminate the specimen at strategic locations thereof. The fluoresced excitation light exits the specimen surface facing the view port 23, and is detected by the imaging sensor of the camera.

[0087] This pinpoint illumination technique is advantageous in that moving the illumination point relative to a fixed fluorescent source provides 3D tomographic localization information. However, since this trans-illumination design is only capable of pinpoint illumination, multiple point illuminations about the surface of the specimen

are necessary to properly scan the entire specimen. Hence, either the specimen and/or specimen platform 26 can be reoriented relative to a beam output end 210 of the trans-illumination device 206 or the beam output end is capable of reorientation relative to the specimen and/or specimen platform 26.

[0088] Referring now to FIGURES 10 and 13, in the preferred form, a trans-illumination device 206 is provided having a low profile illumination output device 207 coupled to a movable translation platform 208. This platform is capable of positioning the output end 210 of the illumination output device 207 at one of a plurality of positions adjacent a surface of the specimen. Briefly, the low profile footprint of the trans-illumination device 206 enables positioning thereof under the specimen platform 26 in a relatively tight access space. In turn, the overall height footprint of the imaging apparatus 21 can also be reduced or minimized.

[0089] The illumination output device 207 includes a housing 211 that defines an interior cavity 212 therein. To reduce the overall height footprint, the excitation light entering the illumination output device is redirected from one direction to another direction toward a window portion 213 of the specimen platform 26. After redirection of the excitation light, the output device 207 includes a lens assembly 215 to focus the redirected excitation light into a smaller beam of light onto a surface of the specimen 145.

[0090] As best viewed in FIGURES 10 and 13, the excitation light is transmitted to the cavity of the housing 211 through a second light transmission unit 216 and a remote excitation light source 37. Similar to the above-mentioned trans-illumination and epi-illumination devices, the light second transmission unit 216 is provided by a plurality or bundle of fiber optic strands having proximal ends positioned in optical communication with a remote light source 37, and distal ends terminating in the interior cavity 212 of the output device housing 211. The composition of the fiber optic strands is selected to minimize or to have low auto-fluorescence properties, such as those containing high purity fused silica (e.g., plastic clad fused silica or silica clad fused silica).

[0091] It is desirable to minimize overall height footprint of the illumination output device 207. Entering the cavity 212 of the housing 211 at a direction toward, and substantially perpendicular, to the window portion 213 would maximize the height footprint rather than minimize it. Moreover, due to the relative stiffness of the fiber optic bundle, the bending radius thereof is relatively large. Accordingly, the overall height footprint has been significantly reduced by optically redirecting the excitation light output from the distal ends of the second transmission unit 216 toward (vertically in this example) and in the direction of the window portion. Essentially, in this specific embodiment, the direction of the excitation light is optically redirected about 90° from a substantially horizontal direction, entering the housing 211, to a substantially vertical direction toward the window portion 213 of the specimen platform 26.

[0092] FIGURE 13 shows that the distal end of the light transmission unit 216 enters the housing 211 and terminates in the cavity 212 generally in a substantially horizontal orientation. Accordingly, the emission of the excitation light from the distal end of the fiber optic strands are also generally in the horizontal direction. To optically redirect the excitation light, an optical element 217 is positioned in the cavity 212, just downstream from the distal ends of the fiber optic strands, and in alignment with the exiting excitation light. Preferably, the redirection of the excitation light is about 90° from the generally substantially horizontal direction towards the substantially vertically direction so as to be substantially perpendicular to the window portion 213 of the specimen platform 26.

[0093] This reflective application structurally facilitates maintaining a low profile footprint of the illumination output device. To reflect the directional beams about 90° from the optical axis of the distal ends of the strands and generally perpendicular to the specimen platform 26, the relatively planar reflective surface 218 should be oriented at about 45° relative to the direction of the optical axis. This enables the entire fiber optic bundle (i.e., the second transmission unit 216) to enter the housing 211 at a substantially horizontal orientation that is essentially parallel to the specimen platform 26. It will be appreciated that depending upon the exact orientation of the optical axis relative to the desired orientation of the output beam along the specimen platform to be illuminated, the angle of the reflective surface can be altered accordingly.

[0094] In accordance with the present invention, the illumination output device 207 is configured to focus the redirected excitation light into a pinpoint beam, through the window portion 213 of the specimen platform, and onto a surface of the specimen. Accordingly, by positioning the pinpoint beam emitted from the output end 210 at a plurality of strategic locations about the surface of the specimen supported by the window portion, strategic trans-illumination can be performed as the diffused light fluoresces the targeted tissue and exits the opposite surface of the specimen.

[0095] To focus the excitation light manipulated by the optical element 217, as mentioned, a lens assembly 215 is disposed in the output device housing 211 in the path of the manipulated light. The lens assembly 215 includes a cylindrical base 204 with a cap member 204a at the output end, and opposite an input end 220 configured to mount to the housing 211. The lens assembly 215, briefly, collects the manipulated light reflecting from the reflective surface 218, in one embodiment, and then output the focused light from the output end 210 thereof.

[0096] FIGURE 13 best illustrates that the lens assembly 215 is comprised of a two lens system containing a proximal plano-convex lens 221 and a bi-convex achromatic lens 222 spaced-apart from the plano-convex lens 221. Briefly, the plano-convex lens 221 functions to collect the light reflected from surface 218, while the spaced bi-convex achromatic lens 222 functions further focus

the light from the plano-convex lens 221 into a pinpoint beam at a selected focal point. Alternatively, the bi-convex achromatic lens can be replaced by a spherical lens to produce a pinpoint beam of a different size. Collectively, these two lenses cooperate to focus the excitation light into a pinpoint beam a predetermined distance from the output end 210 of the illumination output device 207. Accounting for the distance of the output end 210 from the bottom of the window portion 213 of the specimen platform 26, the thickness of the window portion, and the average position of the surface of a specimen from the top surface of the window portion, the position of the focal point of the pinpoint beam can be calculated. For example, this distance can range from about 0.1 mm to about 3.0 mm.

[0097] Disposed within the base 204 of the lens assembly 215, at the input end 220, is a proximal baffle device 223 similar to the light baffle device 70 disclosed above in reference to the filter wheel assembly 47. This proximal baffle is supported between a lower annular support member 209 and a lower annular edge portion of the cylindrical base 204. Similarly, the plano-convex lens 221 is supported atop an annular shoulder portion 209a of the base 204 and a lower portion of the intermediary baffle device 225.

[0098] Further, in between the plano-convex lens 221 and the bi-convex achromatic lens 222 is an intermediary baffle device 225. This intermediary baffle 225 is supported atop a lower annular support device member 214 and the cap member 204a. Collectively, the cap member 204a and the upper portion of the intermediary baffle device 225 sandwiches the bi-convex achromatic lens 222 therebetween for support thereof.

[0099] As indicated above, these baffles devices 223, 225 intercept skewed light rays, and substantially prevent them from reflecting off of the housing interior walls defining the cavity 212. Hence, these skewed light rays are further prevented from leaking around either lens.

[0100] Applying the same concepts, designs and physical properties described in the light baffle device 70, and further incorporated herein, both the proximal baffle device 223 and intermediary baffle device 225 can be provided by one or more plate members. These are disposed substantially adjacent one another, and include centrally disposed apertures having a transverse cross-sectional area smaller than that of the respective plano-convex or bi-convex achromatic lens at their widest cross-sectional dimensions. In both the proximal and intermediary baffle designs 223, 225, the diameter of the respective aperture of each adjacent plate member is smaller than its adjacent, distal plate member. Collectively, as shown, the apertures taper inwardly toward respective lens 221, 222.

[0101] Due in-apart to the application of a pinpoint beam of excitation light, as compared to the diffused light, applied in the embodiments of FIGURES 8 and 9, it is not necessary to size and dimension the window portion 213, so as to form a light-tight seal with a surface of the specimen supported thereatop, but may be sized close to the footprint of the subject specimen to minimize light leakage around the specimen. In other words, the size of the window portion 213 may exceed the size of the footprint of the targeted specimen (FIGURE 10). Not only does this easy placement of the specimen atop the window portion 213, but also facilitates the application of a more universally sized window portion 213. Hence, the window portion 213 can essentially extend nearly across the entire support surface of specimen platform and/or be integrally formed therewith. Of course, faceplates with apertures may be applied, as shown above, if necessary. Further, to reduce reflection, the window portion may include an anti-reflective coating as well.

[0102] While the pinpoint beam of excitation light exiting the output end 210 for the illumination output device 207 is significantly more intense, it does not apply to a large region of the specimen, as compared to a more diffuse light application in the trans-illumination embodiments of FIGURES 8 and 9. Rather, the trans-illumination observed is more local, depending upon the region of entrance relative to the specimen. Accordingly, as mentioned, a plurality of measurements can be performed at strategic locations along the targeted surface of the specimen.

[0103] In accordance with this embodiment of the present invention, however, in order to strategically position the pinpoint beam of excitation light at the predetermined location, the illumination output device 207 cooperates with a translation platform 208 positioned under the specimen platform 26. While a variety of translation mechanisms can be employed, a more conventional X-Y Translation platform is shown and illustrated. As best viewed in FIGURES 10, 14 and 15 the translation platform 208 includes an elongated horizontal or X-arm 226, and an elongated vertical or Y-arm 227, both of which are rail mounted to a translation platform 208. Applying a mounting bracket 228, the low profile illumination output device 207 is secured to one of the translation arms (e.g., Y-arm 227 in FIGURE 15). Accordingly, through conventional control means, the translation arms 226, 227 and the mounting bracket 228 cooperate to selectively position the output end of the illumination output device at one of a plurality of positions adjacent the window portion 213 such that the light beam impinges the targeted surface of the specimen at one of a plurality of positions therealong.

[0104] One example of such a translation platform 208 is that provided by Model No. BG2005A, by Nippon Bearing Co. of Japan.

[0105] In an alternative application of the trans-illumination device 206, an opaque specimen tray 230 may be positioned atop the window portion 213 of the specimen platform 26, containing an array of well plates 231 therein (FIGURE 16). For example, a standard 96 well plate tray 230 may be applied where each well contains a transparent window that enables the passage of the pinpoint beam of excitation light there through. The output end 210 of the illumination output device 207 can be posi-

tioned under one or more selected wells 231 of the array in order to trans-illuminate the specimen contained in the well.

[0106] Referring back to FIGURE 11, another alternative embodiment of the trans-illumination device 206 is shown having a lens system 232 at the distal end of the second light transmission unit 216 that focuses the excitation light onto a movable optical element 233 that redirects the light toward the specimen 145 supported atop the window portion of the specimen stage. In this embodiment, the optical element 233 is preferably provided by a computer-controlled galvanometer having a reflective surface 235. Through precision control of the orientation of the reflective surface 235, via a control system, the reflected excitation light can be directed at strategic locations of the surface of the supported specimen 145 for trans-illumination thereof. While the reflective surface 235 of the optical element 233 is illustrated as substantially planar, it will be appreciated that the surface 235 may be convex or the like to facilitate focusing the light.

[0107] In yet another alternative configuration FIGURE 12 illustrates a trans-illumination device 206 having a plurality of light emitting ends 236 spaced-apart about the transverse cross-sectional dimension of the window portion 213. More preferably, the light emitting ends 236 are provided the distal ends of one or more fiber optic strands 237 aligned in an array. By controlling the output of the excitation light to a selected strand or strands, via a fiber optic switching system 238 strategic regions of the specimen 145 can be trans-illuminated.

[0108] In one specific configuration, both the trans-illumination device 206 and the epi-illumination device 203 of the second illumination assembly 202 are illuminated by remote light sources. Since trans-illumination and epi-illumination devices are separate illumination procedures and are not to be performed simultaneously, a single remote light source 37 can be applied as opposed to requiring an independent light source for each device, as shown in the illumination systems 200 of FIGURES 10-12. Accordingly, as shown generally in FIGURES 17-22, an optical light switch 240 is positioned between the remote excitation light source 37 and the trans-illumination device 206 and the epi-illumination device 203 to selectively direct the passage of the excitation light. Briefly, the optical light switch 240 is selectively movable between a first position (FIGURES 17, 19, 20 and 22), directing the outputted excitation light to one of the epi-illumination device and the trans-illumination device, and a second position (FIGURES 18 and 21), directing the outputted excitation light to the other of the trans-illumination device and the epi-illumination device.

[0109] The light switch 240 includes a support housing 241 defining an enclosed interior cavity 242 upon which the epi-illumination device 203, the trans-illumination device 206, and the remote excitation light source 37 optically communicate. The housing 241 includes a first light path 243 into the cavity 242 that is in optical communication with the epi-illumination device 203, a second light path 245 into the cavity, in optical communication with the trans-illumination device 206, and a third light path 246 into the cavity in optical communication with the excitation light source 37. FIGURES 17, 18 and 21 best illustrate that the light switch 240 includes a switch element 247 that is selectively movable between the first position and the second position. In one configuration, briefly, the switch element 247 permits the passage of the excitation light across the interior cavity 242 from the third light path 246 to the first light path 243, in the first position, while manipulating the direction of excitation light across the interior cavity 242 from the third light path 246 to the second light path 245, in the second position. It will of course be appreciated that the light transmission units coupled to the respective light paths can be easily switched without departing from the true spirit and nature of the present invention.

[0110] In the embodiments illustrated, a proximal end of the first light transmission unit 205 communicates with the interior cavity 242 of the housing 241 along the first light path, while a proximal end of the second light transmission unit 216 communicates with the interior cavity 242 along the second light path. Further, the proximal ends of the light transmission units will often essentially be the proximal ends of the respective bundle of fiber optic strands.

[0111] Moreover, the remote light source 37 is in communication with the housing via a third light transmission unit or the like. In one configuration, a distal end of the third light transmission unit may terminate directly into the interior cavity of the housing, while a proximal end thereof is in communication with the excitation light source. In another arrangement, a lens device 249 or the like is positioned in the interior cavity 242 of the housing and aligned with the third light path 246. Hence, as the excitation light is emitted from the distal end of the third light transmission unit 248, it passes through the lens device that focuses the excitation light across the interior cavity 242. In still another embodiment, a filter wheel assembly 47 or the like can be positioned "in-line" with the third light transmission unit 248 between the light source 37 and the light switch 240. Hence, the light is filtered prior to entering the switch housing 241. Alternatively, of course, separate filtering of the excitation light can be performed downstream from the switch device.

[0112] Preferably, the filter wheel assembly is identical to the filter wheel assembly 47 shown in the embodiments of FIGURES 1-3. These assemblies include a plurality of optical filters contained on a wheel that can be selectably moved into the third light path of the excitation light.

[0113] Referring back to FIGURES 17 and 20, in one specific embodiment, the third light path 246 (i.e., in optical communication with the light source) is in substantial optical alignment with the either of the first light path (i.e., in optical communication with the epi-illumination device 203) or the second light path (i.e., in optical communication with the trans-illumination device 206). Briefly, it will be appreciated that while either the second light path or

the first light path may be optically aligned with the third light path, heretofore, the first light path of the first transmission unit will be described as optically aligned with the third light path for clarity of description. It will further be apparent that neither the first light path 243 nor the second light path 245 need be in optical alignment with the third light path 246. In these configurations, the movable switch element 247 can be shaped to direct the emission of excitation light from the third light path to either the first light path or the second light path.

[0114] More preferably, however, the optical alignment between the first light path 243 and the third light path 246 is a substantially linear, co-axial alignment. As best viewed in FIGURE 17, the first light path 243 is directly across from the third light path 246 in the housing interior cavity 242. Accordingly, to permit passage of the excitation light from the distal end of the third light path 246, across the housing interior cavity 242, to the proximal end of the first light transmission unit 205, the movable switch element 247, in the first position, is positioned out of the optical path of the excitation light transmitted from the third light path distal end. Hence, the excitation light outputted from the distal end of the third light path 246 passes unobstructed across the housing interior cavity 242 and directly into the proximal end of the bundle of fiber optic strands (i.e., the first light transmission unit 205) along the first light path 243.

[0115] In contrast, while the second light path 245 is preferably oriented and contained in substantially the same plane as that of the first light path 243 and the third light path 246 (although it need not be), it is positioned in a non-linear orientation relative to the third light path 246. By angling the second light path 245 relative to the third light path 246, the excitation light exiting the distal end of the third light path will not be directed into the proximal end of the second light transmission unit 216 when the movable switch element 247 is disposed in the first position.

[0116] More preferably, the second light path 245 is oriented substantially perpendicular to both the first light path 243 and the third light path 246, as shown in FIGURES 17, 18 and 221. While the second light path 245 can be off-set from the third light path 246 at nearly any angle between about 20° to about 60° from the optical axis of the third light path, about a 90° off-set is preferred due to the ease of optical manipulation of the excitation light in the direction of the second light path 245.

[0117] In the second position (FIGURES 19 and 21), thus, the movable switch element 247 is configured and oriented to direct the excitation light outputted from the third transmission unit 248 along the third light path 246 and into the proximal end of the second light transmission unit 216. In the embodiment shown in FIGURES 19 and 21, the excitation light is redirected about 90° toward the second light path 245 of the proximal end of the second light transmission unit 216. Preferably, the switch element includes a reflective surface 250 strategically oriented to reflect the outputted excitation light toward the second transmission unit. This reflective surface 250 is preferably mounted to a support flange portion 251 of the switch element 247 at about a 45° angle (since in this embodiment the second light path 245 is off-set about 90° from the third light path 246). Further, the surface of the reflective element is substantially planar, although not need be depending upon the desired reflection characteristics. Accordingly, in the second position, not only is the support flange portion 251 of the switch element 247 obstructively moved between the distal end of the third light transmission unit 248 and the proximal end of the first light transmission unit 205, but also strategically positions the reflective surface 250 to reflect the excitation light into the proximal end of the second light transmission unit 216.

[0118] To control the movement of the flange portion 251, the switch element 247 includes a support arm portion 252 containing an upstanding pivot post 254 that is pivotally coupled to a support bracket 259, which in turn is affixed to the housing 241. This pivot post 254 enables the switch element 247 to pivot about an axis 253 between the first position and the second position. A drive mechanism 255 is coupled to the support arm portion 252 for operation of the switch element 247. In one configuration, the drive mechanism 255 includes a drive rod 256 having one end pivotally mounted to the arm portion 252, and an opposite end mounted to a solenoid device 257 or the like. The solenoid device is then conventionally operated through a control unit or circuitry, reciprocating the drive rod, which in turn rotates the switch element 247 about the axis 253 between the first and second positions.

[0119] In accordance with one specific embodiment of the present invention, the switch element 247 includes a cover unit 258 that is positioned over the proximal end of the second light transmission unit 216 when the switch element 247 is in the first position. This functions to prevent substantially all the diffused or scattered excitation light in the housing interior cavity 242 from entering the proximal end of the second light transmission unit 216 where it would be emitted from the trans-illumination device 206. As best viewed in FIGURES 17, 19 and 20, this cover unit 258 may be a simple plate extension mounted to an end of the support flange portion 251. When the switch element 247 is moved to the first position, the cover unit 258 is moved over the proximal end of the second light transmission unit 216.

[0120] In contrast, when the switch element 247 is moved to the second position, the cover unit 258 is either moved out of the second light path 245 (i.e., from in front to the proximal end of the second light transmission unit 216) or enable entrance of excitation light into the second light transmission unit 216. In this particular design, the cover unit 258 includes an aperture 260 extending through the cover unit 258 that permits the passage of the excitation light after being reflected off of the reflective element. This aperture is sized diametrically at least as large as the proximal end of the second light transmission

unit 216 to allow the passage of the light. This aperture 260 is oriented such that when the switch element is in the second position, the aperture 260 is substantially coaxially aligned with that of the second transmission unit proximal end, but when in the first position, the aperture will not permit the passage of light through to the second transmission unit.

[0121] Although only a few embodiments of the present invention have been described in detail, it should be understood that the present invention might be embodied in many other specific forms without departing from the scope of the appended claims.

Claims

1. A system comprising a dual illumination system (200) and an imaging apparatus (21), the imaging apparatus defining a light-tight imaging compartment (25) with an interior wall having a view port (23) extending into the imaging compartment to enable a camera (31) of the imaging apparatus to acquire data of a specimen contained in the imaging compartment, the dual illumination system comprising:

a first illumination assembly (201) configured to direct structured light onto a side of the specimen facing the view port to enable structured light imaging and surface topography measurements of the specimen; and
a second illumination assembly (202) configured to direct excitation light at the specimen whereby diffused fluorescent light emanates from the surface of the specimen facing the view port for receipt through the view port by the camera, wherein the second illumination assembly comprises:

a trans-illumination device (206) configured to direct excitation light into a surface of the specimen facing away from the view port opposite the viewport whereby the diffused fluorescent light exits the surface facing the view port for receipt through the view port by the camera, wherein the trans-illumination device comprises:

a lens assembly (215) configured to focus the excitation light from the trans-illumination device onto the specimen; and
a translation platform (208) configured to position an output end of the trans-illumination device at one of a plurality of positions adjacent the surface of the specimen facing away from the view port; and

an epi-illumination device (203) configured to direct excitation light onto the surface of the specimen facing the viewport.

2. The system as defined by claim 1, further comprising:

a specimen platform (26) positioned in the imaging compartment, wherein:

the epi-illumination device (203) comprises a first light transmission unit (205) having a proximal end for optical communication with an excitation light source and a distal end which terminates proximate the view port, and
the trans-illumination device (206) comprises a second light transmission unit (216) having a respective proximal end thereof for optical communication with the excitation light source and a respective distal end thereof for directing the excitation light into a side surface of the specimen on the specimen platform (26).

3. The system as defined by claim 2, further comprising:

a window portion (213) defined in the specimen platform for enabling the passage of light there through; and
the distal end of the second light transmission unit (216) being configured to emit the excitation light in a beam toward the window portion.

4. The system as defined by claim 2, wherein the second illumination assembly (202) comprises an optical light switch (240) coupled to the proximal ends of the respective first (205) and second (216) light transmission units for selectively directing the excitation light to one of the epi-illumination device (203) and the trans-illumination device (206).

5. The system defined by claim 4, wherein:

the light switch (240) comprises a housing (241) defining a cavity (242) and having a first light path (243) into the cavity in optical communication with the epi-illumination device (203), a second light path (245) into the cavity in optical communication with the trans-illumination device (206), and a third light path (246) into the cavity, and
a switch element selectively movable between a first position in which the switch element couples the first and third light paths and a second position in which the switch element couples the second and third light paths.

6. The system as defined by claim 2, wherein the distal end of the first light transmission unit (205) is disposed in the imaging compartment, and peripherally encircles the view port.

7. A method for performing 3D diffuse fluorescence tomography reconstruction, using a system as defined by any of claims 1 to 6, for a specimen disposed in the light-tight imaging compartment (25) of the imaging apparatus, the method comprising:

applying structured light to a surface of the specimen in the imaging compartment and determining the 3D surface topography data thereof;
applying trans-illumination fluorescence excitation light to the specimen using the trans-illumination device (206) and acquiring trans-illumination fluorescence imaging data of the specimen;
applying epi-illumination fluorescence excitation light to the specimen using the epi-illumination device (203) and acquiring epi-illumination fluorescence imaging data of the specimen; and
calculating the 3D diffuse fluorescence tomography reconstruction using the 3D surface topography data and the epi-illumination fluorescence imaging data and the trans-illumination fluorescence imaging data, acquired from the system.

8. The method of claim 7 wherein applying trans-illumination fluorescence excitation light to the specimen comprises moving the trans-illumination device (206) to a plurality of positions relative to the specimen in the imaging compartment (25), and at each of the plurality of positions, directing focused excitation light onto a portion of a surface of the specimen.

9. The method of claim 7 wherein acquiring trans-illumination fluorescence imaging data comprises acquiring the trans-illumination fluorescence imaging data at a plurality of excitation or emission wavelengths.

10. The method of claim 7, wherein acquiring epi-illumination fluorescence imaging data comprises acquiring the epi-illumination fluorescence imaging data at a plurality of excitation or emission wavelengths.

Patentansprüche

1. System umfassend ein duales Illuminationssystem (200) und eine Bildgebungsvorrichtung (21), wobei die Bildgebungsvorrichtung ein lichtdichtes Bildgebungsfach (25) mit einer Innenwand definiert, welche ein sich in das Bildgebungsfach erstreckendes Sichtfenster (23) aufweist, um eine Kamera (31) der Bildgebungsvorrichtung zu befähigen, Daten einer in dem Bildgebungsfach aufgenommenen Probe zu erfassen, wobei das duale Illuminationssystem umfasst:

eine erste Illuminationsbaugruppe (201), welche dazu eingerichtet ist, strukturiertes Licht auf eine dem Sichtfenster zugewandte Seite der Probe zu richten, um Bildgebungs- und Oberflächentopografiemessungen der Probe mit strukturiertem Licht zu ermöglichen; und
eine zweite Illuminationsbaugruppe (202), welche dazu eingerichtet ist, Anregungslicht auf die Probe zu richten, wodurch Fluoreszenzstreulicht von der dem Sichtfenster zugewandten Oberfläche der Probe zum Empfang durch die Kamera durch das Sichtfenster hindurch ausgeht, wobei die zweite Illuminationsbaugruppe umfasst:

eine Trans-Illuminationsvorrichtung (206), welche dazu eingerichtet ist, Anregungslicht in eine von dem Sichtfenster abgewandte, dem Sichtfenster gegenüberliegende Oberfläche der Probe zu richten, wodurch das Fluoreszenzstreulicht die dem Sichtfenster zugewandte Oberfläche zum Empfang durch die Kamera durch das Sichtfenster hindurch verlässt, wobei die Trans-Illuminationsvorrichtung umfasst:

eine Linsenbaugruppe (215), welche dazu eingerichtet ist, das Anregungslicht von der Trans-Illuminationsvorrichtung auf die Probe zu fokussieren; und
eine Translationsplattform (208), welche dazu eingerichtet ist, ein Ausgabeende der Trans-Illuminationsvorrichtung an einer von einer Vielzahl von Positionen zu positionieren, welche zu der von dem Sichtfenster abgewandten Oberfläche der Probe benachbart ist; und

eine Epi-Illuminationsvorrichtung (203), welche dazu eingerichtet ist, Anregungslicht auf die dem Sichtfenster zugewandte Seite der Probe zu richten.

2. System wie in Anspruch 1 definiert, ferner umfassend:

eine in dem Bildgebungsfach positionierte Probenplattform (26), wobei:

die Epi-Illuminationsvorrichtung (203) eine erste Lichttransmissionseinheit (205) um-

fasst, welche ein proximales Ende für eine optische Verbindung mit einer Anregungslichtquelle und ein distales Ende, welches nahe dem Sichtfenster mündet, aufweist und
die Trans-Illuminationsvorrichtung (206) eine zweite Lichttransmissionseinheit (216) umfasst, welche ein entsprechendes proximales Ende davon für eine optische Verbindung mit der Anregungslichtquelle und ein entsprechendes distales Ende davon zum Richten des Anregungslichts in eine seitliche Oberfläche der Probe auf der Probenplattform (26) aufweist.

3. System wie in Anspruch 2 definiert, ferner umfassend:

einen in der Probenplattform definierten Fensterabschnitt (213) zum Ermöglichen eines Lichtdurchgangs dadurch; und
wobei das distale Ende der zweiten Lichttransmissionseinheit (216) dazu eingerichtet ist, das Anregungslicht in einem Strahl zu dem Fensterabschnitt hin abzugeben.

4. System wie in Anspruch 2 definiert, wobei die zweite Illuminationsbaugruppe (202) einen optischen Lichtschalter (240) umfasst, welcher an die proximalen Enden der entsprechenden ersten (205) und zweiten (216) Lichttransmissionseinheiten gekoppelt ist zum selektiven Richten des Anregungslichts auf eine aus der Epi-Illuminationsvorrichtung (203) und der Trans-Illuminationsvorrichtung (206).

5. System wie in Anspruch 4 definiert, wobei:

der Lichtschalter (240) ein Gehäuse (241) umfasst, welches einen Hohlraum (242) definiert, und einen ersten Lichtpfad (243) in den Hohlraum in optischer Verbindung mit der Epi-Illuminationsvorrichtung (203), einen zweiten Lichtpfad (245) in den Hohlraum in optischer Verbindung mit der Trans-Illuminationsvorrichtung (206), und einen dritten Lichtpfad (246) in den Hohlraum aufweist, und
ein Schalterelement, welches selektiv zwischen einer ersten Position, in welcher das Schalterelement die ersten und dritten Lichtpfade koppelt und einer zweiten Position, in welcher das Schalterelement die zweiten und dritten Lichtpfade koppelt, beweglich ist.

6. System wie in Anspruch 2 definiert, wobei das distale Ende der ersten Lichttransmissionseinheit (205) in dem Bildgebungsfach angeordnet ist und das Sichtfenster peripher umschließt.

7. Verfahren zum Durchführen von 3D-Fluoreszenzstreutomographierekonstruktion mittels eines Systems wie in jedem der Ansprüche 1 bis 6 definiert, für eine in einem lichtdichten Bildgebungsfach (25) der Bildgebungsvorrichtung angebrachten Probe, wobei das Verfahren umfasst:

Applizieren von strukturiertem Licht auf eine Oberfläche der Probe in dem Bildgebungsfach und Bestimmen der 3D-Oberflächentopographiedaten davon;
Applizieren von Trans-Illuminationsfluoreszenzanregungslicht auf die Probe mittels der Trans-Illuminationsvorrichtung (206) und Erfassen von Trans-Illuminationsfluoreszenzbildgebungsdaten der Probe;
Applizieren von Epi-Illuminationsfluoreszenzanregungslicht auf die Probe mittels der Epi-Illuminationsvorrichtung (203) und Erfassen von Epi-Illuminationsfluoreszenzbildgebungsdaten der Probe; und
Berechnen der 3D-Fluoreszenzstreu-Tomographierekonstruktion mittels der 3D-Oberflächentopographiedaten und der Epi-Illuminationsfluoreszenzbildgebungsdaten und der Trans-Illuminationsfluoreszenzbildgebungsdaten, welche von dem System erfasst wurden.

8. Verfahren gemäß Anspruch 7, wobei ein Applizieren von Trans-Illuminationsfluoreszenzanregungslicht auf die Probe ein Bewegen der Trans-Illuminationsvorrichtung (206) an eine Vielzahl von Relativpositionen zu der Probe in dem Bildgebungsfach (25) umfasst, und an jeder der Vielzahl von Positionen ein Richten von fokussiertem Anregungslicht auf einen Abschnitt einer Oberfläche der Probe.

9. Verfahren gemäß Anspruch 7, wobei ein Erfassen von Trans-Illuminationsfluoreszenzbildgebungsdaten ein Erfassen der Trans-Illuminationsfluoreszenzbildgebungsdaten bei einer Vielzahl von Anregungs- oder Emissionswellenlängen umfasst.

10. Verfahren gemäß Anspruch 7, wobei ein Erfassen von Epi-Illuminationsfluoreszenzbildgebungsdaten ein Erfassen der Epi-Illuminationsfluoreszenzbildgebungsdaten bei einer Vielzahl von Anregungs- oder Emissionswellenlängen umfasst.

Revendications

1. Système comprenant un double système d'illumination (200) et un appareil d'imagerie (21), l'appareil d'imagerie définissant un compartiment d'imagerie opaque (25) avec une paroi intérieure ayant un orifice de visualisation (23) s'étendant dans le compartiment d'imagerie pour permettre à une caméra (31)

de l'appareil d'imagerie d'acquérir des données d'un spécimen contenu dans le compartiment d'imagerie, le double système d'illumination comprenant :

un premier ensemble d'illumination (201) configuré pour diriger une lumière structurée sur un côté du spécimen faisant face à l'orifice de visualisation pour permettre des mesures topographiques de surface et d'imagerie à lumière structurée du spécimen ; et
un deuxième ensemble d'illumination (202) configuré pour diriger une lumière d'excitation sur le spécimen moyennant quoi une lumière fluorescente diffuse émane de la surface du spécimen faisant face à l'orifice de visualisation pour être reçue par la caméra à travers l'orifice de visualisation, où le deuxième ensemble d'illumination comprend :

un dispositif de transillumination (206) configuré pour diriger une lumière d'excitation vers une surface du spécimen opposée à l'orifice de visualisation à l'opposé de l'orifice de visualisation moyennant quoi la lumière fluorescente diffuse sort de la surface faisant face à l'orifice de visualisation pour être reçue par la caméra à travers l'orifice de visualisation, où le dispositif de transillumination comprend :

un ensemble de lentilles (215) configuré pour focaliser la lumière d'excitation provenant du dispositif de transillumination sur le spécimen ; et
une plate-forme de translation (208) configurée pour positionner une extrémité de sortie du dispositif de transillumination à l'une d'une pluralité de positions adjacentes à la surface du spécimen opposée à l'orifice de visualisation ; et

un dispositif d'épi-illumination (203) configuré pour diriger une lumière d'excitation sur la surface du spécimen faisant face à l'orifice de visualisation.

2. Système tel que défini par la revendication 1, comprenant en outre :

une plate-forme de spécimen (26) positionnée dans le compartiment d'imagerie, où :

le dispositif d'épi-illumination (203) comprend une première unité de transmission de lumière (205) ayant une extrémité proximale pour une communication optique avec une source de lumière d'excitation et une extrémité distale qui se termine à proximité de l'orifice de visualisation, et
le dispositif de transillumination (206) comprend une deuxième unité de transmission de lumière (216) ayant une extrémité proximale respective de celle-ci pour une communication optique avec la source de lumière d'excitation et une extrémité distale respective de celle-ci pour diriger la lumière d'excitation vers une surface latérale du spécimen sur la plate-forme de spécimen (26).

3. Système tel que défini par la revendication 2, comprenant en outre :

une partie de fenêtre (213) définie dans la plate-forme de spécimen pour permettre le passage de lumière à travers celle-ci ; et
l'extrémité distale de la deuxième unité de transmission de lumière (216) étant configurée pour émettre la lumière d'excitation sous forme d'un faisceau vers la partie de fenêtre.

4. Système tel que défini par la revendication 2, dans lequel le deuxième ensemble d'illumination (202) comprend un commutateur optique de lumière (240) couplé aux extrémités proximales des première (205) et deuxième (216) unités respectives de transmission de lumière pour diriger de manière sélective la lumière d'excitation vers l'un parmi le dispositif d'épi-illumination (203) et le dispositif de transillumination (206).

5. Système tel que défini par la revendication 4, dans lequel :

le commutateur de lumière (240) comprend un boîtier (241) définissant une cavité (242) et ayant un premier trajet de lumière (243) dans la cavité en communication optique avec le dispositif d'épi-illumination (203), un deuxième trajet de lumière (245) dans la cavité en communication optique avec le dispositif de transillumination (206), et un troisième trajet de lumière (246) dans la cavité, et
un élément de commutation pouvant être déplacé de manière sélective entre une première position dans laquelle l'élément de commutation couple les premier et troisième trajets de lumière et une deuxième position dans laquelle l'élément de commutation couple les deuxième et troisième trajets de lumière.

6. Système tel que défini par la revendication 2, dans lequel l'extrémité distale de la première unité de transmission de lumière (205) est disposée dans le compartiment d'imagerie, et entoure de manière pé-

riphérique l'orifice de visualisation.

7. Procédé pour effectuer une reconstruction 3D par tomographie diffuse de fluorescence, en utilisant un système tel que défini par l'une des revendications 1 à 6, pour un spécimen disposé dans le compartiment d'imagerie opaque (25) de l'appareil d'imagerie, le procédé comprenant le fait :

d'appliquer une lumière structurée à une surface du spécimen dans le compartiment d'imagerie et de déterminer les données de topographie de surface 3D de celui-ci ;
d'appliquer une lumière d'excitation fluorescente par transillumination au spécimen en utilisant le dispositif de transillumination (206) et d'acquérir des données d'imagerie de fluorescence par transillumination du spécimen ;
d'appliquer une lumière d'excitation fluorescente par épi-illumination au spécimen en utilisant le dispositif d'épi-illumination (203) et d'acquérir des données d'imagerie de fluorescence par épi-illumination du spécimen ; et
de calculer la reconstruction 3D par tomographie diffuse de fluorescence en utilisant les données de topographie de surface 3D et les données d'imagerie de fluorescence par épi-illumination et les données d'imagerie de fluorescence par transillumination, acquises à partir du système.

8. Procédé de la revendication 7, dans lequel le fait d'appliquer une lumière d'excitation fluorescente par transillumination au spécimen comprend le fait de déplacer le dispositif de transillumination (206) vers une pluralité de positions par rapport au spécimen dans le compartiment d'imagerie (25) et de diriger, à chacune de la pluralité de positions, une lumière d'excitation focalisée sur une partie d'une surface du spécimen.

9. Procédé de la revendication 7, dans lequel le fait d'acquérir des données d'imagerie de fluorescence par transillumination comprend le fait d'acquérir les données d'imagerie de fluorescence par transillumination à une pluralité de longueurs d'onde d'excitation ou d'émission.

10. Procédé de la revendication 7, dans lequel le fait d'acquérir des données d'imagerie de fluorescence par épi-illumination comprend le fait d'acquérir les données d'imagerie de fluorescence par épi-illumination à une pluralité de longueurs d'onde d'excitation ou d'émission.

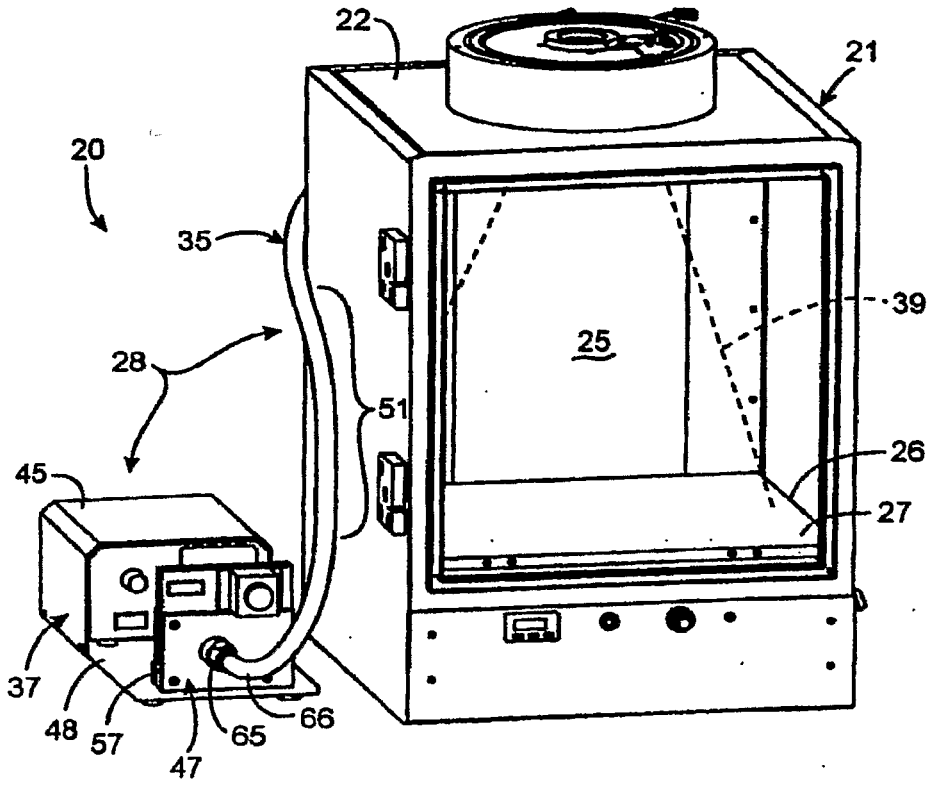

FIG. 1

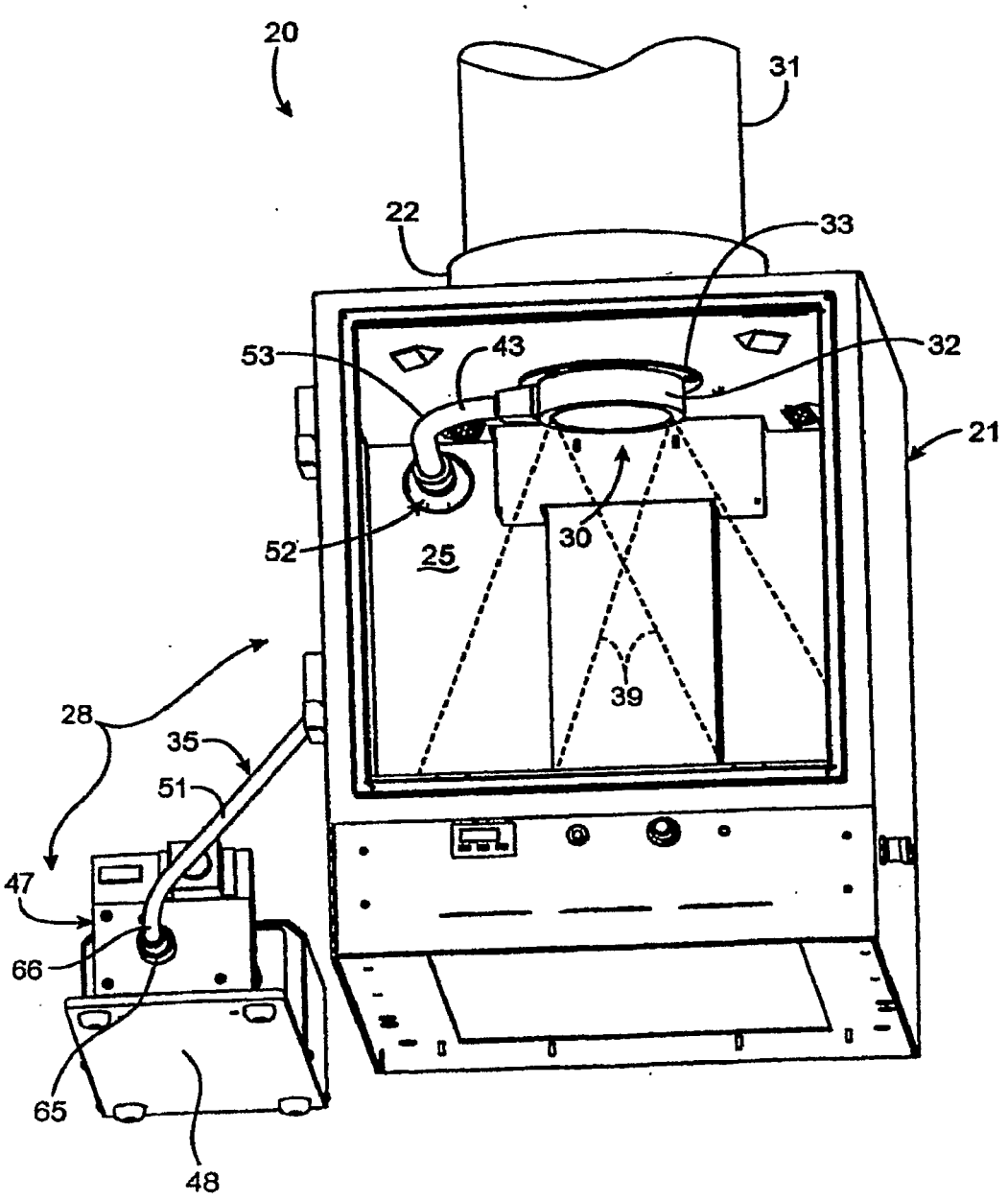

FIG. 2

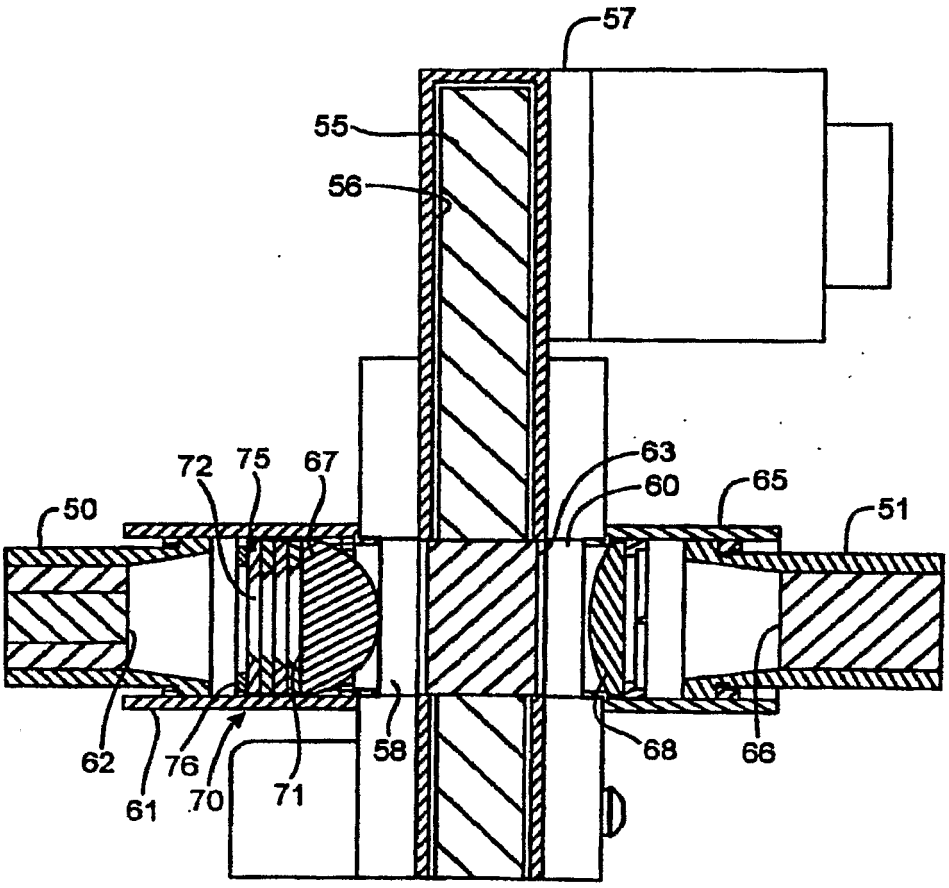

FIG. 3

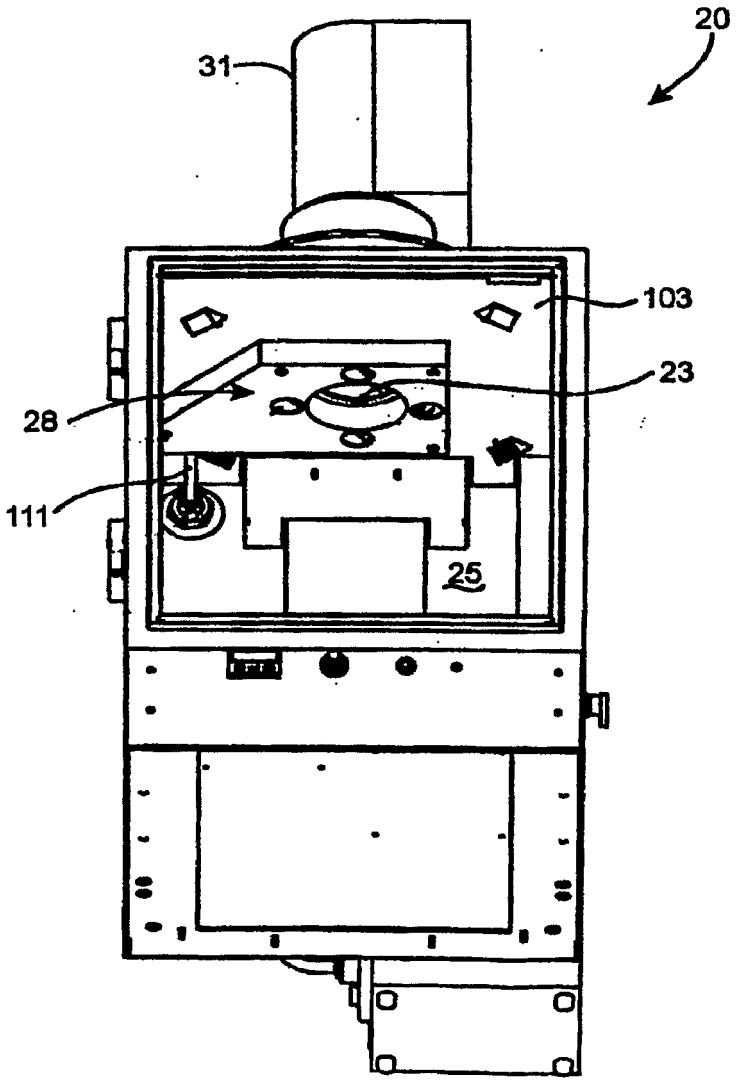

FIG. 4

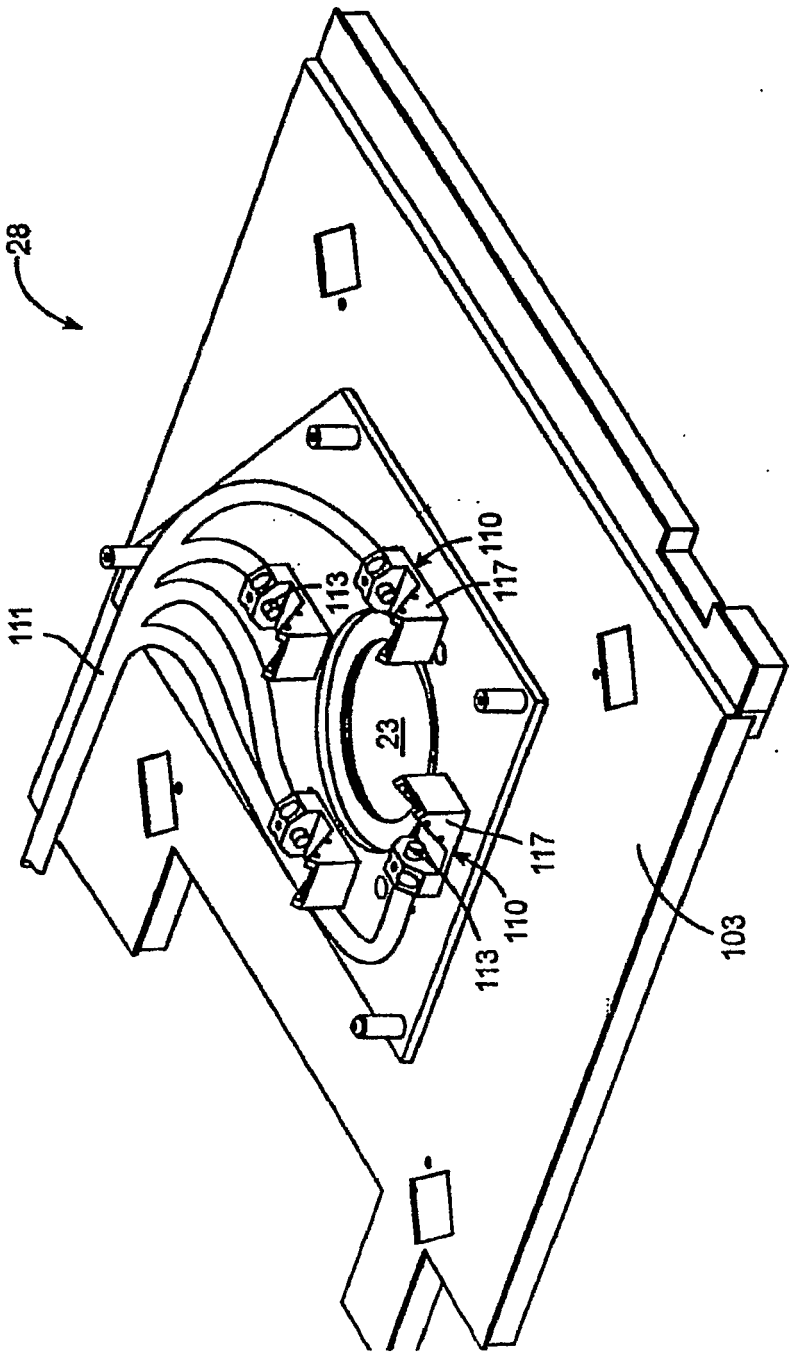

FIG.—5

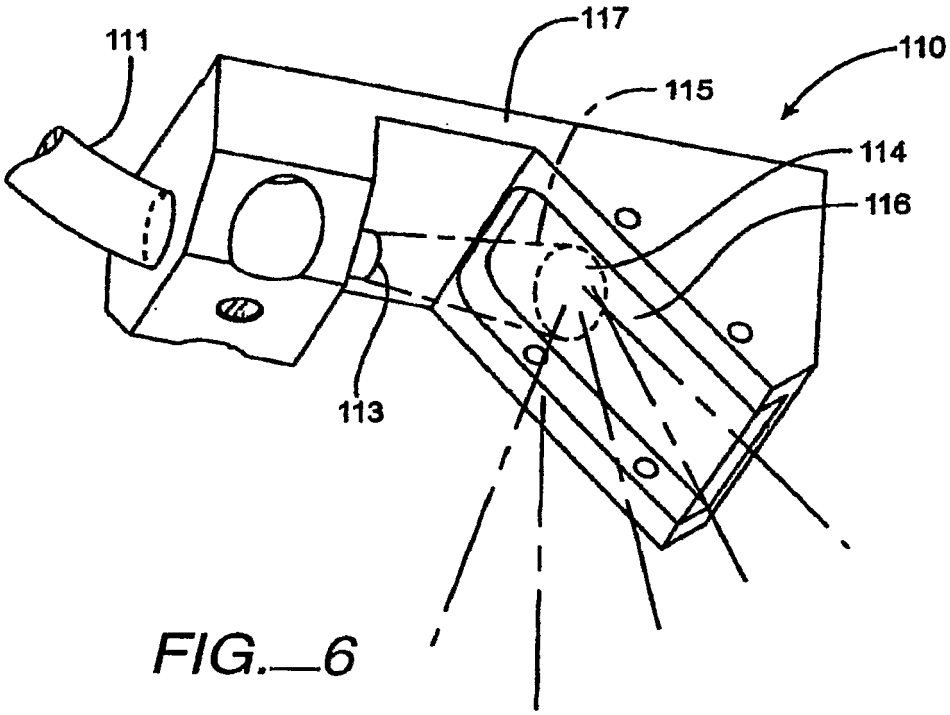

FIG._6

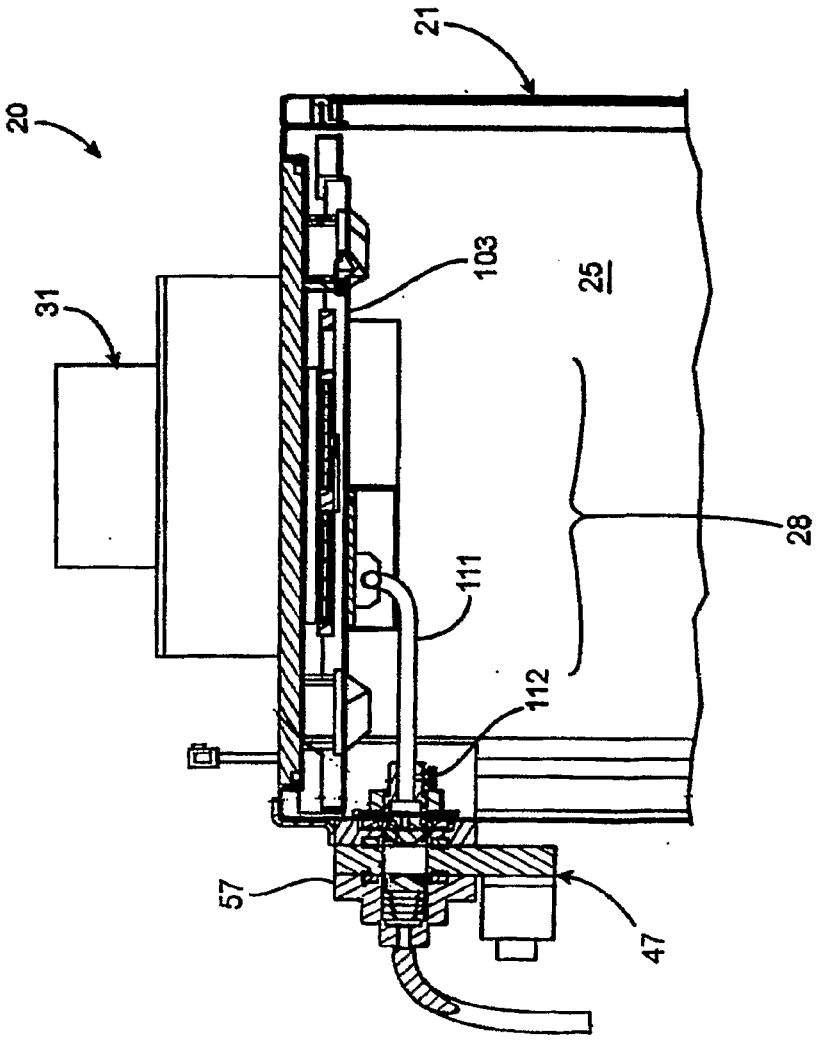

FIG.—7

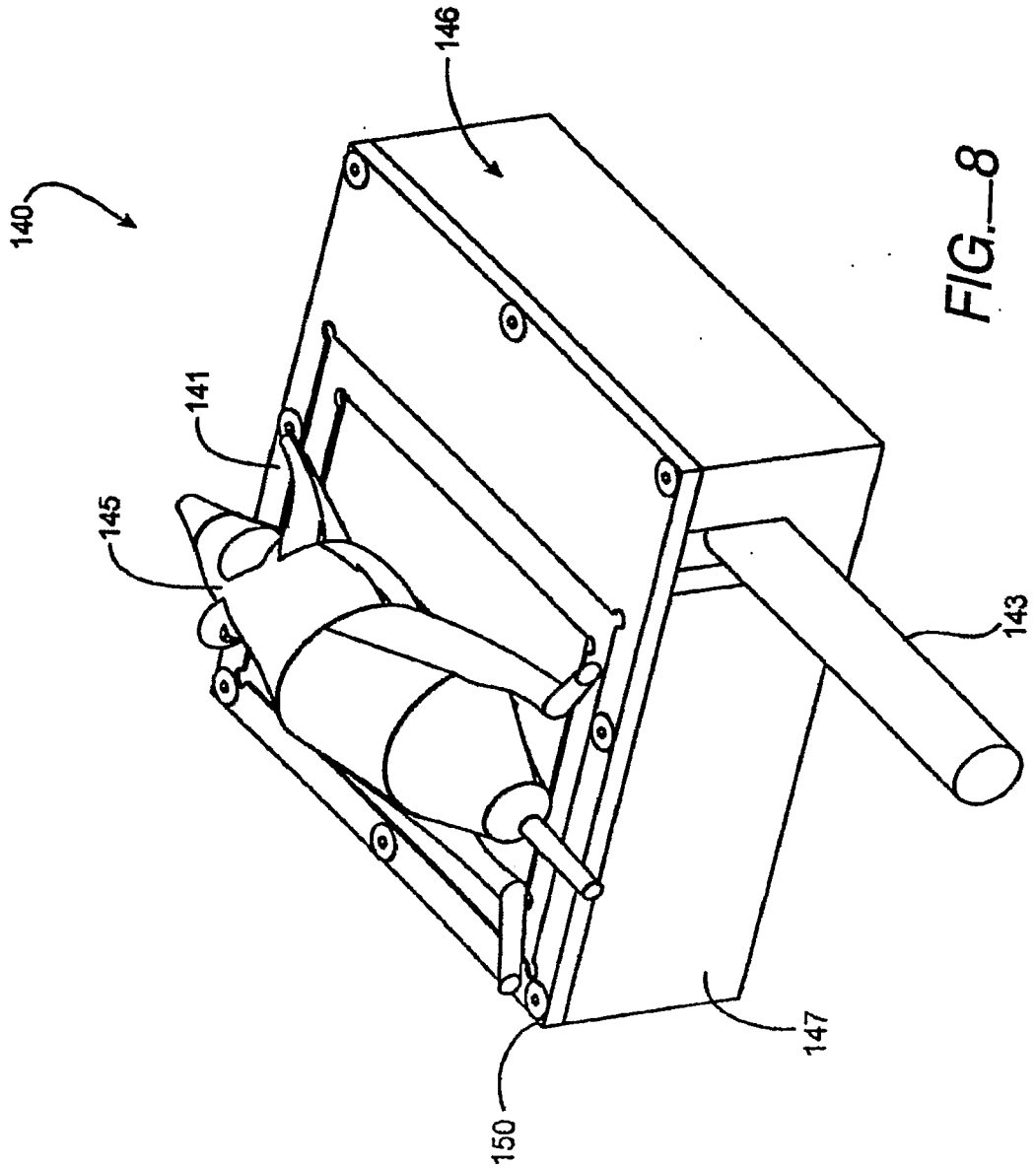

FIG.—8

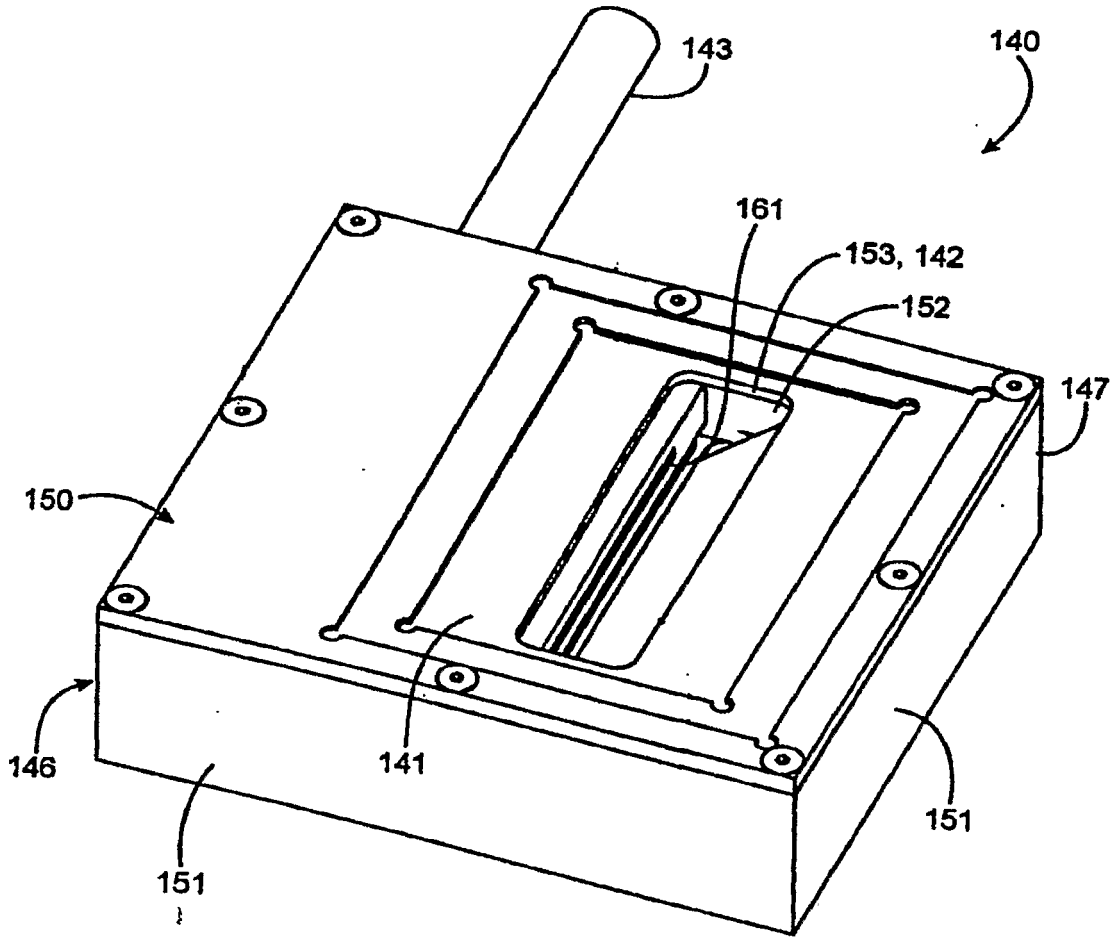

FIG._9

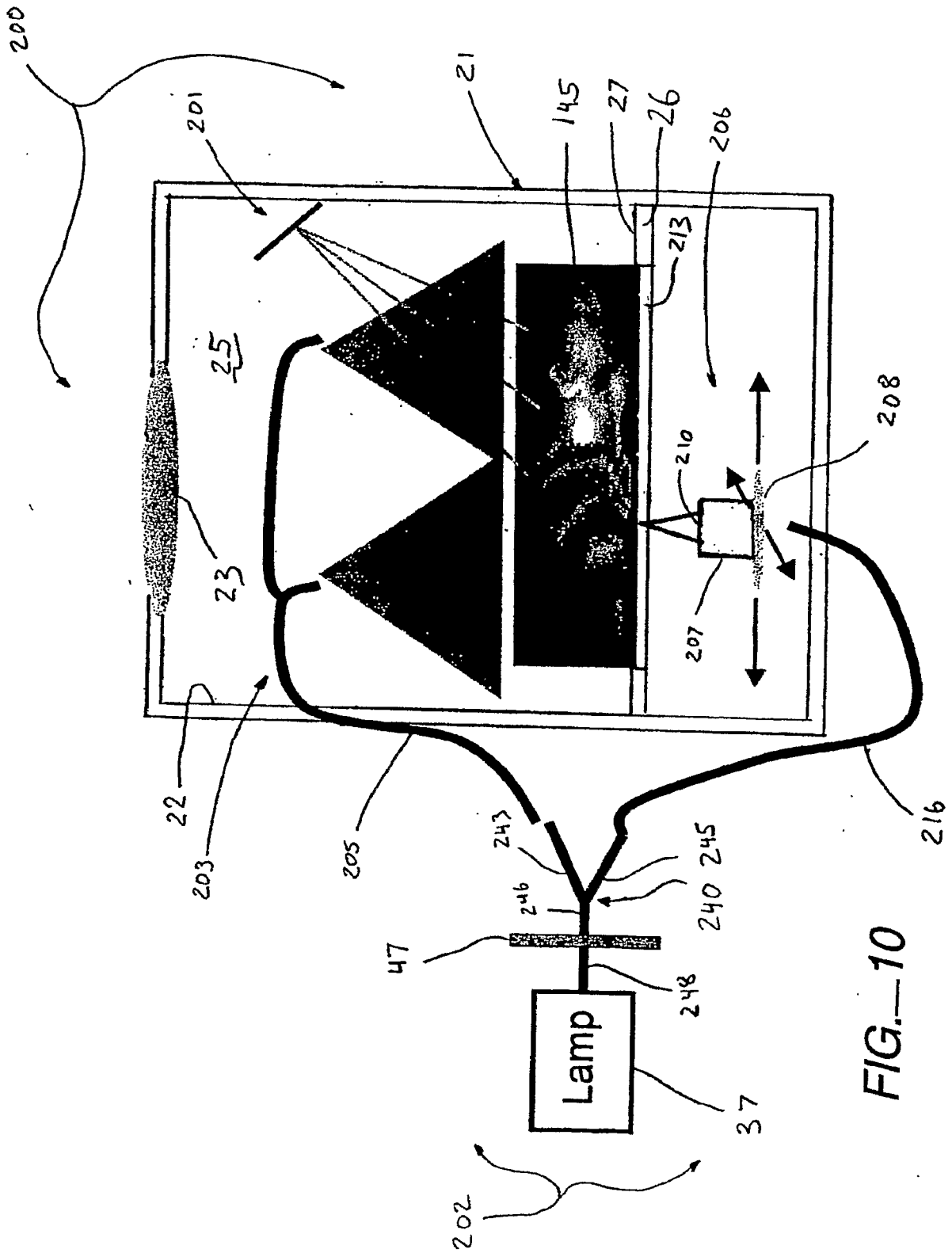
200
201
21
145
27
26
206
213
25
23
208
210
207
22
203
205
243
245
240
246
47
248
Lamp
37
202
216

FIG._10

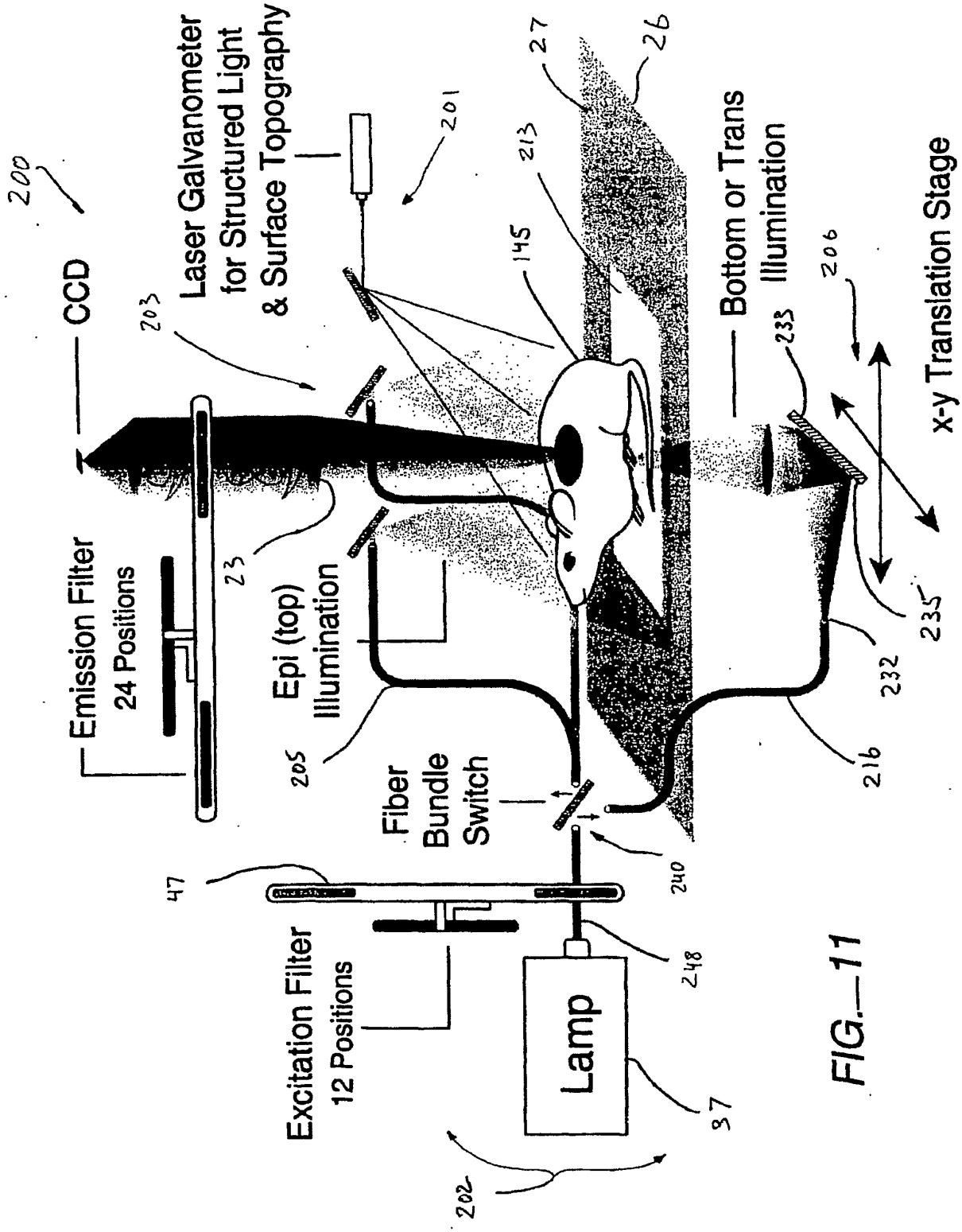
200
CCD
Laser Galvanometer for Structured Light & Surface Topography
203
201
27
26
213
145
Bottom or Trans Illumination
233
206
Emission Filter 24 Positions
23
Epi (top) Illumination
205
Fiber Bundle Switch
47
240
Excitation Filter 12 Positions
248
Lamp
37
202
216
232
235
x-y Translation Stage

FIG._11

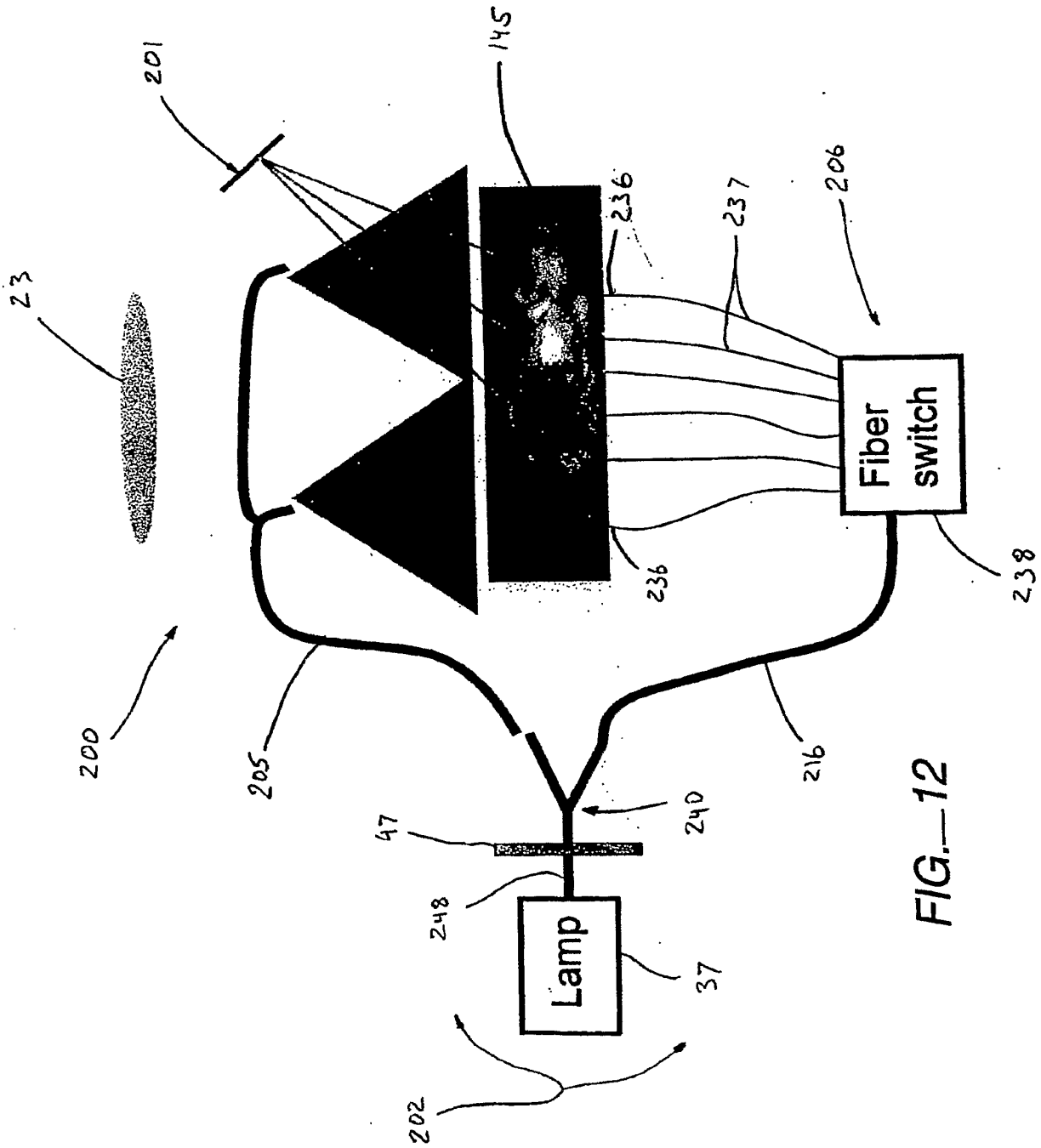
201
145
23
236
237
206
Fiber switch
238
200
205
216
240
47
248
Lamp
37
202

FIG. 12

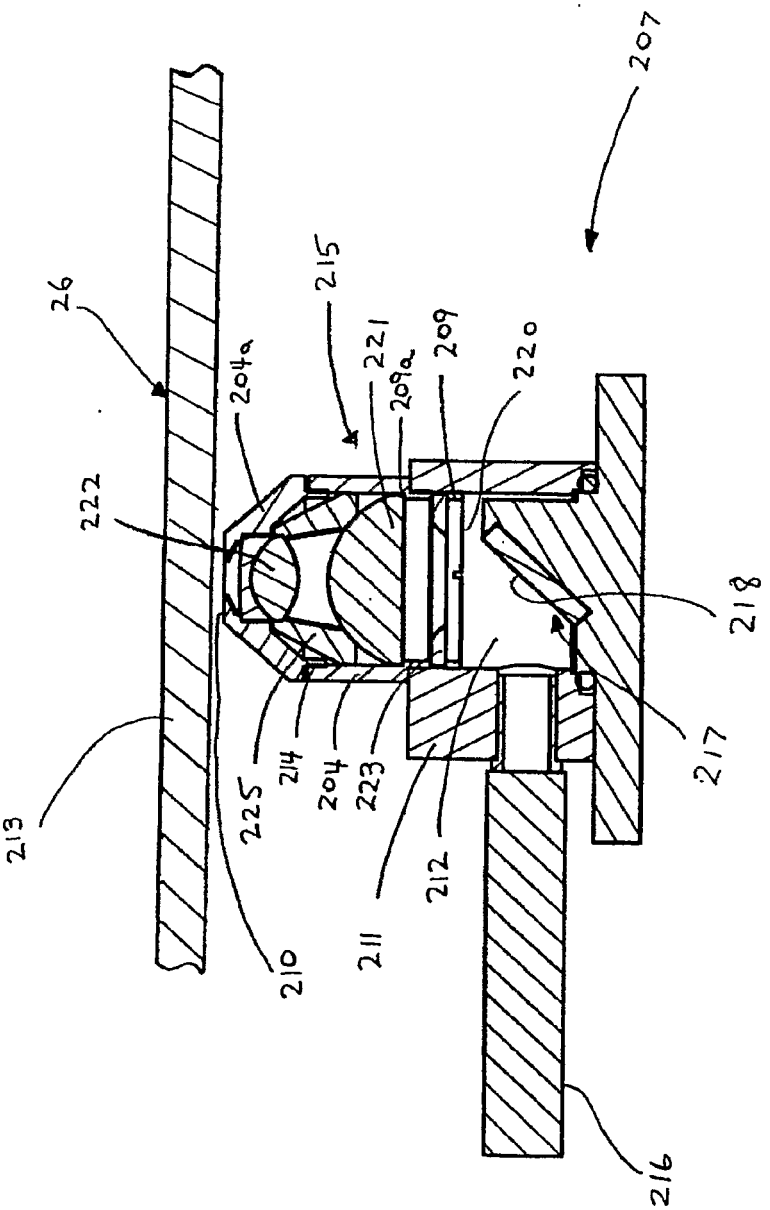
207
26
215
221
209a
209
220
204a
222
218
213
225
214
204
223
217
212
211
210
216

FIG._13

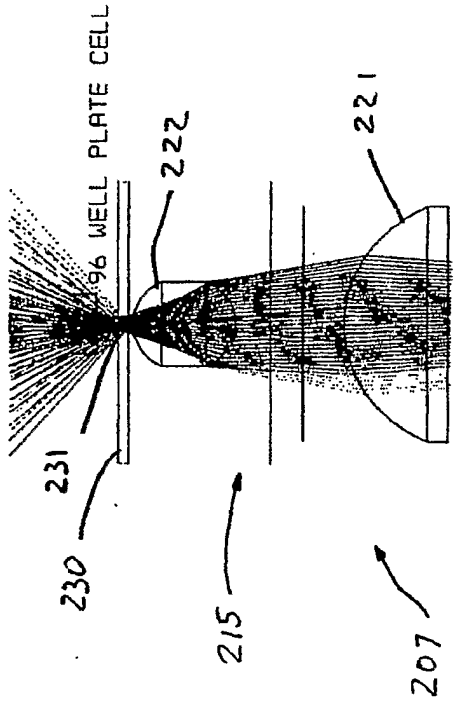

FIG._16

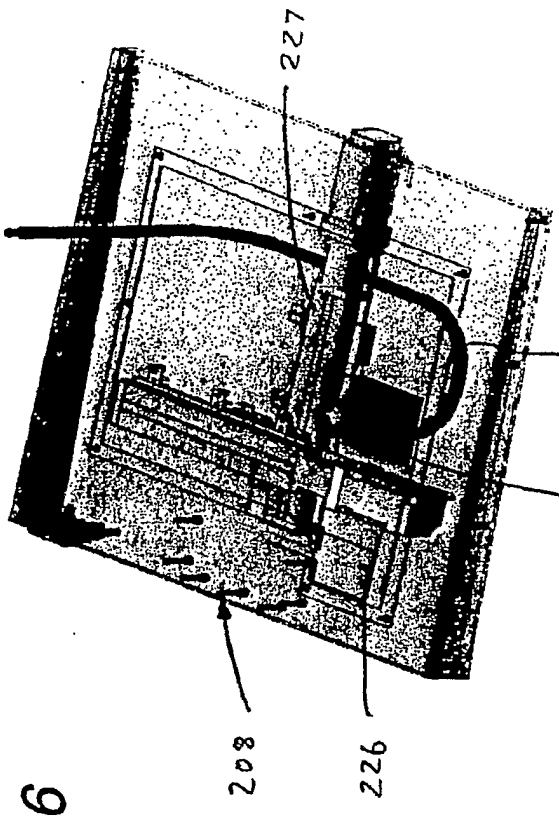

FIG._14

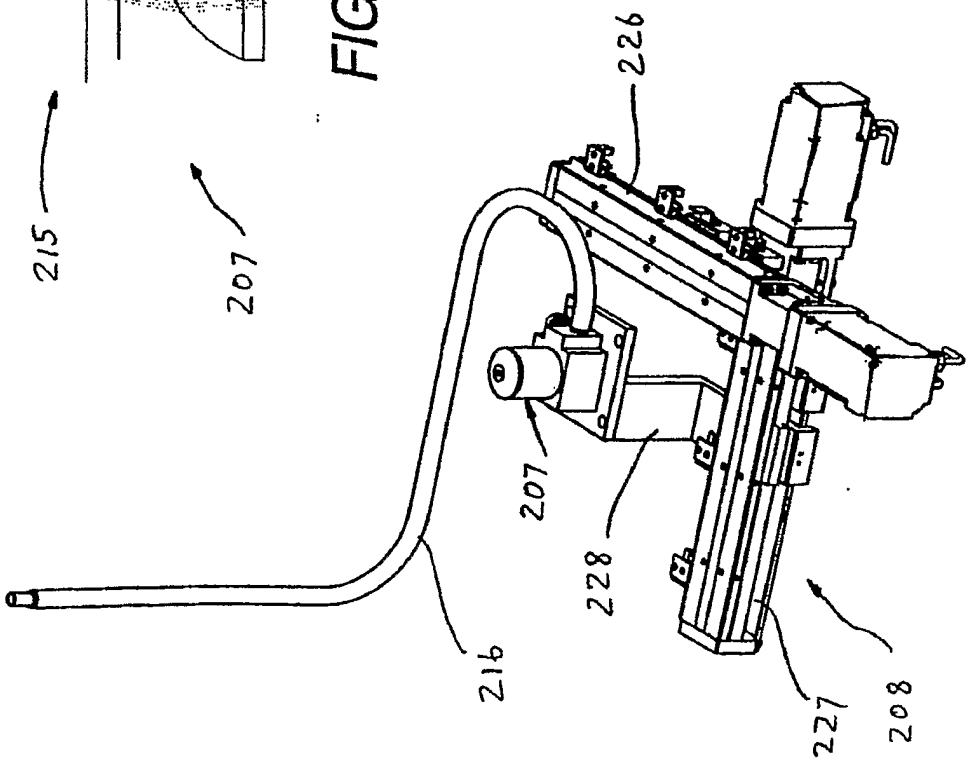

FIG._15

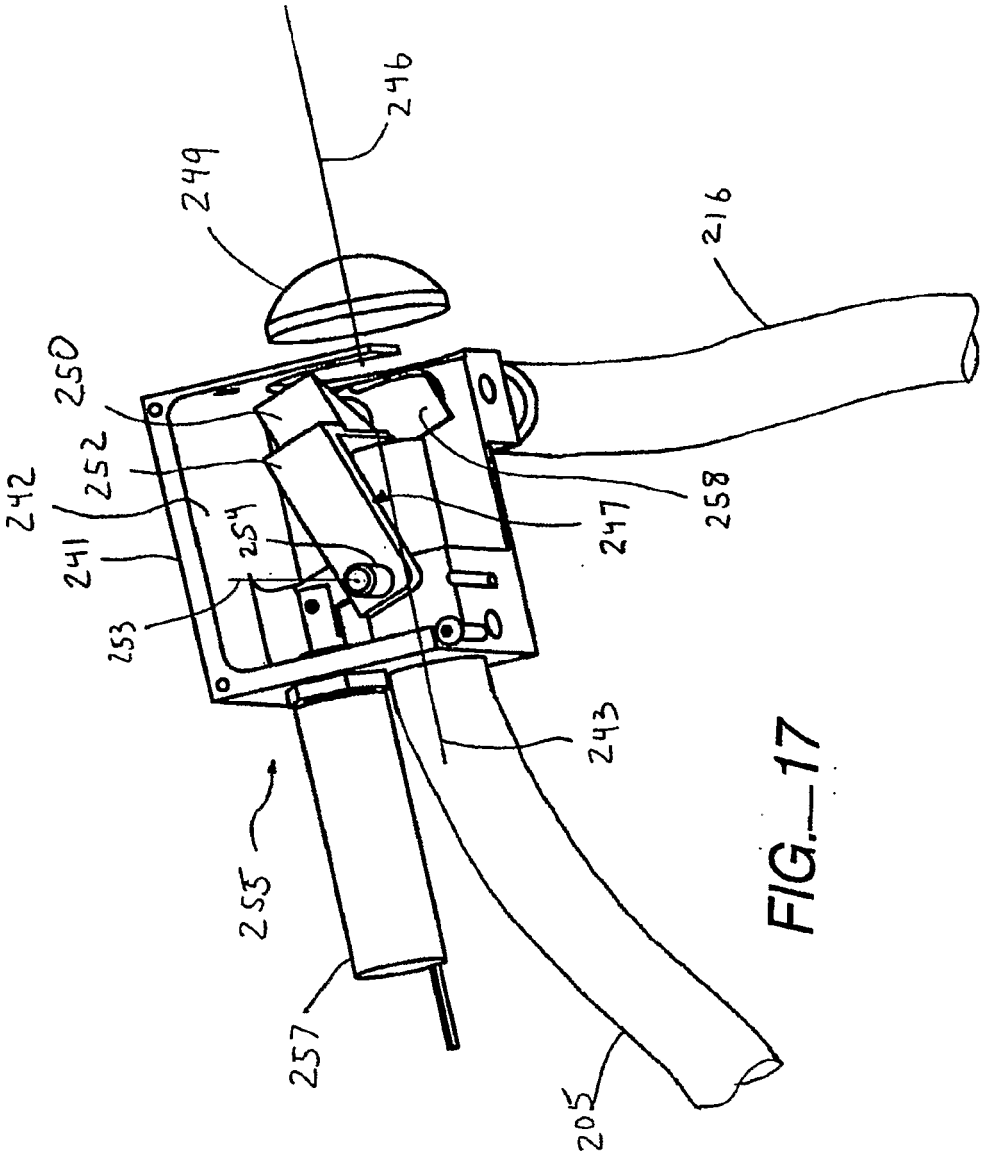

FIG._17

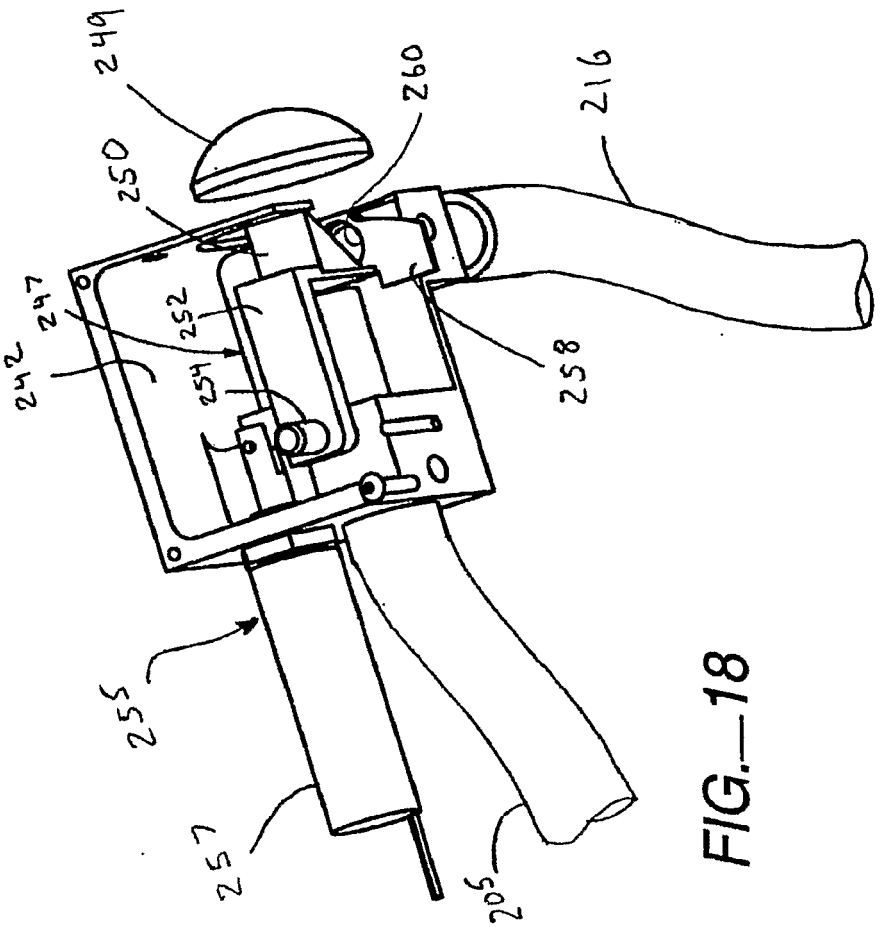

FIG.—18

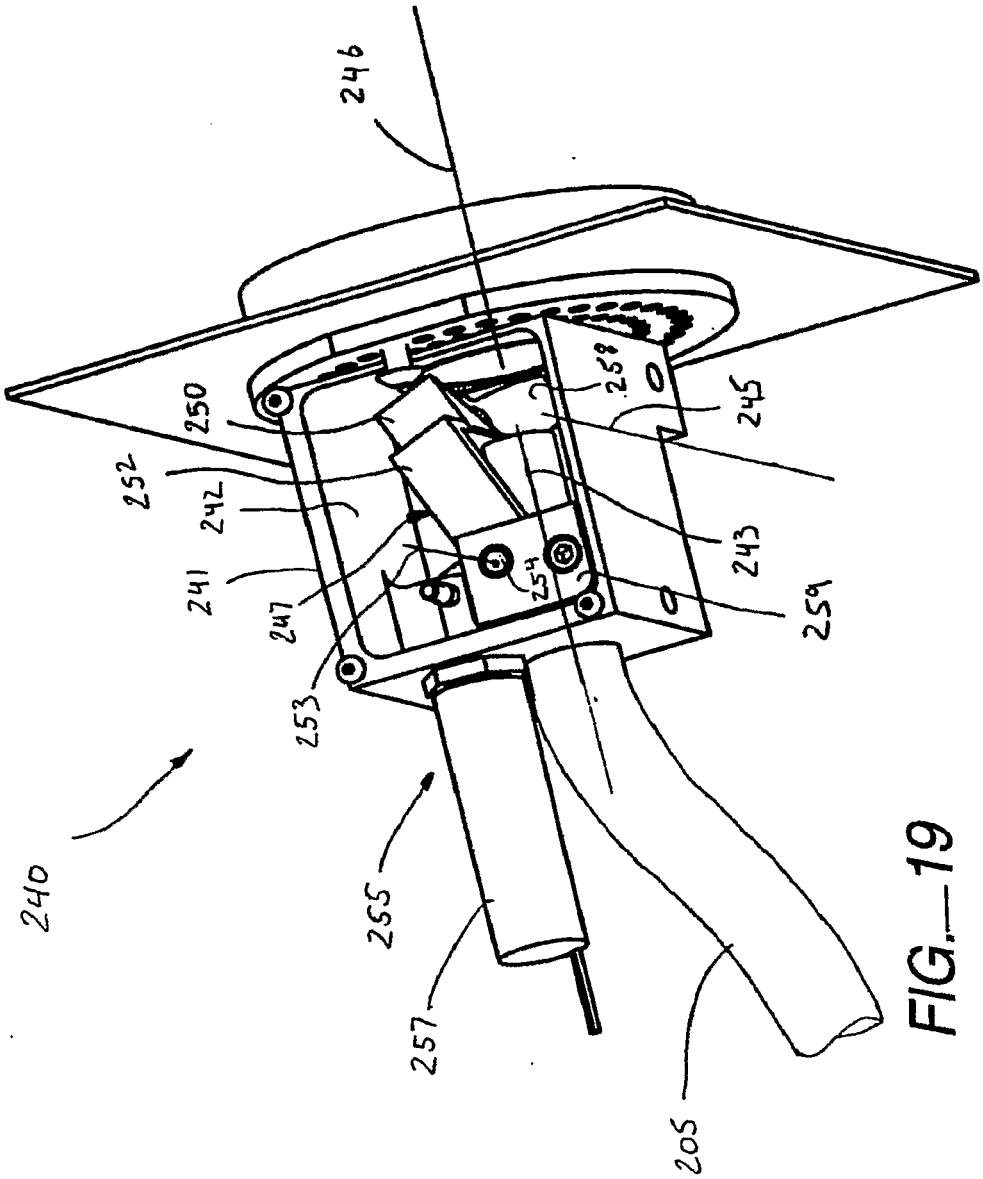

FIG.—19

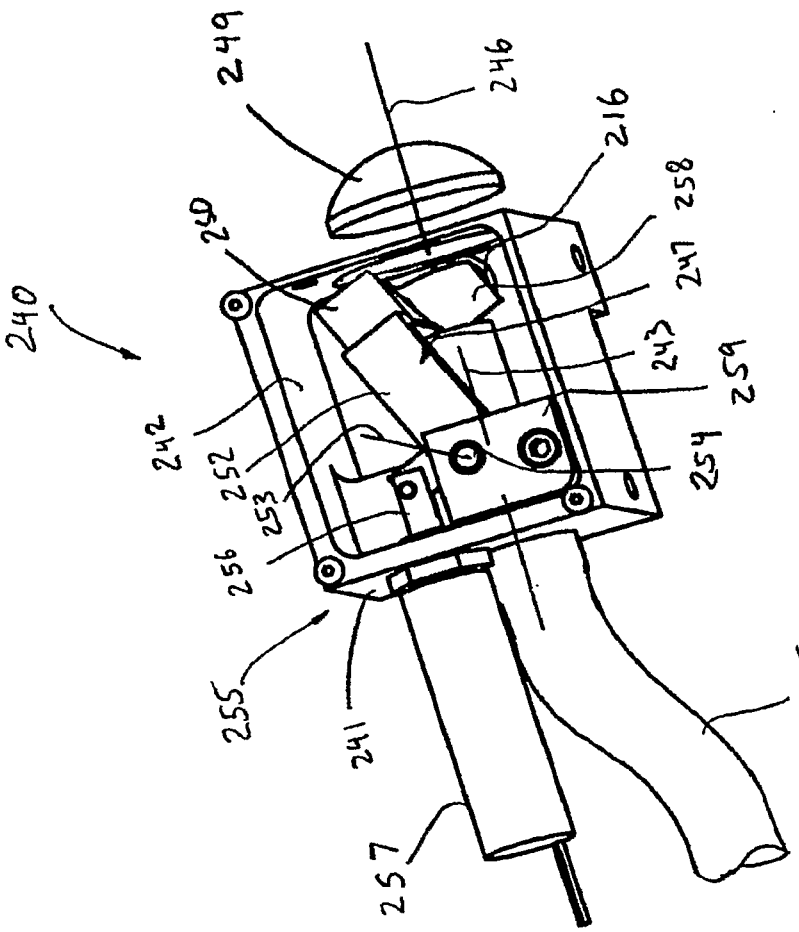
240
242
249
246
216
250
258
247
243
259
254
252
253
256
255
241
257
205

FIG.—20

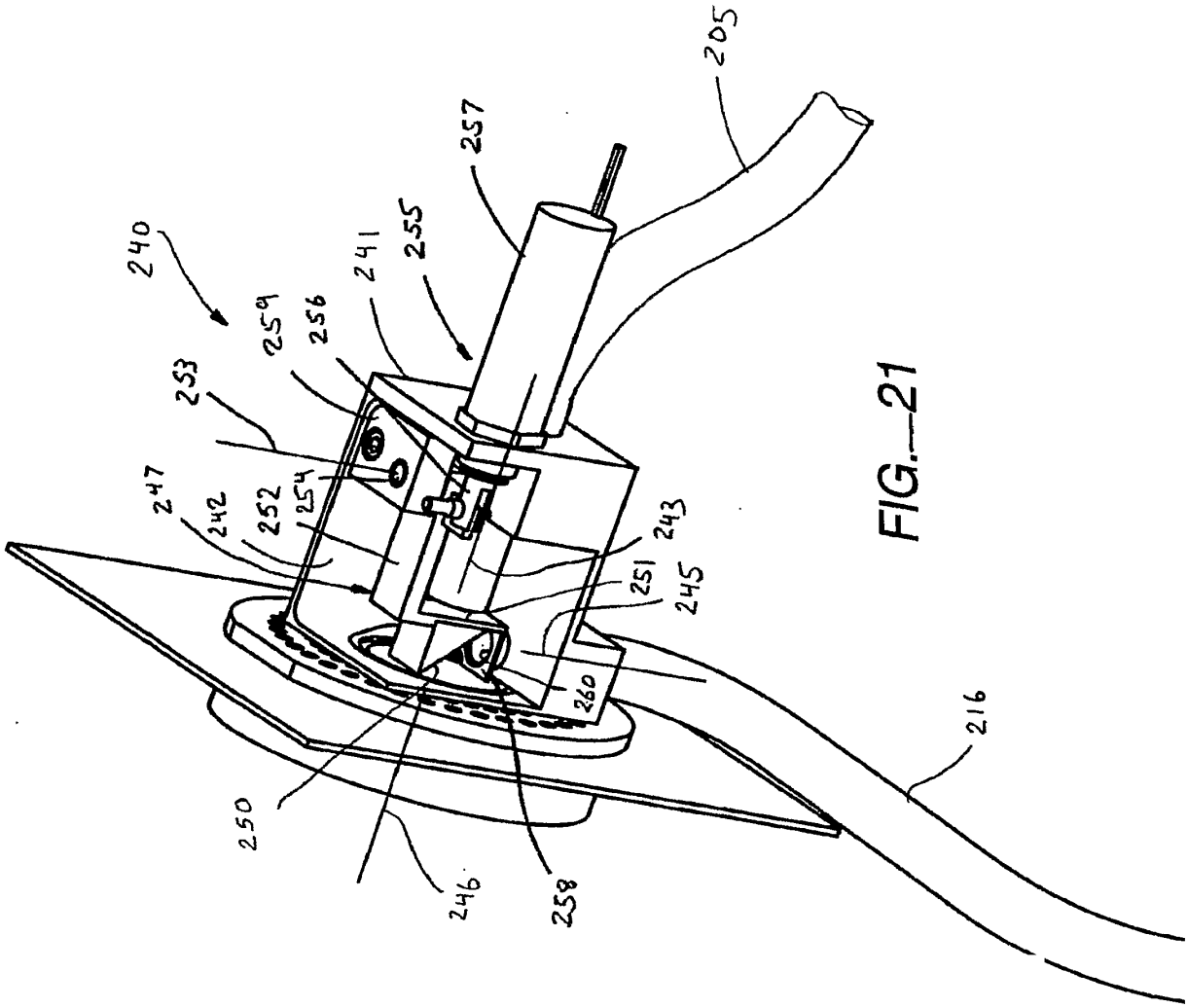
205
257
240
241
255
259
256
253
247
242
252
254
243
251
245
260
216
250
246
258

FIG._21

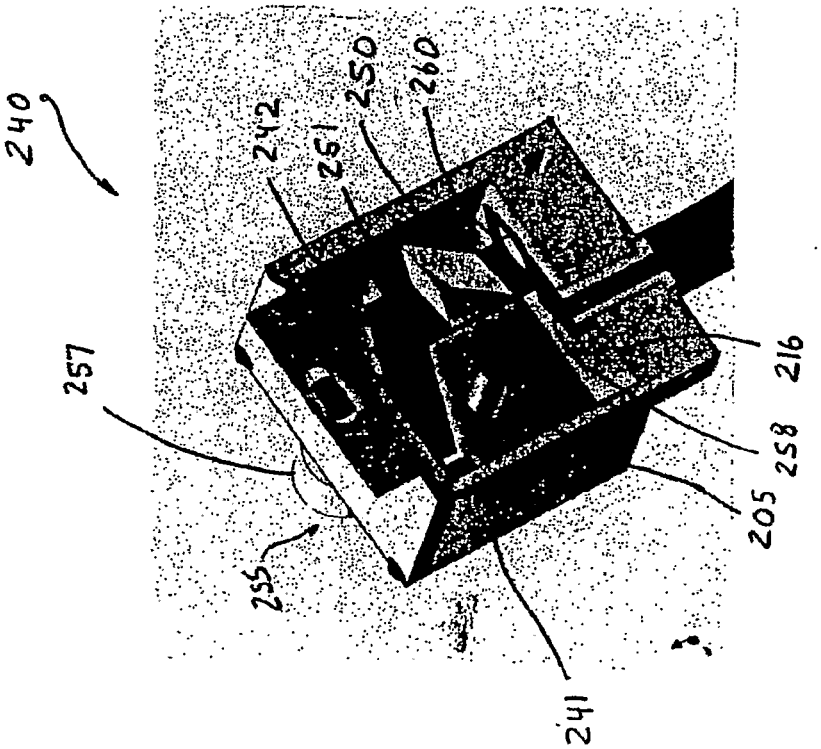

FIG.—22

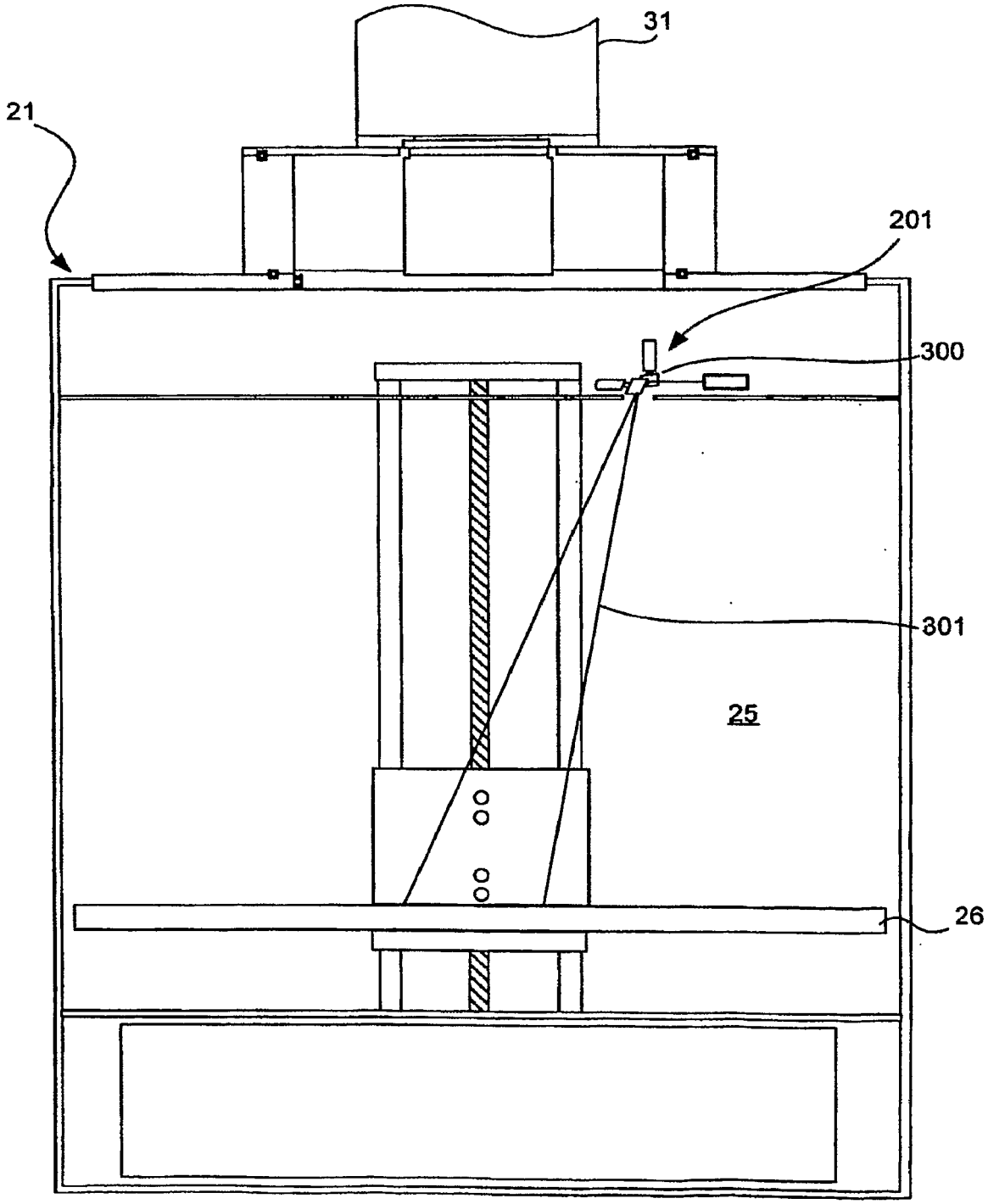

FIG. 23

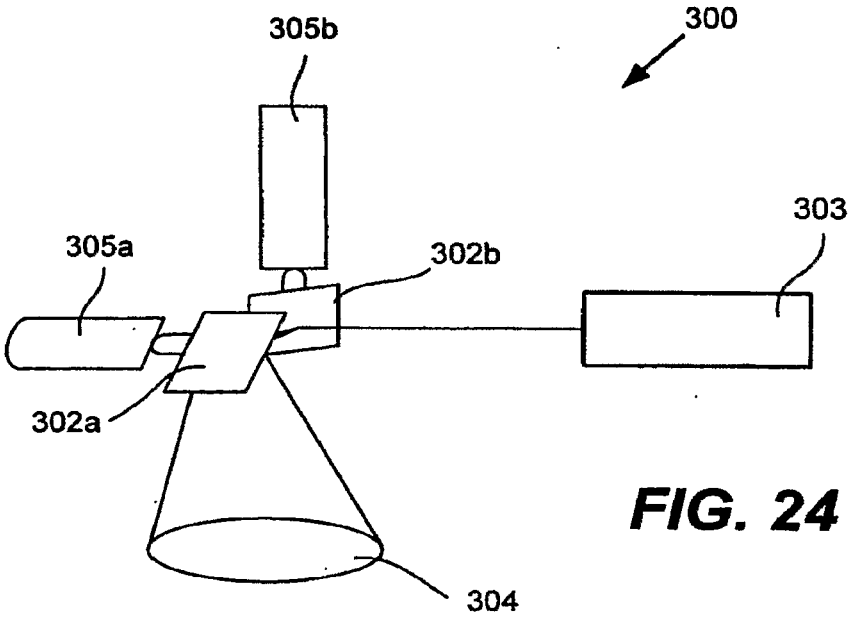

FIG. 24

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	一种系统，包括双照明系统和使用所述系统的成像设备和方法		
公开(公告)号	EP2021774B1	公开(公告)日	2016-07-27
申请号	EP2007777063	申请日	2007-05-14
申请(专利权)人(译)	精诺CORPORATION		
当前申请(专利权)人(译)	精诺CORPORATION		
[标]发明人	NILSON DAVID RICE BRAD TROY TAMARA		
发明人	NILSON, DAVID RICE, BRAD TROY, TAMARA		
IPC分类号	A61B5/00 G01N21/47 G01N21/64 G01N21/17		
CPC分类号	G01N21/6456 A61B5/0059 A61B5/0064 A61B5/0073 A61B2503/40 G01N21/4795 G01N2021/1787 G01N2201/0635		
优先权	11/434606 2006-05-15 US 11/434605 2006-05-15 US		
其他公开文献	EP2021774A2		
外部链接	Espacenet		

摘要(译)

公开了一种用于成像设备的双照明系统。成像设备限定了具有内壁的不透光成像隔间，内壁具有延伸到成像隔室中的观察口。该观察口能够获取包含在成像室中的生物样本的数据。双照明系统包括第一照明组件，第一照明组件构造成将结构光引导到样本的第一侧上，以实现其结构光和表面形貌测量。然后，第二照明组件将光导向样本，其中漫射的荧光从其表面发出，以通过观察口接收，以获取样本的荧光数据。结构光成像和荧光成像的组合使得能够进行荧光探针定位和浓度的3D漫射断层摄影重建。

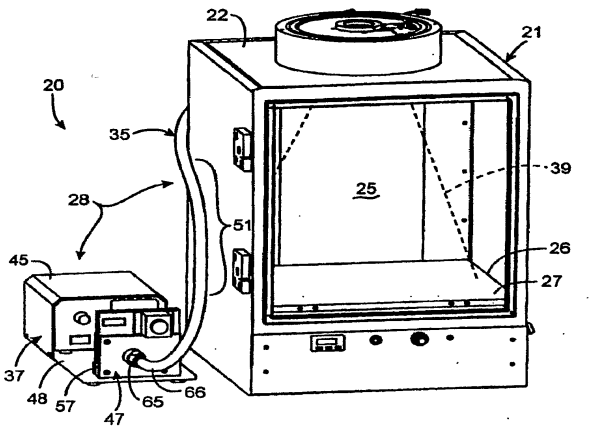

FIG. 1