(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 April 2007 (12.04.2007)

PCT

(10) International Publication Number
WO 2007/041382 A1

(51) International Patent Classification:
A61B 5/00 (2006.01) *G01B 9/02* (2006.01)
G02B 21/00 (2006.01) *G01N 21/64* (2006.01)

(21) International Application Number:
PCT/US2006/038223

(22) International Filing Date:
29 September 2006 (29.09.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/721,802 29 September 2005 (29.09.2005) US

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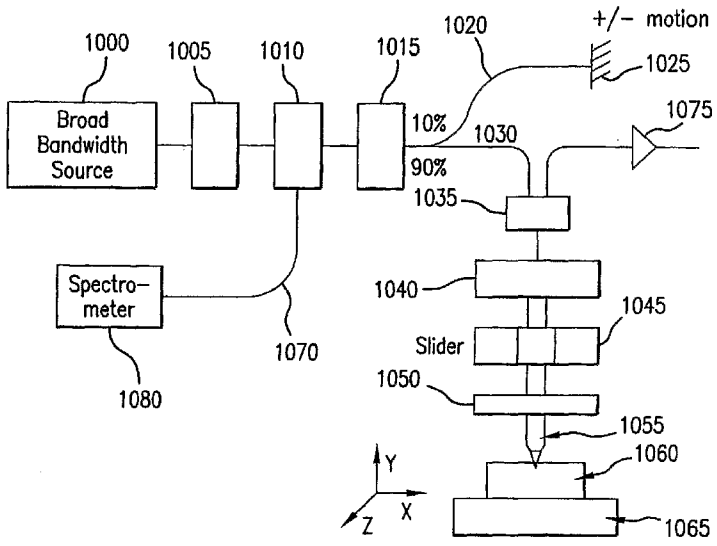
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ,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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) **Title:** ARRANGEMENTS AND METHODS FOR PROVIDING MULTIMODALITY MICROSCOPIC IMAGING OF ONE OR MORE BIOLOGICAL STRUCTURES



(57) **Abstract:** Method and apparatus according to an exemplary embodiment of the present invention can be provided. For example, first data associated with a first signal received from at least one region of at least one sample can be provided based on a first modality, and second data associated with a second signal received from the at least one sample can be provided based on a second modality which is different from the first modality. Third data associated with a reference can be received. Further data can be generated based on the first, second and third data. In addition, third data associated with a second signal received from the at least one sample can be obtained. Each of the third data can be based on a further modality which is different from the first modality and the second modality, and the further data can be further determined based on the third data. Further, the first modality can be a spectral-encoded modality, and the second modality can be a non-spectral-encoding modality.

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ARRANGEMENTS AND METHODS FOR PROVIDING MULTIMODALITY MICROSCOPIC IMAGING OF ONE OR MORE BIOLOGICAL STRUCTURES

CROSS-REFERENCE TO RELATED APPLICATION(S)

[0001] This application is based upon and claims the benefit of priority from U.S. Patent Application Serial No. 60/721,802, filed September 29, 2005, the entire disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention generally relates to arrangements and methods for providing multimodality microscopic imaging of one or more biological structures, and particularly to, e.g., conducting reflectance and/or fluorescence microscopy of biological specimens using spectrally encoded confocal microscopy ("SECM"), fluorescence SECM, optical coherence tomography ("OCT"), spectral domain ("SD")-OCT, optical frequency domain interferometry ("OFDI"), and optical coherence microscopy ("OCM") procedures.

BACKGROUND OF THE INVENTION

[0003] A determination of the relationship between the molecular basis of genetic alterations and phenotype generally utilizes accurate two- and three-dimensional characterization of microstructure of biological specimens. However, motion and small dimensions make many living biological specimens can be more difficult to evaluate.

[0004] Optical techniques offer the potential to image the biological specimens at a high resolution. For certain applications, optical imaging based on endogenous contrast can be advantageous over techniques that require exogenous agents, since such beneficial procedures can allow the analysis of the specimen in its native state and at multiple time points, with a small amount of preparation. As an example, several endogenous-contrast imaging modalities are described herein for visualizing embryonic heart microstructure: two exemplary forms of optical coherence tomography ("OCT") as described in D.

Huang et al., "Optical coherence tomography," *Science* 254, pp. 1178-1181 (1991), time-domain optical coherence tomography ("TD-OCT") as described in S. A. Boppart et al., "Investigation of developing embryonic morphology using optical coherence tomography," *Dev Biol* 177, pp. 54-63 (1996), and optical frequency domain imaging ("OFDI") as described in M. A. Choma et al., "Sensitivity advantage of swept source and Fourier domain optical coherence tomography," *Optics Express* 11, pp. 2183-2189 (2003); and S. H. Yun et al., "High-speed optical frequency-domain imaging," *Optics Express* 11, pp2953-2963 (2003).

[0005] Additional examples can be provided and utilized including two reflectance microscopy techniques, e.g., full-field optical coherence microscopy ("FFOCM") as described in E. Beaurepaire et al., "Full-field optical coherence microscopy," *Optics Letters* 23, pp. 244-246 (1998); A. Dubois et al., "Ultrahigh-resolution full-field optical coherence tomography," *Appl Opt* 43, pp. 2874-2883 (2004); and G. Moneron et al., "Stroboscopic ultrahigh-resolution full-field optical coherence tomography," *Opt Lett* 30, pp. 1351-1353 (2005), and spectrally encoded confocal microscopy ("SECM") as described in G. J. Tearney et al., "Spectrally encoded confocal microscopy," *Optics Letters* 23, pp. 1152-1154 (1998); and C. Boudoux et al., "Rapid wavelength-swept spectrally encoded confocal microscopy," *Optics Express* 13, pp. 8214-8221 (2005).

[0006] For example, the TDOCT techniques can use low-coherence interferometry to obtain cross-sectional images with ~10 μm resolution and at depths of up to 2 mm. (See S. A. Boppart et al., "Noninvasive assessment of the developing Xenopus cardiovascular system using optical coherence tomography," *Proc Natl Acad Sci U S A* 94, pp. 4256-4261 (1997); S. Yazdanfar et al., "High resolution imaging of *in vivo* cardiac dynamics using color Doppler optical coherence tomography," *Optics Express* 1, pp. 424-431 (1997); T. M. Yelbuz et al., "Optical coherence tomography: a new high-resolution imaging technology to study cardiac development in chick embryos," *Circulation* 106, pp. 2771-2774 (2002); V. X. D. Yang et al., "High speed, wide velocity dynamic range Doppler optical coherence tomography (Part II): Imaging in vivo cardiac dynamics of Xenopus laevis," *Optics Express* 11, pp. 1650-1658 (2003); and W. Luo et al., "Three-

dimensional optical coherence tomography of the embryonic murine cardiovascular system " *Journal of biomedical optics* 11, 021014 (2006).

[0007] The exemplary OFDI technique can be considered as a derivative of the TDOCT techniques that may enable an acquisition of images at significantly higher frame rates as described in R. Huber et al., "Three-dimensional and C-mode OCT imaging with a compact, frequency swept laser source at 1300 nm," *Optics Express* 13, pp. 10523-10538 (2005). The high speed of the OFDI techniques can enable an implementation of a true four-dimensional (4D) microscopy (e.g., three-dimensional microscopy as a function of time). Full-field optical coherence microscopy ("FFOCM") techniques can utilize low-coherence interferometry and higher numerical aperture objective lenses to attain resolution at the subcellular level in all three dimensions. Such FFOCM techniques are likely considerably slower than the OFDI techniques. The exemplary SECM techniques can have a form of the reflectance confocal microscopy using which it may be possible to obtain two-dimensional images with micron-level resolution, at significantly higher speeds than possibly obtained using the FFOCM techniques.

[0008] While each of these natural-contrast procedures can individually be used for imaging a microstructure of the embryonic heart, when combined, these procedures can provide a powerful set of tools for two-, three-, and four-dimensional characterization of early myocardial morphology and dynamics. A combination of these different modalities into one single microscopy device may have additional advantages such as, e.g., (a) a comparison of images in different formats, different resolutions, and fields of view, (b) a simultaneous acquisition of both structural and function information, and/or (c) these tasks can be accomplished using one instrument without requiring moving or altering the specimen.

OBJECTS AND SUMMARY OF THE INVENTION

[0009] One of the objects of the present invention is to overcome certain deficiencies and shortcomings of the prior art systems (including those described herein above), and provide exemplary embodiments of providing multimodality microscopic imaging of one

or more biological structures. Such exemplary embodiments can conduct reflectance and/or fluorescence microscopy of biological specimens using spectrally encoded confocal microscopy ("SECM"), fluorescence SECM, optical coherence tomography ("OCT"), spectral domain ("SD")-OCT, optical frequency domain interferometry ("OFDI"), and optical coherence microscopy ("OCM") procedures.

[0010] For example, an analysis of biological specimens generally employs a visualization of its microstructure and functions, preferably with small alterations to the specimen. According to one exemplary embodiment of the present invention, a combination of multiple different imaging modalities can be provided in a single microscope device. Each exemplary technique according to certain exemplary embodiments of the present invention can provide distinct and complementary imaging capabilities, including high-speed (e.g., 1000 frames per second) and high axial resolution (4-16 μm) cross-sectional imaging in vivo, true four-dimensional imaging in vivo, three-dimensional microscopy with isotropic cellular (e.g., 1-2 μm) resolution in vitro, and two-dimensional subcellular imaging in vivo. When combined, these exemplary imaging modalities can effectuate a more complete picture of the morphologic and dynamics of biological specimens.

[0011] Thus, the exemplary embodiments of the present invention include arrangements and methods for acquiring multimodality microscopic data. For example, according to one exemplary embodiment, it is possible to use a combination of unique broad bandwidth or rapid wavelength swept sources and optics interposed between a scanning mechanism and an imaging lens. Data can be acquired simultaneously and/or serially, e.g., without moving the specimen. For example, data obtained from different modalities can be co-registered so that it can be displayed side-by-side and/or overlaid on top of each other. Quantitative information can be obtained from all of the datasets in a complementary manner.

[0012] Thus, in accordance with the exemplary embodiments of the present invention, method and apparatus can be provided. For example, first data associated with a first signal received from at least one region of at least one sample can be provided

based on a first modality, and second data associated with a second signal received from the at least one sample can be provided based on a second modality which is different from the first modality. Third data associated with a reference can be received. Further data can be generated based on the first, second and third data. In addition, third data associated with a second signal received from the at least one sample can be obtained. Each of the third data can be based on a further modality which is different from the first modality and the second modality, and the further data can be further determined based on the third data. Further, the first modality can be a spectral-encoded modality, and the second modality can be a non-spectral-encoding modality.

[0013] In another exemplary embodiment of the present invention, the first modality can be florescence imaging. A microscope arrangement and/or a beam-scanning arrangement can be provided. The beam-scanning arrangement may be configured to forward electro-magnetic radiation to the at least region. Further, a two-dimensional image and/or a three-dimensional image can be produced as a function of the further data. The first and second data may be obtained substantially simultaneously. In addition, the first and second data may be associated with approximately the same location on the sample, and/or can be obtained using another one of the first and second data.

[0014] According to a further exemplary embodiment of the present invention, the apparatus can be provided in a probe and/or a single enclosure. It is also possible to obtain spectral encoding microscopy information using such exemplary apparatus and method, as well as bright field, dark field, phase contrast, polarization, epireflectance and/or reflectance microscopy information. It is further possible to use such exemplary apparatus and method change from the first modality to the second modality. Optical coherence tomography information associated with a signal provided by a source arrangement having a plurality of wavelengths can be obtained. A plurality of detectors can be provided to detect a spectral interference between the second and third signals as a function of the wavelengths.

[0015] Optical coherence tomography information associated with a signal provided by a source arrangement can be obtained whose wavelength varies over time. At least

one image can be generated based on the first and second data. In addition, a first image can be generated based on the first data and a second image can be generated based on the second data. The first and second images may be associated with one another as a function of the first and second data. It is possible to obtain optical coherence tomography information and/or optical frequency domain interferometry information.

[0016] Other features and advantages of the present invention will become apparent upon reading the following detailed description of embodiments of the invention, when taken in conjunction with the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Further objects, features and advantages of the present invention will become apparent from the following detailed description taken in conjunction with the accompanying figures showing illustrative embodiments of the present invention, in which:

[0018] Figure 1 is a schematic diagram of an exemplary SECM system that utilizes a broad bandwidth source;

[0019] Figure 2 is a schematic diagram of an exemplary SD-OCT system;

[0020] Figure 3 is a schematic diagram of an exemplary OCM system that utilizes a broad bandwidth source;

[0021] Figure 4 is a schematic diagram of an exemplary FFOCM system that utilizes a broad bandwidth source;

[0022] Figure 5 is a schematic diagram of an exemplary fluorescence SECM system that utilizes a broad bandwidth source;

[0023] Figure 6 is a schematic diagram of an exemplary SECM system that utilizes a wavelength tuning source;

[0024] Figure 7 is a schematic diagram of an exemplary OFDI system that utilizes a wavelength tuning/modulated source;

[0025] Figure 8 is a schematic diagram of an exemplary OCM system that utilizes a wavelength modulated/tuning source;

[0026] Figure 9 is a schematic diagram of an exemplary FFOCM system that utilizes a wavelength modulated/tuning source;

[0027] Figure 10 is a schematic diagram of an exemplary combined SECM/SD-OCT/OCM system that utilizes a broad bandwidth source according to a first exemplary embodiment of the present invention;

[0028] Figure 11 is a schematic diagram of an exemplary combined SECM/SD-OCT/FFOCM system that utilizes a broad bandwidth source according to a second exemplary embodiment of the present invention;

[0029] Figure 12 is a schematic diagram of exemplary multimodality microscope sliders according to a particular exemplary embodiment of the present invention;

[0030] Figure 13 is a schematic diagram of an exemplary combined SECM/OFDI/OCM system that utilizes a wavelength tuning source according to a third exemplary embodiment of the present invention;

[0031] Figure 14 is a schematic diagram of an exemplary combined SECM/OFDI/FFOCM system that utilizes a wavelength tuning source according to a third exemplary embodiment of the present invention;

[0032] Figures 15a-15m are various exemplary images of Xenopus laevis hearts (stage 49) in vivo using exemplary embodiments of the TDOCT and OFDI procedures.

[0033] Figures 16a-16m are various exemplary three-dimensional images of Xenopus heart in vitro using exemplary embodiments of the FFOCM procedure.

[0034] Figures 17a-17h are exemplary high-resolution confocal images obtained in vivo using the exemplary embodiments of the SECM procedure;

[0035] Figures 18a-18e are exemplary images of an aneurismal dilatation in the Xenopus heart obtained using the exemplary embodiments of the method and arrangements according to the present invention; and

[0036] Figures 19a-19x are exemplary images of abnormal heart formation due to ethanol exposure using the exemplary embodiments of the method and arrangements according to the present invention.

[0037] Throughout the figures, the same reference numerals and characters, unless otherwise stated, are used to denote like features, elements, components or portions of the illustrated embodiments. Moreover, while the subject invention will now be described in detail with reference to the figures, it is done so in connection with the illustrative embodiments. It is intended that changes and modifications can be made to the described embodiments without departing from the true scope and spirit of the subject invention as defined by the appended claims.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

[0038] Exemplary SECM techniques are capable of providing subcellular level resolution images in tissue or biological specimens. SECM images can alternatively represent fluorescence from the sample or reflectance from the sample. Figure 1 depicts a schematic diagram of an exemplary SECM arrangement which utilizes a broadband source. In this exemplary configuration, a quasimonochromatic or broadband light 100 illuminates a circulator 110, which alternatively may be a beam splitter. In one embodiment this circulator or beam splitter is fiber-optic coupled. The core of the optical fiber can serve as the pinhole for the confocal microscope system. The fiber may alternatively have multiple claddings that transmit light such that for example the light exciting the sample is single mode and the collected light is multimode. Light from this element may be incident on a scanning mechanism 115 that scans the angle of the beam so as to produce one or more transverse scans on the sample. The scanning mechanism

may alternatively be one of a resonant scanner, galvanometer scanner, polygon scanning mirror, acousto-optic scanner or the like. A telescope apparatus may be used to image the scan axis to the back focal plane of the objective lens 130. Light from the scanning mechanism can then be directed towards a wavelength dispersing element 120 such as a transmission diffraction grating, prism, grating prism, dual prism grating prism (DP-GRISM) or the like. This exemplary element may disperse the different wavelengths in the broad bandwidth source so that it is incident on the objective lens 130 with varying angles that depend on wavelength.

[0039] In one exemplary embodiment, the lens can have a numerical aperture that may produce a small focused spot or alternatively the lens has a high NA >0.2. The objective lens 130 focuses each wavelength region onto the sample where each wavelength region on the sample 160 that can be located at a different spatial location. For a diffraction grating and an objective lens, these exemplary elements may form a wavelength encoded line 140 on the sample where each position on the line is encoded by a different wavelength region. Light from the sample 160 can be reflected back through the exemplary system of Figure 1. Out-of-focus light may be rejected by the cladding of the optical fiber and in focus (e.g., confocal) light is transmitted back through the circulator/beam splitter 110 to a spectrometer that measures the spectral content of the returned light 145. Confocal remittance as a function of spatial location is decoded by measuring this spectrum, forming one line on an image. Successive lines are formed for each angular position of the scanning mechanism 115, forming a spectrally-encoded confocal microscopy image.

[0040] Figure 2 depicts a schematic diagram of an exemplary spectral-domain OCT system. Contrary to the exemplary SECM system, the exemplary SD-OCT can provide cross-sectional images of a biological specimen by using coherence gating in the Fourier domain. SD-OCT images can typically have a lower resolution (~3-10 μm), and may have a larger field of view (several mm's). In this exemplary SD-OCT system, a broad bandwidth or quasimonochromatic source 200 can be input into an interferometer, which may be fiber optic-based. The fiber-coupled light can be transmitted to a circulator 210 and a beam splitter 220. When coupled into the circulator 210, the light can preferably be

subsequently split by a beam splitter 220 so that a portion thereof can be transmitted to a reference arm 225 and a portion is transmitted to a sample arm 235. Light from the reference arm 225 can be reflected off a mirror 230 (e.g., a reference) to the beam splitter 220 or alternatively transmitted back to the beam splitter 220. In one exemplary embodiment, the splitter 220 can be configured so that the majority of light is transmitted to the sample arm 235. Light from the sample arm fiber can then be directed towards a lens and a scanning mechanism 240. The scanning mechanism can scan the light of the sample arm 235 in arbitrary one- or two-dimensional patterns. Light can be transmitted from the scanning mechanism to a lens 250 which, in one exemplary embodiment, can have a NA so that the confocal parameter is sufficiently large to allow cross-sectional imaging in the biological specimen or sample 260.

[0041] Light remitted from the sample may be transmitted back through the apparatus to the circulator/beam splitter 210, and directed to a spectrometer 280. The reflectance as a function of depth (A-line) within the tissue may be reconstructed by, e.g., a background subtraction, remapping λ-space to k-space, and inverse Fourier transformation of the spectral interference signal in a central processing unit or computer 290. Successive A-lines are obtained for each scanning mechanism position, thereby reconstructing a cross-sectional image of the sample. Alternative exemplary embodiments known in the art, including the capability to obtain spectral information from the sample by short-time-Fourier transformation ("STFT") of the spectral interference, Doppler-sensitive SD-OCT and polarization-sensitive SD-OCT, may be also utilized to extract additional information from the biological specimen, such as absorption, flow, and birefringence.

[0042] Figure 3 depicts a schematic diagram of an exemplary optical coherence microscopy ("OCM") system. The exemplary OCM system can utilize a combination of confocal microscopy and OCT techniques that may be advantageous, as the axial point spread functions of both such exemplary techniques may be multiplied so as to provide a greater degree of optical sectioning. In one exemplary embodiment of the OCM system, light from a broad bandwidth source can be input into a modulating element 310 so that the modulation frequency approximates that of the spectral interference in the interferometer. This exemplary modulation element may be one of a Michelson

interferometer, pulse shaping apparatus, spectral filter, etc. The modulation may also shift the spectral phase by some amount over time so that successive spectra may be subtracted to extract only the spectral interference term. Following the modulating element, the light can be transmitted to a circulator/beam splitter 320 and then, if a circulator is used, to a beam splitter 330. Light can again be transmitted to a reference arm 335 and a sample arm 345. Light from the reference arm 335 is reflected by a mirror 340. Light from the sample arm 345 can be transmitted to an x-y scanner 350, which can alternatively be one of a or a combination of a resonant scanner, galvanometer scanner, polygon scanning mirror, acousto-optic scanner or the like.

[0043] Light from the scanner 350 can be directed to an objective lens 355 so that a tightly focused spot can be scanned within the sample. The objective lens or sample 360 may be alternatively scanned in any of three dimensions to facilitate data collection from different portions within the sample. Light is transmitted back from the sample 360 to the circulator/beam splitter 320 and subsequently to a detection apparatus. In one embodiment, the detector is a spectrometer and OCM data is obtained by obtaining A-lines from the sample in a similar manner as performed by the exemplary SD-OCT. In the spectral modulation embodiment the detector can alternatively be a photodiode or other single detector that is synchronized to the source modulation element 310. Exemplary lock-in or subtraction techniques can be utilized to extract the OCM signal.

[0044] Full-field optical coherence microscopy is typically a free-space interferometric technique that utilizes a broad bandwidth source to obtain transverse, high-resolution optical sections of biological specimens. Figure 4A depicts a schematic diagram of an exemplary FFOCM system, where broad bandwidth light 400 is transmitted to a beam splitter 410. Light is split into the sample arm 423 and the reference arm 422. Light in the reference arm 422, according to one exemplary embodiment, may be directed 415 towards a reference objective lens 420 and to a mirror 425, which is capable of an axial motion. Light in the sample arm 423 may be directed towards a sample objective lens 430 and to the sample 440. In one exemplary embodiment, the reference and sample objectives 420, 430 have the similar characteristics.

[0045] In another exemplary embodiment, the objective lenses 420, 430 may be optimized for use with immersion fluid that has a refractive index that is similar to the sample. The sample can be coupled to a stage 443 that provides motion in any of three-dimensions. Light from the reference arm 422 and the sample arm 423 can be imaged using a lens 445 onto a CCD camera 450. Fringes are detected by the CCD camera 445 resulting from the interference of the sample arm 422 and the reference arm 423. Multiple images can be typically detected for different positions of the reference arm mirror 425. The exemplary images may be arithmetically combined to extract the information from an optical section within the sample.

[0046] In another exemplary embodiment of the FFOCM system as depicted in Figure 4B, a broad bandwidth light source 451 can be coupled into a modulating element, such as a Michelson interferometer or other interferometer (e.g. Mach-Zehnder, Sagnac) or spectrum altering unit. For the Michelson interferometer case, light from the source 451 can be transmitted to a beam splitter 452. Light may then be split into two arms for exemplary arm A 453 and arm B 455. Light from arm A 453 is transmitted to a mirror and backreflected back to a beam splitter. Light from arm B 455 is likewise transmitted to a mirror 456, and backreflected back to a beam splitter 452. The difference between the path length L_a in arm A and the path length L_b in arm B, $|L_a - L_b|$, can be set to be substantially equal to the path length difference between the reference and sample arms in the second interferometer. At least one of the arms A or B can be configured to change path lengths or produce a phase shift in the light therein. In one exemplary embodiment, the path length may be changed by a motion of one of the mirrors or a rapidly scanning optical delay line. The motion may be actuated by a piezoelectric transducer, galvanometer, linear motor or the like. Alternatively, path length changes may be generated by one of an acousto-optic modulator or electro optic modulator.

[0047] Both reference and sample arm light may be combined at the beam splitter, and transmitted to another static interferometer with beam splitter 459, separating light into a reference arm 458 and a sample arm 457, respectively. Light from both arms 457, 458 can illuminate objective lenses 460, 470, respectively, which are substantially similar. In the reference arm 458, the reference objective lens 460 can be brought to a

focus on a reflector 465, which is typically not moving, whereas in the sample arm the sample objective lens 470 focuses the sample arm light on or within the sample 480. The sample 480 or the sample objective lens 470 may be mounted to a stage 481, capable of moving the sample 480 in any of three-dimensions, under manual control or computer control.

[0048] The path length difference between the path lengths of the reference arm 458 and the sample arm 457 may be substantially equal to $|L_a - L_b|$ of the first interferometer. Light from reference and sample arms 458, 457, respectively, can be combined at a beam splitter 459, and imaged onto a CCD array 490 or array of detectors via a lens 485. A FFOCM image or data can be generated by a linear combination of images acquired by CCD 490 and while moving or at different positions of mirror 456. Processing, display and storage of FFOCM images is provided by a CPU 495. Accumulations or averages are utilized to increase signal to noise ratio.

[0049] Figure 5 depicts an exemplary embodiment of a SECM system configured for a fluorescence detection and using a broad bandwidth source. For example, light from the source 500 can be transmitted to a beam splitter 510, which splits light into two paths 515 and 520. Both arms/paths terminate on mirrors 520 and 525, with at least one arm having a path length or phase that changes over time. Light returned from both arms 530 can be coupled to the beam splitter 510 and directed 535 towards a SECM probe containing a grating or dispersive element 540, an objective lens 550. As discussed herein, the arrangement of the grating and the objective lens 550 focuses a spectrally encoded line 560 on or within the specimen 562 which may be mounted to a three-dimensional stage. Fluorescent light within the sample can be excited by the illuminating light, transmitted back through the objective lens 550, imaged by another lens 565 onto a detector 570. Detected light can be digitized and converted to a line in an image by a processing arrangement (e.g., CPU) 580. Additional lines in the image may be generated at different positions of the beam scanning mechanism 537. Nonlinearities in the moving mirror can be corrected by an exemplary interferometer 521 that has a narrow bandwidth source that illuminates the same moving mirror 520. \

[0050] Figure 6 depicts a schematic diagram of an exemplary embodiment of an SECM system that uses a wavelength tuning source 600. For example, the source 600 can be coupled into a circulator/beam splitter 610. According to one exemplary embodiment, light from the splitter 610 is transmitted via an optical fiber to a scanner, which alternatively may also contain a telescope lens imaging system that projects the scan axis to the back focal plane of the objective lens 625. Light from the scanning mechanism is transmitted to a dispersive element 620 (such as a diffraction grating, prism, GRISM, or DP-GRISM, etc.). Light from 620 is transmitted to an objective lens 625, with preferably a high NA, which can focus the beam within the sample 635. At any point in time, one wavelength from the wavelength swept source 600 can illuminate a distinct portion of the sample. As the wavelength of the swept source 600 changes over time, the beam can be scanned along a line 630 within the sample 635. Remitted light from the sample 635 can be transmitted back through the elements 625, 620, and 615, respectively, spatially filtered by the optical fiber or a pinhole and transmitted back to the circulator beam splitter 610. Light from the splitter 610 can be directed to a detector 640, and digitized by a processing arrangement (e.g., CPU) 650, displayed and digitally stored. A single line in the image is obtained following one full sweep of the wavelength-tuning source. Lines may be acquired at different positions of the scanning mechanism to form the image. Fluorescent light excited by the wavelength-tuning source 600 remitted from the sample can be alternatively detected by a detector 660 to form a fluorescent image.

[0051] Figure 7 depicts a schematic diagram of an exemplary OFDI system. In one exemplary embodiment of this exemplary OFDI system, a wavelength tuning source may be coupled to an optical fiber-based circulator 705 and a beam splitter 705. Light from the circulator 705 can be transmitted to the beam splitter 705, configured to send a majority of light, in the preferred embodiment, to the sample arm 725. Such split light forwarded to the reference arm 715 can be terminated by a reflector 720, and sent back to the beam splitter 710 and the circulator 705. Light in the sample arm 725 is transmitted to a scanning mechanism 730 and an imaging lens 735 that has a NA sufficiently low to allow cross-sectional imaging of the biological specimen 740. Light is reflected from the reference mirror 720 and the sample 740, recombined at the circulator 705, and directed

by an optical fiber 750 to a detector apparatus 755, which in an exemplary embodiment can contain dual-balanced detectors.

[0052] Light is digitized by the detector apparatus 755 and the digital signal is transmitted to a CPU 760. Spectral interference is processed in a manner similar to the processing using the exemplary SD-OCT system/procedure, e.g., the background is subtracted, λ-space is converted to k-space, and an inverse Fourier transform is performed to produce an A-line. A-lines can be acquired as a function of scanning mechanism position, creating a cross-sectional OFDI image. Alternative exemplary embodiments known in the art, including the capability to obtain spectral information from the sample by short-time-Fourier transformation (STFT) of the spectral interference, complex spectral domain processing, Doppler-sensitive OFDI and polarization-sensitive OFDI, may be also utilized to extract additional information from the biological specimen, such as absorption, flow, and birefringence.

[0053] Figure 8 depicts a schematic diagram of an exemplary embodiment of an OCM system which utilizes a wavelength tuning/modulated source. For example, a wavelength modulation arrangement 805 may produce a spectral pattern on the source, for example, a sinusoidal modulation of the spectrum, which may be altered over time to correspond to spectral interference modulation produced by interference between the sample and reference arms. Light from the source 800 and/or the modulation arrangement 805 can be coupled into a fiber-optic circulator/ beam splitter 810, and subsequently transmitted to a beam splitter 815 which preferably directs a majority of light to the sample arm 830.

[0054] Light in the reference arm 820 is directed towards a reference reflector 825 or a transmission element. Light in the sample arm 830 can be transmitted to an x-y scanner, which may comprise one or more of galvanometers, resonant scanners, polygon scanners, acousto-optic scanners, electro optic scanners, etc. Light from the scanner can be alternatively transmitted to a telescope 837 and an objective lens 840 with preferably a high NA. The objective lens 840 focuses the light within the sample 845, which is alternatively affixed to a three-dimensional stage 847. Light is returned from the sample

back through the elements 840, 837 and 835 and coupled back into preferable the core of an optical fiber or pinhole in the sample arm 831 to reject out-of-focus light. Light is directed to the circulator 810 and transmitted to a detector 855, digitized and transmitted to a CPU 860.

[0055] In one exemplary embodiment, OCM data can be obtained by obtaining A-lines from the sample in a similar manner to the way it is performed using the exemplary OFDI system and procedure. For example, in the exemplary spectral modulation system and procedure, the detector can be synchronized to the source modulation element 805. Lock-in or subtraction techniques can be utilized to extract the OCM signal in this case. An exemplary image can be generated by acquiring data for each position of the x-y scanning mechanism 835. Fluorescent light remitted from the sample can be further detected by use of a dichroic mirror or filter 853 and a second detector 865.

[0056] Figure 9 depicts an exemplary embodiment of an FFOCM system that utilizes a wavelength-tuning/modulated source 900. The light source may be tuned over its bandwidth or alternatively be modulated to contain a spectral modulation frequency substantially similar the frequency provided by spectral interference modulation of the interferometer. Light from the source 900 may be coupled into a beam splitter 905, and directed to a sample arm 910 and a reference arm 915, respectively, which are terminated by respective objectives 920, 930. The reference arm objective lens 920 focuses reference arm light onto a reflector, which is subsequently returned to the beam splitter 905. Sample arm light is focused by 930 onto or within the specimen 935. Light remitted from the sample is combined with the reference arm light at 905, and imaged by a lens 940 onto a CCD array 950. Images can be obtained for each wavelength of the wavelength swept source or different modulation patterns of the source and arithmetically combined by a CPU 960 to reconstruct an exemplary FFOCM optical section.

[0057] According to one exemplary embodiment of the present invention , the exemplary systems described above and alternative exemplary embodiments thereof may be combined to form a multimodality imaging system. This exemplary combination of systems and/or devices can be provided by creating separate systems, and configuring

their optics so that they can obtain images from the same portions of the biological specimen. Different wavelength, scanning, and detection mechanisms may be provided in such combined modality system. Alternatively, the different devices can be implemented using many common components, which they share to provide a more efficient, cost-effective apparatus.

[0058] Figure 10 depicts a schematic diagram of a multimodality system according to an exemplary embodiment of the present invention that utilizes a broad bandwidth source 1000 and spectrometer 1080 to provide simultaneous and co-registered SD-OCT, OCM, SECM, and fluorescence SECM data and/or images. For example, light from the broad bandwidth source 1000 can be coupled alternatively to a spectral modulation unit 1005. Light from the spectral modulation unit 1005 is coupled into a circulator 1010 and a beam splitter 1015. If a circulator is utilized, light from the circulator 1010 is transmitted to the beam splitter 1015 that preferably directs a majority of light to the sample. Light in the reference arm 1020 is transmitted to a reference reflector 1025 that may move or otherwise change the path length of 1020, and/or which can be non-movable. If the reference arm is allowed to move, conventional time-domain OCT (e.g., TD-OCT) arrangement and/or procedures may be implemented or complex spectral domain may be obtained using the exemplary SD-OCT arrangement and/or procedures using processes that are known in the art.

[0059] Light in the sample arm 1030 is transmitted to a filter/dichroic/WDM apparatus 1035 that transmits the sample arm light in the direction from the beam splitter to the sample. Light from 1035 is directed to a beam scanning mechanism 1040 that is capable of scanning the beam in two directions at high or slow speeds. The beam scanning mechanism 1040 may also contain a telescope for imaging the scanners onto the back focal plane of the lens 1055. Light from the scanning mechanism 1040 can be transmitted to a slider 1045 that contains multiple optical elements. For example, when the slider 1045 is positioned at a distinct position, either one or more or a combination of SD-OCT, OCM, SECM and/or fluorescence OCM arrangements/procedures can be implemented. Light from the slider 1045 can be transmitted to an objective lens 1055 mounted to a lens turret in one embodiment that is capable of changing objective lenses.

The slider 1045 and/or turret 1050 may be under computer control for automatic selection of imaging modality. Light is focused by objective lens 1055 onto or within the sample 1060, which may be mounted to a computer-controlled three-dimensional translation stage 1065. Reflected light is transmitted back through the apparatus to 1010, which redirects the light to a spectrometer. Detected reflected light is processed to form exemplary SD-OCT, OCM, SECM images using the arrangements and/or procedures described herein.

[0060] As shown in Figure 10, fluorescent light may be redirected to a second detector via the filter/dichroic mirror/WDM apparatus 1035 to a second detector 1075. Fluorescent light from 1075 is utilized to reconstruct a fluorescent confocal image of the biological sample 1060. In the case where invisible near-infrared light is utilized, a visible aiming beam may be coupled into the exemplary system, coincident with the near-infrared light, to allow visualization of the locations of imaging. Alternatively or in addition, a white light image of the specimen under investigation may be provided by use of an alternative imaging port on the microscope. Alternative exemplary embodiments known in the art, including the capability to obtain spectral information from the sample by short-time-Fourier transformation (STFT) of the spectral interference, Doppler-sensitive SD-OCT and polarization-sensitive SD-OCT, may be also utilized to obtain additional information from the biological specimen, such as, e.g., absorption, flow, and birefringence.

[0061] An alternative exemplary multimodality embodiment configured to provide SD-OCT, OCM, SECM, and FFOCM images and data according to the present invention at a different wavelength from the other three modalities is depicted in Figure 11. In this exemplary embodiment a broad bandwidth source 1100 is coupled alternatively to a spectral modulation unit 1105. Light from the spectral modulation unit 1105 is coupled into a circulator 1110 and a beam splitter 1115. If the circulator 1110 is utilized, light from the circulator 1110 can be transmitted to the beam splitter 1115 that preferably directs a majority of light to the sample. Light in the reference arm 1120 is transmitted to a reference reflector 1125 that can be stationary and/or may or otherwise change the path length of the reference arm 1120. In case the reference arm 1120 is allowed to move,

exemplary conventional time-domain OCT (TD-OCT) procedures or complex spectral domain may be utilized for SD-OCT by methods known in the art. Light in the sample arm 1130 is transmitted a beam scanning mechanism 1135 that is capable of scanning the beam in two directions at high or slow speeds. The beam scanning mechanism 1135 may also include a telescope for imaging the scanners onto the back focal plane of the lens 1160. Light from the scanning mechanism 1135 is transmitted to a dichroic splitter/WDM 1140 that transmits the excitation light for SD-OCT, OCM, and SECM modalities, and can reflect FFOCM light.

[0062] For example, an exemplary FFOCM system similar to that shown in Figure 3 can be coupled into the beam path via 1140. Light from 1140 is directed to a slider 1150 that contains multiple optical elements; when the slider may be positioned at a distinct position, either one or a combination of SD-OCT, OCM, SECM or FFOCM is provided. Light from the slider 1150 is transmitted to an objective lens 1160 mounted to a lens turret 1155 in one embodiment that is capable of changing objective lenses. The slider 1150 and/or turret 1155 may be under computer control for automatic selection of imaging modality. Light is focused by the objective lens 1160 onto or within the sample 1165, which may be mounted to a computer-controlled three-dimensional translation stage 1170. Reflected light is transmitted back through the apparatus to the circulator 1110, which redirects the light to a spectrometer. Detected reflected light may be processed to form exemplary SD-OCT, OCM, SECM images by methods described herein. FFOCM light may be redirected to the FFOCM system 1175 via the filter/dichroic mirror/WDM apparatus 1140.

[0063] In the case where invisible near-infrared light is utilized, a visible aiming beam may be coupled into the exemplary system shown in Figure 11, coincident with the near-infrared light, to allow visualization of the locations of imaging. Alternatively or in addition, a white light image of the specimen under investigation may be provided by use of an alternative imaging port on the microscope. Alternative exemplary embodiments known in the art, including the capability to obtain spectral information from the sample by short-time-Fourier transformation (STFT) of the spectral interference, Doppler-sensitive SD-OCT and polarization-sensitive SD-OCT, may be also utilized to extract

additional information from the biological specimen, such as, e.g., absorption, flow, and birefringence.

[0064] Figure 12 depicts an exemplary embodiment of an arrangement of sliders that may be utilized for the multimodality imaging according to the present invention. For example, optical elements can be contained in a housing 1200 that may be translated manually, or under computer or automatic control. Each slider position can terminate in different slider positions 1205, 1210, 1230, 1260 that provide one or more imaging modalities. The slider position 1205, 1210, 1230, 1260 may be coupled to the objective lens turret. In one exemplary embodiment, the slider position 1205 contains no optical elements (air) or optical element windows. In this exemplary configuration, the microscope is configured to perform FFOCM. For the slider position 1210, a lens apparatus 1212 and 1213 can be configured to expand the beam and illuminate a DP-GRISM containing two prisms 1215 and 1225 that surround a transmission grating 1220. This exemplary configuration provides an ability to perform the SECM imaging. Exemplary OCM procedures can also be conducted in this position using a scanning mechanism that scans the spectrally-encoded line across the sample. For the slider position 1230, a lens apparatus 1240, 1250 can be configured to image beam angle, with or without beam magnification. This slider position 1230 can provide imaging using exemplary SDOCT procedures. For the slider position 1260, a lens apparatus 1270, 1280 is configured to expand the scanned beam to allow imaging using the exemplary OCM procedures.

[0065] While certain embodiments of the multimodality imaging systems have utilized a broad bandwidth source, exemplary embodiments of combined systems can also include wavelength tuning/modulated sources and single or multiple detector configurations, and such exemplary embodiment is shown in Figure 13. For example, in Figure 13, a wavelength tuning/modulated source 1300 is coupled into a circulator 1305 and a beam splitter 1310. If a circulator is utilized, light from the circulator 1305 is transmitted to a beam splitter 1310 that preferably directs a majority of light to the sample. Light in the reference arm 1315 is transmitted to a reference reflector 1320 that may be stationary, and may or otherwise change the path length of 1315. In case the

reference arm is allowed to move, conventional exemplary time-domain OCT (TD-OCT) procedures may be provided or complex spectral domain may be utilized for implementing OFDI modalities by methods known in the art. Light in the sample arm 1325 is transmitted to a filter/dichroic/WDM apparatus 1330 that transmits the sample arm light in the direction from the beam splitter to the sample. Light from 1330 is directed to a x-y beam scanning mechanism 1335 that is capable of scanning the beam in two directions at high or slow speeds.

[0066] The beam scanning mechanism 1335 may also include a telescope for imaging the scanners onto the back focal plane of the lens 1353. Light from the scanning mechanism 1335 is transmitted to a slider 1340 that contains multiple optical elements; when the slider is positioned at a distinct position, either one or a combination of OFDI, OCM, SECM or fluorescence OCM modalities can be provided. Light from the slider 1340 is transmitted to an objective lens 1353 mounted to a lens turret 1350 in one embodiment that is capable of changing objective lenses. The slider 1340 and/or turret 1350 may be manual, under computer control for automatic selection of imaging modality. Light is focused by objective lens 1353 onto or within the sample 1355, which may be mounted to a computer-controlled three-dimensional translation stage 1360. Reflected light is transmitted back through the apparatus to 1305, which redirects the light to a detector apparatus 1380 suitable for detecting OFDI, wavelength tuning OCM or SECM signals, images and/or data. Detected reflected light is processed by a CPU 1385 to form exemplary OFDI, OCM, SECM images by methods described above.

[0067] Fluorescent light may be redirected to a second detector via the filter/dichroic mirror/WDM apparatus 1330 to a second detector 1370. Fluorescent light from 1370 is utilized to reconstruct a fluorescent confocal image of the biological sample 1355. In the case where invisible near-infrared light is utilized, a visible aiming beam may be coupled into the system, coincident with the near-infrared light, to allow visualization of the locations of imaging. Alternatively or in addition, a white light image of the specimen under investigation may be provided by use of an alternative imaging port on the microscope. Alternative embodiments known in the art, including the capability to obtain spectral information from the sample by short-time-Fourier transformation (STFT) of the

spectral interference, Doppler-sensitive SD-OCT and polarization-sensitive SD-OCT, may be also utilized to extract additional information from the biological specimen, such as absorption, flow, and birefringence.

[0068] Another exemplary multimodality embodiment of a system according to the present invention which is configured to provide OFDI, OCM, SECM, and FFOCM images, data and other information, where FFOCM signal is provided at a different wavelength from the other three modalities, is depicted in Figure 14. In this exemplary embodiment, a wavelength tuning source 1400 is coupled alternatively to a spectral modulation unit 1405. Light from the modulation unit 1405 is coupled into a circulator 1410 and a beam splitter 1415. If the circulator 1410 is utilized, light from the circulator 1410 is transmitted to the beam splitter 1415 that preferably directs a majority of light to the sample. Light in the reference arm 1420 is transmitted to a reference reflector 1425 that may be stationary, or can move or otherwise change the path length of 1420. In case the reference arm 1420 is allowed to move, conventional time-domain OCT (TD-OCT) procedures and modalities may be provided or complex spectral domain may be obtained for the OFDI data by methods known in the art. Light in the sample arm 1430 is transmitted a beam scanning mechanism 1435 that is capable of scanning the beam in two directions at high or slow speeds. The beam scanning mechanism 1435 may also contain a telescope for imaging the scanners onto the back focal plane of the lens 1465. Light from the scanning mechanism 1435 is transmitted to a dichroic splitter/WDM 1445 that transmits the excitation light for OFDI, OCM, and SECM, but reflects FFOCM light.

[0069] An exemplary FFOCM system similar to the system(s) of Figure 3 and/or Figure 4 can be coupled into the beam path via the dichroic splitter/WDM 1445. Light from the dichroic splitter/WDM 1445 is directed to a slider 1455 that contains multiple optical elements; when the slider 1455 is positioned at a distinct position, either one or a combination of OFDI, OCM, SECM or FFOCM data and/or images is provided. Light from the slider 1455 is transmitted to an objective lens 1465 mounted to a lens turret 1460 in one exemplary embodiment that is capable of changing the objective lenses. The slider 1455 and/or a turret 1460 may be under computer control for an automatic selection of imaging modality. Light can be focused by objective lens 1465 onto or

within the sample 1470, which may be mounted to a computer-controlled three-dimensional translation stage 1475.

[0070] Reflected light is transmitted back through the apparatus to 1410, which redirects the light to a spectrometer. Detected reflected light is processed to form OFDI, OCM, SECM images by methods described herein. FFOCM light may be redirected to the FFOCM system 1450 via the filter/dichroic mirror/WDM apparatus 1445. In the case where invisible near-infrared light is utilized, a visible aiming beam may be coupled into the exemplary system, coincident with the near-infrared light, to allow visualization of the locations of imaging. Alternatively or in addition, a white light image of the specimen under investigation may be provided by use of an alternative imaging port on the microscope. Alternative exemplary embodiments known in the art, including the capability to obtain spectral information from the sample by short-time-Fourier transformation (STFT) of the spectral interference, Doppler-sensitive OFDI and polarization-sensitive OFDI may be also utilized to extract additional information from the biological specimen, such as absorption, flow, and birefringence.

[0071] In another exemplary embodiment of the present invention, the microscope can be configured to allow imaging from both sides of the sample. For example, SDOCT, SECM and OCM procedures can be performed from above the sample, and FFOCM procedures may be performed with the imaging lens illuminates the sample from below. In such exemplary configuration, the sample can be mounted between a microscope slide and a thin cover glass, to allow imaging from both sides.

[0072] The exemplary systems described herein can provide a multimodality imaging of biological specimens in a variety of different formats, speeds, resolutions, fields of view, and contrast mechanisms. Each image data set may be two- or three-dimensional, and may be co-registered to the data sets of the other respective imaging modalities. Computer processing methods known in the art may be utilized to display the different data sets in a variety of different imaging formats including three-dimensional volume visualization, four-dimensional representations, and processed two-, three- and four-dimensional data sets, where the processing apparatus is configured to highlight

important areas of interest. Any one or more datasets may be displayed with respect to the other and a comprehensive, all-inclusive dataset may be derived from a combination of the individual data sets. Quantitative information may be derived from the data sets in their two-, three-, and four-dimensional contexts. Image data may also be combined with conventional fluorescent or brightfield images of the biological specimen.

EXAMPLES

[0073] Provided below are examples conducted to investigate using exemplary multiple imaging modalities according to the present invention to image the developing Xenopus laevis heart.

Exemplary methods

Bench-Top Exemplary OCT and OFDI Systems

[0074] In the exemplary TDOCT configuration, axial ranging is performed by use of low coherence reflectometry where the individual depth points are probed sequentially in time. A broad bandwidth (50 nm) source centered at 1.3 μm was used, providing an axial resolution of ~10 μm in tissue (n=1.4). The frame rate was 20 per second (2 kHz A-line rate, 100 x 500 pixels).

[0075] Exemplary OFDI procedures and systems can use a frequency domain reflectometry in which all depth points are acquired simultaneously. This technique provides a several-hundred-fold improvement in signal-to-noise ratio (SNR) as described in M. A. Choma et al. "Sensitivity advantage of swept source and Fourier domain optical coherence tomography," *Optics Express* 11, pp 2183-2189 (2003); and S. H. Yun et al., "High-speed optical frequency-domain imaging," *Optics Express* 11, pp. 2953-2963 (2003). The exemplary OFDI systems and procedures can use a rapidly swept, wavelength tunable laser as a light source. An extended-cavity semiconductor laser employing an intracavity spectral filter, as described in M. A. Choma et al. "Sensitivity advantage of swept source and Fourier domain optical coherence tomography," *Optics Express* 11, pp 2183-2189 (2003), C. Boudoux et al., "Rapid wavelength-swept spectrally encoded confocal microscopy," *Optics Express* 13, pp. 8214-8221 (2005).

[0076] The laser featured a sweep repetition rate of up to 64 kHz, a wide tuning range of 111 nm centered at 1320 nm, and a high average output power of 30 mW (7 mW on the tissue). The axial resolution was 10 μm in tissue. The system further comprised an acousto-optic frequency shifter (25 MHz) to remove the depth degeneracy inherent in the frequency-domain reflectometry, as described in S. H. Yun et al., "Removing the depth-degeneracy in optical frequency domain imaging with frequency shifting," *Optics Express* 12, pp. 4822-4828 (2004). Polarization-diversity detection was implemented to eliminate polarization artifacts in the fiber-based OFDI system. Dual-balanced photoreceivers were used to improve imaging sensitivity through the reduction of laser intensity noise. The photoreceiver outputs were digitized with a 2-channel analog-to-digital converter at a sampling rate of 100 MHz with 14-bit resolution.

[0077] Exemplary TDOCT and high-speed OFDI configuration were incorporated into a dissecting light microscope. The scanning system was comprised of a collimating lens (5 mm beam diameter), two synchronized galvanometric scanners for transverse scanning, a focusing lens (50 mm focal length), and a small mirror that deflected the beam downward toward the sample. For exemplary TDOCT and OFDI configuration, the transverse resolution was 16 μm with a confocal parameter of 330 μm.

[0078] Displacements associated with local cardiac motion were determined directly from the volumetric data by subtracting the heart surface locations at end diastole from those at end systole on a frame-by-frame basis. Displacement was displayed using a color look up table. Volumetric rendering and three-dimensional visualization was accomplished by using OsiriX software.

[0079] High-resolution OFDI procedure was performed using a laser source with 200 nm tuning range, centered at 1250 nm, in which two semiconductor optical amplifiers were utilized as the gain media, as described in W. Y. Oh et al., "Wide tuning range wavelength-swept laser with two semiconductor optical amplifiers," *IEEE Photonics Technology Letters* 17, pp. 678-680 (2005). An axial resolution of 4 μm in tissue was achieved. The transverse resolution was 2 μm with NA=0.2 objective lens. The imaging rate was 40 frames per second with an A-line rate of 20 kHz (500 A-lines per frame).

Polarization-diversity and dual-balanced detection was performed and the photoreceiver outputs were digitized with a 2-channel analog-to-digital converter at a sampling rate of 10 MHz with 12-bit resolution.

Exemplary FFOCM system

[0080] For example, FFOCM is an interferometric technique that utilizes two-dimensional parallel detection to provide subcellular resolution images of reflected light within biological specimens, as described in A. Dubois et al., "Ultrahigh-resolution full-field optical coherence tomography," *Appl Opt* 43, pp. 2874-2883 (2004), and A. Dubois et al., "Three-dimensional cellular-level imaging using full-field optical coherence tomography," *Phys Med Biol* 49, pp. 1227-1234 (2004). The exemplary FFOCM system used spatially incoherent broadband light from a xenon arc lamp to illuminate the sample and the reference mirror of a Linnik interference microscope using two identical NA=0.3 water-immersion microscope objective lenses. Interference images were captured with a CMOS area scan camera with spectral response centered at 650 nm. The transverse resolutions were 2 μm and axial resolution, 1.1 μm. Acquisition time was 2 seconds per frame for a transverse field of view of approximately 700 μm x 700 μm. Three-dimensional data was obtained by moving the sample through the focus at 1 μm increments. Volumetric rendering and visualization was accomplished by using OsiriX software.

Exemplary SECM system

[0081] For example, SECM is a reflectance confocal microscopy technique, which uses near-infrared light that allows deeper penetration into tissue, as described in R. R. Anderson et al., "The optics of human skin," *J Invest Dermatol* 77, pp. 13-19 (1981), compared with confocal microscopes that utilize visible light. Exemplary SECM technique differs from conventional laser scanning confocal microscopy in that it projects different wavelengths onto distinct locations on the sample, as described in G. J. Tearney et al., "Spectrally encoded confocal microscopy," *Optics Letters* 23, pp. 1152-1154 (1998). Rapid acquisition of spectra returned from the sample enables high-speed reconstruction of the image. In the SECM system, as described in C. Boudoux et al., "Rapid wavelength-swept spectrally encoded confocal microscopy," *Optics Express* 13,

pp. 8214-8221 (2005), light from a rapid wavelength tuning source in the near-infrared (center wavelength = 1.32 μm, instantaneous line width = 0.1 nm, total bandwidth = 70 nm, repetition rate up to 15.7 kHz), was collimated onto a diffraction grating (1100 lines per mm) and focused using a 1.2 NA, 60x objective (Olympus UPlanApo/IR 60X/1.20W). A multimode fiber was used for signal collection, resulting in 0.9 μm transverse and 2.5 μm axial resolutions. Images comprised of 500 x 500 pixels were acquired at 10 frames per second. The maximum imaging depth was limited to the 280 μm working distance of the objective lens.

Specimen preparation, ethanol treatment and histology

[0082] Xenopus laevis frogs were purchased from Nasco (Fort Atkinson, Wisconsin). Animal procedures were performed according to the approved protocols of Massachusetts General Hospital Subcommittee on Research Animal Care. Embryos were obtained by in vitro fertilization, incubated in 0.1x Marc's modified Ringer's medium (MMR)(as described in J. Newport et al., "A major development transition in early Xenopus embryos: I. Characterization and timing of cellular changes at the midblastula stage," *Cell* 30, pp. 675-686, 1982), and staged according to Nieuwkoop and Faber tables. (see P. D. Nieuwkoop and J. Faber, *Normal table of Xenopus laevis, Daudin,* North-Holland Publishing Company, Amsterdam, 1967).

[0083] Ethanol treatments were performed in 0.1X MMR (vol/vol), soon after Mid Blastula Transition (stage 8.5) (as described in R. Yelin et al., "Ethanol exposure affects gene expression in the embryonic organizer and reduces retinoic acid levels," *Dev Biol* 279, pp. 193-204 (2005).) until imaging. Prior to in vivo imaging, embryos were anesthetized using 0.02% 3-aminobenzoic acid ethyl ester (A-5040, Sigma). For TDOCT and OFDI imaging techniques and systems, embryos were positioned on a 1.5% agarose gel plate with their ventral side facing up, covered by the anesthesia working solution. For imaging with the exemplary SECM system, embryos were placed on a cover slip, lying on their ventral side in an anesthesia buffer, and imaged from below. In vitro imaging by the exemplary FFOCM procedures and/or systems commenced following fixation in MEMFA (0.1M MOPS [pH7.4], 2mM EGTA, 1mM MgSO4 and 3.7% formaldehyde) for greater than one hour. Prior to imaging, the fixed embryos were

transferred into a Petri dish with 1x PBS (8gr NaCl, 0.2gr KCl, 1.44gr Na2HPO4, 0.24gr KH2PO4), with its ventral side facing up, supported by clay.

[0084] Plastic Histology sections (as described in A. M. Glauert, *Fixation, Dehydration and Embedding of Biological Specimens.*, North-Holland Publishing Company Amsterdam, 1986) were obtained after additional fixation in Karnovsky's Fixative (KII) and embedding in tEpon-812 (Tousimis). Sections of 1μm thick were cut on a Reichert Ultracut Microtome and stained with methylene blue/toluidine blue in borate buffer (Tousimis). Paraffin sections (5 μm thickness) were stained with Hematoxylin & Eosin.

Exemplary results

Four-dimensional imaging of embryonic heart with OFDI techniques in vivo

[0085] Rapid volumetric imaging of the beating heart enables the evaluation of three-dimensional morphology and function during the cardiac cycle. Compared with TDOCT, which provides cross-sectional imaging in vivo (as shown in Figures 15a and 15b), the exemplary OFDI system and procedure can image at much higher frame rates, making four-dimensional imaging of the beating heart without cardiac gating possible. Volumetric OFDI images of the Xenopus heart (stage 49) were acquired at a rate of 20 three-dimensional data sets per second (as shown in Figures 15c-15g). At end systole, the use of the OFDI procedure demonstrated that the ventricle was at its smallest volume; the volumes of the atrium and truncus arteriosus (TA) were conversely at their maxima (as shown in Figures 15c and 15d). At end diastole, the ventricle was dilated to its greatest volume, whereas the volumes of the atrium and TA were at their minima (as shown in Figures 15e and 15f). A three-dimensional rendering of the heart (as shown in Figure 15g), taken from the four-dimensional data set, corresponds to a brightfield photograph of the same heart following its dissection (as shown in Figure 15h).

High-resolution OFDI procedure on embryonic heart in vivo

[0086] While the exemplary OFDI system was capable of four-dimensional imaging, there are cases where higher resolution is required to identify subtle morphological and functional abnormalities. In order to increase resolution, OFDI cross-sections of a stage

49 Xenopus heart were obtained in vivo (as shown in Figures 15i-15m) using a broadband (e.g., 200 nm) wavelength-swept source, as described in W. Y. Oh et al., "Wide tuning range wavelength-swept laser with two semiconductor optical amplifiers," *IEEE Photonics Technology Letters* 17, pp. 678-680 (2005) Compared to the 16 μm transverse and 10 μm axial resolutions of the previously described TDOCT and OFDI procedures and systems, the transverse and axial resolutions of high resolution OFDI results were 2 μm and 4 μm, respectively. Details within the three-chamber Xenopus heart can be clearly resolved with the high-resolution OFDI procedures and systems, including atrioventricular valve dynamics (as shown in Figures 15i-15k), ventricular contractions, and trabecular dynamics (Fig. 15m). Individual blood cells can also be seen, flowing from the atrium to the ventricle through the atrioventricular valve (as shown in Figure 15k).

High-resolution three-dimensional imaging of embryonic heart using FFOCM procedures in vitro

[0087] Exemplary FFOCM procedures and systems offer the capability to image microstructure of the embryonic heat with nearly isotropic cellular level resolution. Volumetric FFOCM images spanned a field of view of 700 x 700 x 1000 μm (axial). The transverse and axial resolutions were 2 μm and 1.1 μm, respectively. Acquisition time was 2 seconds for a single en face section, and 33 minutes for the entire volume. Exemplary FFOCM sections of the Xenopus heart (stage 49) allow visualization of ventricular trabeculae (as shown in Figures 16a and 16c), the spiral valve (as shown in Figures 16b and 16d, see arrows), and the partial atrial septum (as shown in Figure 16d, see arrow head) with greater detail than generated using the exemplary TDOCT or OFDI procedures or systems. Partially transparent volumetric rendering of the heart (as shown in Figures 16e-16h), reveals the looping-compression structure with the angled TA (as shown in Figure 16e), the aortic arches (as shown in Figures 16f and 16g), and the thin wall of the atrium (as shown in Figures 16g and 16h), in their three-dimensional context. Cut-away views of (as shown in Figure 16e) show fine three-dimensional internal structures, including the trabeculae (as shown in Figures 16i and 16j) and the atrioventricular valve (as shown in Figure 16k). A magnified view of the atrioventricular

valve shown (as shown in Figure 16l) next to a corresponding histology section of the same embryo (as shown in Figure 16m), demonstrates its bicuspid morphology.

High-speed imaging of embryonic heart with SECM procedures in vivo

[0088] Exemplary SECM procedures and systems provide a transverse resolution comparable to those associated with FFOCM, but at higher frame rates, enabling microscopy of the heart in vivo. The Xenopus myocardium (stage 49) was imaged in vivo using the exemplary SECM procedures and systems at a frame rate of 10/s, a field of view of 220 x 220 μm, and transverse and axial resolutions of 1.2 and 6 μm, respectively. The maximum penetration depth was 280 μm. Exemplary of the same tadpole (stage 49) visualized by TD-OCT (as shown in Figures 15a and 15b) and FFOCM (as shown in Figures 16a-16m) procedures and systems, show the thin cusps of the atrioventricular valve (as shown in Figure 17a), approximately 280 μm below the ventral surface, and parts of the ventricle and TA (as shown in Figures 17c), containing individual blood cells within the intratrabecular spaces. SECM images correlated well with corresponding histology sections (as shown in Figures 17b and 17d). A series of frames from a different tadpole (stage 47), demonstrates the spiral valve as it closes (as shown in Figure 17e) and opens (as shown in Figures 17f and 17g), regulating blood flow, seen at the single-cell level, from the TA to the aortic bifurcation. Blood cells are also apparent within the trabeculae (as shown in as shown in Figures 17h). Intracellular features within individual myocytes that may represent nuclei and organelles can be observed.

Aneurismal dilatation in the Xenopus embryo

[0089] In one of the embryos (stage 47), a protrusion emanating from the TA wall has been identified. SECM sections obtained in vivo at two different depths (as shown in Figures 18a and 18b), reveal its saccular shape, its location with respect to spiral valve, as well the flow of individual blood cells through the defect. This abnormality was also observed using the exemplary TDOCT procedures and systems in vivo (as shown in Figure 18a, see inset). The embryo was then fixed and imaged with the exemplary FFOCM procedures and systems. An FFOCM section (as shown in Figure 18c) and a three-dimensional rendering of the FFOCM volumetric data set (as shown in Figure 18d) show the dilatation in the context of the entire heart. Difficult to see under conventional brightfield microscopy (as shown in Figure 18e), but clearly visualized using exemplary TDOCT, FFOCM and SECM procedures and systems, this protrusion may represent a

saccular aneurismal dilatation of the TA, in a heart that otherwise appeared to have a normal phenotype.

Heart Abnormalities due to ethanol exposure

[0090] Cardiovascular malformation can be caused by genetic (as described in K. L. Clark et al., "Transcription factors and congenital heart defects," *Annu Rev Physiol* 68, pp. 97-121 (2006)) and teratogenic factors (as described in S. M. Mone et al., "Effects of environmental exposures on the cardiovascular system: prenatal period through adolescence," *Pediatrics* 113, pp. 1058-1069 (2004)). Ethanol is a well-known teratogen; exposure of human embryo during pregnancy to alcohol (ethanol) is associated with Fetal Alcohol Syndrome (FAS). (See K. L. Jones et al., "Recognition of the fetal alcohol syndrome in early infancy," *Lancet* 2, pp. 999-1001 (1973), and J. D. Chaudhuri, "Alcohol and the developing fetus--a review," *Med Sci Monit* 6, pp. 1031-1041 (2000)). One estimate indicates that 54% of the children with FAS have heart defects. (See E. L. Abel, *Fetal Alcohol Syndrome*, Medical Economics Books, Oradell, NJ, 1990).

[0091] In order to study the teratogenic effect of ethanol on Xenopus heart development, embryos were exposed to different concentrations of ethanol (0.5%-2.5%) from the mid blastula transition (stage 8.5). (See R. Yelin et al., "Ethanol exposure affects gene expression in the embryonic organizer and reduces retinoic acid levels," *Dev Biol* 279, pp. 193-204 (2005)). Siblings developing under the same conditions, but not exposed to ethanol were used as controls. During the developmental process we screened the heart area of the embryos using the exemplary TDOCT procedures and systems to identify and qualitatively evaluate the extent of the teratogenic effect. We did not observe morphologic differences between the 0.5% ethanol treated group (n=16) and the control group (n=42). Moderate teratogenic effects, defined as complete maturation with a substantial change in morphology compared to the controls, was found in a minority (25%) of embryos that were exposed to 1% ethanol (n=28), and in a majority (74%) of embryos that were exposed to 1.5% ethanol (n=27). Severe effect, defined as grossly abnormal rotation of the heart tube and/or incomplete maturation, was found in all the embryos in the 2.0% and 2.5% groups (n=17, n=7, respectively). Cardiac motion was evident in all embryos, even those with the most severe malformations.

[0092] Using the exemplary TDOCT procedures and systems, a tadpole (stage 48) has been selected from each of the control, 0.5%, 1.5%, and 2.0% ethanol treated groups to demonstrate typical phenotypes (as shown in Figures 19a-19d). It was determined that the four tadpoles' hearts were in advanced developmental stages by identifying the existence of a partial atrial septum (as shown in Figures 19a-19d, see right images, septa marked by arrows) and an atrioventricular valve. The TDOCT images provided the first indication of damaged looping in the 1.5% and 2.0% groups. Further observed were lower TDOCT signal from within the ventricle in the 1.5% and 2.0% groups, which may be attributed to diminished blood flow in these embryos. Photographs of the tadpoles, taken in vivo from the ventral aspect, are shown in Figures 19e-19h.

[0093] Three-dimensional rendering of data acquired with the exemplary FFOCM systems and procedures in vitro allowed evaluation of myocardial structure at high-resolution, revealing the similarity between the control and the 0.5% tadpoles and clearly showing defective heart tube looping in the tadpoles from the 1.5% and 2.0% groups (as shown in Figures 19i-19l). Sections through the FFOCM volumetric data sets demonstrated smaller, distorted TA's and spiral valves (marked by arrows) in the 1.5% (as shown in Figure 19o) and 2.0% embryos (as shown in Figure 19p) compared with the control (as shown in Figure 19m) and the 0.5% (as shown in Figure 19n) embryos. Pericardial edema was present in the 1.5% and 2.0% groups (as shown in Figures 19o, 19p, 19s and 19t), compared with control and 0.5% groups. Ethanol also affected the ventricle; the developed trabeculae in the control (as shown in Figure 19q) and 0.5% (as shown in Figure 19r) hearts contrast the less developed trabeculae in the 1.5% group (as shown in Figure 19s) and the large ventricular cavity with sparse, stunted trabeculae in embryos exposed to 2.0% ethanol (as shown in Figure 19t). Corresponding histological sections confirmed some of our findings, including the less developed trabeculae (as shown in Figures 19u-19x) in embryos with the greater ethanol exposure.

Discussion of Exemplary Results

[0094] A common paradigm in developmental biology research is to manipulate the genotype and monitor the phenotype. Morphology is an important aspect of the phenotype. In the heart, even slight morphological and dynamical abnormalities may be

critical for proper myocardial function. An ability to identify subtle morphological and dynamical variations in two and three dimensions can significantly improve the sensitivity of this paradigm.

[0095] In the Xenopus tadpole, heart structures such as the myocardium wall, septum and valves may only be a few cells thick. Evaluating the morphological phenotype not only requires resolving such fine structures, but also the capability to visualize these microscopic features within the beating heart, where typical displacement velocities are on the order of 1 mm/sec. If the imaging speed is sufficiently high, three-dimensional images of the embryo heart can be obtained at different times within the cardiac cycle. This exemplary four-dimensional imaging could allow reliable measurements of dynamic physiological parameters, such as stroke volume and ejection fraction, as well as valve opposition, stiffness and modularity, which have close analogs in human pathophysiology. High resolution and high speed are not the only requirements for effective imaging of the heart. In the Xenopus embryo, the heart extends from between 200 μm and 800 μm beneath the ventral surface. An effective imaging method should therefore also be capable of imaging at these depths without substantial loss of signal and resolution.

[0096] The morphology of the developing Xenopus laevis heart has been studied in vitro and described in detail, using three-dimensional rendering of histology sections. (See T. J. Mohun et al., "The morphology of heart development in Xenopus laevis," *Dev Biol* 218, 74-88 (2000)). For histologic studies, however, sample preparation and sectioning make preserving structural fidelity difficult. As a result, imaging of intact embryos in their natural environment is preferred. Structural imaging of the heart in vivo has been demonstrated using a variety of non-invasive imaging modalities such as micro-MRI (see D. L. Kraitchman et al., "In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction," *Circulation* 107, pp. 2290-2293 (2003), and F. Wiesmann et al., "Developmental changes of cardiac function and mass assessed with MRI in neonatal, juvenile, and adult mice," *Am J Physiol Heart Circ Physiol* 278, pp. H652-657 (2000)), micro-CT (see M. Malyar et al., "Relationship between arterial diameter and perfused tissue volume in myocardial microcirculation: a micro-CT-based

analysis," *Am J Physiol Heart Circ Physiol* 286, pp. H2386-2392 (2004), and C. T. Badea et al., "4-D micro-CT of the mouse heart," *Mol Imaging* 4, pp. 110-116 (2005)), ultrasound (see S. Srinivasan et al., "Noninvasive, in utero imaging of mouse embryonic heart development with 40-MHz echocardiography," *Circulation* 98, pp. 912-918 (1998)), and PET (see L. W. Dobrucki et al., "Molecular cardiovascular imaging," *Curr Cardiol Rep* 7, pp. 130-135 (2005), and L. Stegger et al., "Monitoring left ventricular dilation in mice with PET," *J Nucl Med* 46, pp. 1516-1521 (2005)).

[0097] Optical techniques enable imaging of the embryonic heart at higher resolution. Confocal microscopy has been used to image early Xenopus heart development, in vitro (as described in S. J. Kolker et al., "Confocal imaging of early heart development in Xenopus laevis," *Dev Biol* 218, pp. 64-73 (2000)), and to study the role of intracardiac fluid forces in zebrafish embryonic cardiogenesis, in vivo (as described in J. R. Hove et al., "Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis," *Nature* 421, pp. 172-177 (2003)). Doppler TDOCT procedures and systems were used to study blood flow in the Xenopus tadpole, allowing quantitative velocity measurements under the tissue surface. (See J. R. Hove et al., "Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis," *Nature* 421, pp. 172-177 (2003), and V. X. D. Yang, M. L. Gordon, E. Seng-Yue et al., "High speed, wide velocity dynamic range Doppler optical coherence tomography (Part II): Imaging in vivo cardiac dynamics of Xenopus laevis," *Optics Express* 11, pp. 1650-1658 (2003)). Due to its limited imaging speed, three-dimensional heart imaging using TDOCT has primarily only been previously demonstrated in vitro. (See S. A. Boppart et al., "Noninvasive assessment of the developing Xenopus cardiovascular system using optical coherence tomography," *Proc Natl Acad Sci U S A* 94, pp. 4256-4261 (1997), T. M. Yelbuz et al., "Optical coherence tomography: a new high-resolution imaging technology to study cardiac development in chick embryos," *Circulation* 106, pp. 2771-2774 (2002), and W. Luo et al., "Three-dimensional optical coherence tomography of the embryonic murine cardiovascular system " *Journal of biomedical optics* 11, 021014 (2006).

[0098] Gating or post-acquisition synchronization techniques have been employed to circumvent the limited speed of conventional imaging methods, enabling the

reconstruction of three-dimensional images of embryo hearts at different stages in the cardiac cycle. (See M. W. Jenkins et al., "4D embryonic cardiography using gated optical coherence tomography," *Optics Express* 14, pp. 736-748 (2006). M. Liebling et al., "Four-dimensional cardiac imaging in living embryos via postacquisition synchronization of nongated slice sequences," *J Biomed Opt* 10, 054001 (2005). For some of the experiments, we utilized TDOCT as it was more readily available in our laboratory, however the exemplary OFDI procedures and systems were capable of providing all of the functionality of the exemplary TDOCT procedures and systems at much higher speeds. The exemplary OFDI procedures and systems provided real-time, true four-dimensional imaging of a beating heart without requiring cardiac gating and was found to be useful for assessing myocardial wall displacement during the cardiac cycle (as shown in Figures 15c-15f).

[0099] By modifying the OFDI light source, we were also able to conduct real-time cross-sectional imaging with higher axial resolution (4 μm), enabling visualization of valve dynamics (as shown in Figures 15i-15k) and single-cell blood flow. For subcellular-level resolution imaging of the embryonic heart, we investigated the use of the exemplary FFOCM and SECM procedures and systems. The FFOCM modality was found to be capable of providing high quality three-dimensional imaging with isotropic cellular (1-2 μm) resolution. The SECM modality demonstrated comparable resolution to the FFOCM modality , but was capable of imaging at higher speeds, enabling visualization of myocyte, blood, and valve motion in vivo at the subcellular level. Table 1 summarizes the different capabilities of each procedure, highlighting their complementary nature.

Table 1. Comparison of endogenous-contrast modalities for optical imaging of the embryonic heart. Cells shaded in gray denote the imaging technologies with the best transverse resolution, axial resolution, and frame rate characteristics.

	OCT*	FFOCM	SECM
Transverse resolution	2-16 μm	2 μm	0.9 μm
Axial resolution	4-10 μm	1.1 μm	2.5 μm
Speed [frames per second]	10-1000	0.5	10
Three dimension in vivo (4D)	Yes	No	No
Applications	Architectural dynamics	Whole organ microscopic morphology	Subcellular dynamics

* Includes TDOCT and OFDI modalities.

[00100] The large penetration depth of the exemplary TDOCT and FFOCM procedures and systems allowed imaging of the heart through pericardial edema that developed as part of the ethanol teratogenic phenotype. Our preliminary results suggest that ethanol interferes with the process of heart looping (Figs. 19i-l), in agreement with a study in quail. (See W. O. Twal et al., "Retinoic acid reverses ethanol-induced cardiovascular abnormalities in quail embryos," *Alcohol Clin Exp Res* 21, pp. 1137-1143 (1997)). The reduction in TA size that is reported in this work was predicted by Cavierres and Smith, (see M. F. Cavieres et al., "Genetic and developmental modulation of cardiac deficits in prenatal alcohol exposure," *Alcohol Clin Exp Res* 24, pp. 102-109 (2000)), but not observed. It is believed that the less developed ventricular trabeculae described here (as shown in Figures 19q-t) have not been previously developed. Since in

Xenopus and zebrafish (Danio rerio), the ventricular trabeculae serve as a functional equivalent of the His-Purkinje system (see D. Sedmera et al., "Functional and morphological evidence for a ventricular conduction system in zebrafish and Xenopus hearts," *Am J Physiol Heart Circ Physiol* 284, pp. H1152-1160 (2003)), .a determination of less developed trabeculae could be associated with the slower heart rate that has been reported in ethanol treated quail (see W. O. Twal et al., "Retinoic acid reverses ethanol-induced cardiovascular abnormalities in quail embryos," *Alcohol Clin Exp Res* 21, pp. 1137-1143 (1997)), and zebrafish embryos (see J. Bilotta et al., "Ethanol exposure alters zebrafish development: a novel model of fetal alcohol syndrome," *Neurotoxicol Teratol* 26, pp. 737-743 (2004)). Interruption of active blood circulation due to ethanol treatment (W. O. Twal et al., "Retinoic acid reverses ethanol-induced cardiovascular abnormalities in quail embryos," *Alcohol Clin Exp Res* 21, pp. 1137-1143 (1997), and X. Wang et al., "Japanese medaka (Oryzias latipes): developmental model for the study of alcohol teratology," *Birth Defects Res B Dev Reprod Toxicol* 77, pp. 29-39 (2006)) may explain the loss of signal from within the heart cavities, which is also consistent with the determinations.

[00101] Despite their relatively high penetration depth, none of the conventional optical imaging procedures could image the heart at the onset of cardiac organogensis (heart tube formation, stage 29), due to high scattering at these earlier stages. The initiation of cardiac movements (stage 35), however, was observed and detailed structural images at the onset of chamber formation (around stage 40) were obtained as the embryo became optically transparent. Especially for the FFOCM and SECM modalities, it was difficult to match histology to the microscopy data sets. The embryos were quite fragile when processed and embedded, making preservation of morphology challenging. Furthermore, images should be registered to histology with a precision on the order of 10 μm, which is difficult to achieve with conventional sectioning techniques.

[00102] For the imaging procedures according to exemplary embodiments of the present invention, contrast was generated by endogenous scattering. Still, molecular imaging may be important for relating gene and protein expression to phenotype. Thus, the exemplary systems and methods described herein can be used for imaging fluorescent

labels and molecular species. It has been described that fluorescence imaging can be conducted via spectral encoding by modification of the source and detection electronics. (See J. T. Motz et al., "Spectral- and frequency-encoded fluorescence imaging," *Opt Lett* 30, pp. 2760-2762 (2005)). The same principles used in fluorescence SECM procedures and systems can likewise be utilized for endoscopic two-photon and second harmonic imaging. With the coherent detection used in the exemplary TDOCT, OFDI, and FFOCM procedures and systems, it may be difficult to directly detect fluorescence. However, several molecular contrast methods have already been described for the OCT modality. (See C. Yang, "Molecular contrast optical coherence tomography: a review," *Photochem Photobiol* 81, pp. 215-237 (2005) and S. A. Boppart, et al., "Optical probes and techniques for molecular contrast enhancement in coherence imaging," *J Biomed Opt* 10, 41208 (2005)).

[00103] The natural contrast optical imaging modalities presented in this work allow evaluation of the embryonic heart from different vantage points. Combining OFDI, SECM, and FFOCM modalities can leverage their strengths (see Table 1), and provide a ability for obtaining a more comprehensive morphological and functional myocardial phenotype. This multi-modality paradigm can be extended to other systems and animal models as well. Since these non-invasive imaging techniques do not alter the specimen, they can be used sequentially or in parallel. Furthermore, while we have used separate imaging systems in this work, there is no fundamental barrier preventing their combination into one imaging system that uses a single wavelength swept source. (See S. H. Yun et al., "High-speed optical frequency-domain imaging," *Optics Express* 11, pp. 2953-2963 (2003); C. Boudoux et al., "Rapid wavelength-swept spectrally encoded confocal microscopy," *Optics Express* 13, pp. 8214-8221 (2005); and W. Y. Oh et al., "Wide tuning range wavelength-swept laser with two semiconductor optical amplifiers," *IEEE Photonics Technology Letters* 17, pp. 678-680 (2005)).

[00104] The foregoing merely illustrates the principles of the invention. Various modifications and alterations to the described embodiments will be apparent to those skilled in the art in view of the teachings herein. Indeed, the arrangements, systems and methods according to the exemplary embodiments of the present invention can be used

with any OCT system, OFDI system, SD-OCT system or other imaging systems, and for example with those described in International Patent Application PCT/US2004/029148, filed September 8, 2004, U.S. Patent Application No. 11/266,779, filed November 2, 2005, and U.S. Patent Application No. 10/501,276, filed July 9, 2004, the disclosures of which are incorporated by reference herein in their entireties. It will thus be appreciated that those skilled in the art will be able to devise numerous systems, arrangements and methods which, although not explicitly shown or described herein, embody the principles of the invention and are thus within the spirit and scope of the present invention. In addition, to the extent that the prior art knowledge has not been explicitly incorporated by reference herein above, it is explicitly being incorporated herein in its entirety. All publications referenced herein above are incorporated herein by reference in their entireties.

What Is Claimed Is:

1. An apparatus comprising:

at least one first arrangement configured to provide first data associated with a first signal received from at least one region of at least one sample based on a first modality, and second data associated with a second signal received from the at least one sample based on a second modality which is different from the first modality, wherein the at least one first arrangement is further configured to receive third data associated with a reference; and

at least one second arrangement configured to generate further data based on the first, second and third data.

2. The apparatus according to claim 1, wherein the first modality is spectrally-encoded confocal microscopy.

3. The apparatus according to claim 1, wherein the second modality is florescence imaging.

4. The apparatus according to claim 1, further comprising a microscope arrangement which is associated with the first and second arrangements.

5. The apparatus according to claim 1, further comprising a beam-scanning arrangement which is configured to forward electro-magnetic radiation to the at least region.

6. The apparatus according to claim 1, wherein the at least one second arrangement generates at least one of (i) a two-dimensional image or (ii) a three-dimensional image as a function of the further data.

7. The apparatus according to claim 1, wherein the first and second data are obtained substantially simultaneously.

8. The apparatus according to claim 1, wherein the first and second data are associated with approximately the same location on the at least one sample.

9. The apparatus according to claim 1, wherein at least one of the first and second data is obtained using another one of the first and second data.

10. The apparatus according to claim 1, wherein the first and second arrangements are provided in at least one of a probe or a single enclosure.

11. The apparatus according to claim 1, wherein the first and second arrangements include common components.

12. The apparatus according to claim 11, wherein the common components are provided in at least one of a source arrangement or a detector arrangement.

13. The apparatus according to claim 1, wherein the at least one first arrangement is configured to obtain spectral encoding microscopy information.

14. The apparatus according to claim 1, wherein the at least one first arrangement is configured to obtain at least one of bright field, dark field, phase contrast, polarization, epireflectance or reflectance microscopy information.

15. The apparatus according to claim 1, further comprising a further arrangement which is configured to change from the first modality to the second modality.

16. The apparatus according to claim 1, wherein the at least one first arrangement is configured to obtain optical coherence tomography information associated with a signal provided by a source arrangement having a plurality of wavelengths, and further comprising a plurality of detectors configured to detect a spectral interference between the second and third signals as a function of the wavelengths.

17. The apparatus according to claim 1, wherein the at least one first arrangement is configured to obtain optical coherence tomography information associated with a signal provided by a source arrangement whose wavelength varies over time.

18. The apparatus according to claim 1, wherein the at least one first arrangement is further configured to receive third data associated with a reference, and the at least one second arrangement is configured to generate the further data further the third data

19. The apparatus of claim 1, further comprising:

at least one third arrangement configured to control at least one of the first arrangement based on at least one of the first data or the second data.

20. The apparatus of claim 1, further comprising:

at least one fourth arrangement configured to generate an image based on the first and second data.

21. The apparatus of claim 1, further comprising:

at least one fifth arrangement configured to generate at least one first image based on the first data and at least one second image based on the second data, wherein the first and second images are associated with one another as a function of the first and second data.

22. The apparatus according to claim 1, wherein the at least one first arrangement is configured to obtain optical coherence tomography information.

23. The apparatus according to claim 1, wherein the at least one first arrangement is configured to obtain optical frequency domain interferometry information.

24. An apparatus comprising:

at least one first arrangement configured to provide first data associated with a first signal received from at least one region of at least one sample based on a first modality, second data associated with a second signal received from the at least one

sample based on a second modality which is different from the first modality, and least one third data associated with a second signal received from the at least one sample, wherein each of the at least one third data is based on a further modality which is different from the first modality and the second modality; and

at least one second arrangement configured to generate further data based on the first, second and third data.

25. The apparatus according to claim 24, wherein the at least one first arrangement is configured to obtain optical coherence tomography information.

26. The apparatus according to claim 24, wherein the at least one first arrangement is configured to obtain optical coherence microscopy information.

27. The apparatus according to claim 24, wherein the at least one first arrangement is configured to obtain full field optical coherence microscopy information.

28. An apparatus comprising:

at least one first arrangement configured to provide first data associated with a first signal received from at least one region of at least one sample based on a first spectral-encoding modality, and second data associated with a second signal received from the at least one sample based on a second non-spectral-encoding modality; and

at least one second arrangement configured to generate further data based on the first and second data.

29. A method comprising:

providing first data associated with a first signal received from at least one region of at least one sample based on a first modality, and second data associated with a second signal received from the at least one sample based on a second modality which is different from the first modality;

receiving third data associated with a reference; and

generating further data based on the first, second and third data.

30. A method comprising:

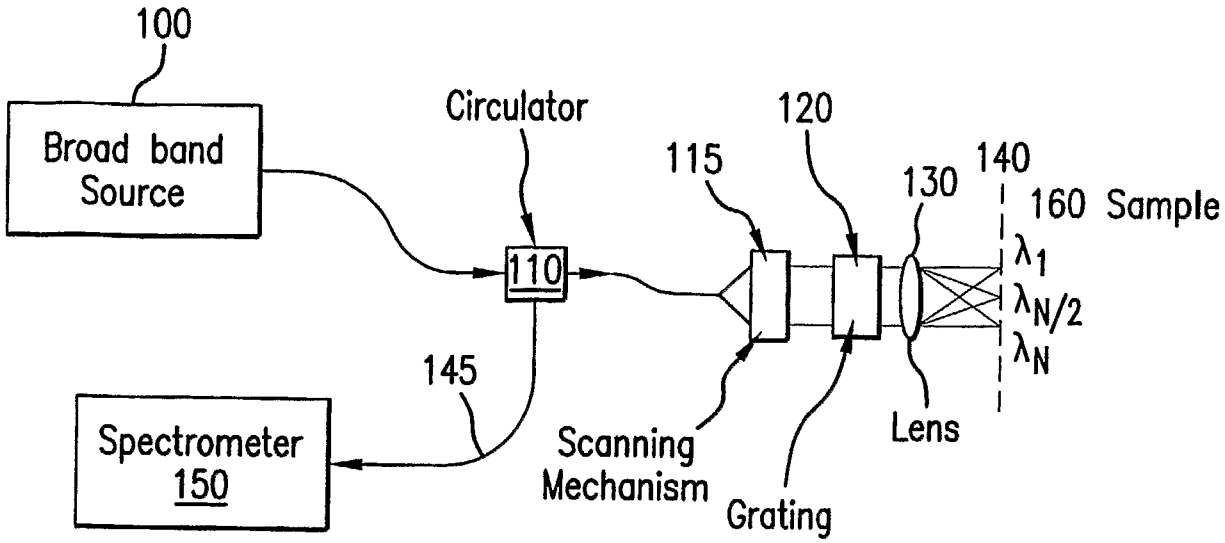
providing first data associated with a first signal received from at least one region of at least one sample based on a first modality, second data associated with a second signal received from the at least one sample based on a second modality which is different from the first modality, and least one third data associated with a second signal received from the at least one sample, wherein each of the at least one third data is based on a further modality which is different from the first modality and the second modality; and

generating further data based on the first, second and third data.

31. A method comprising:

providing first data associated with a first signal received from at least one region of at least one sample based on a first spectral-encoding modality, and second data associated with a second signal received from the at least one sample based on a second non-spectral-encoding modality; and

generating further data based on the first and second data.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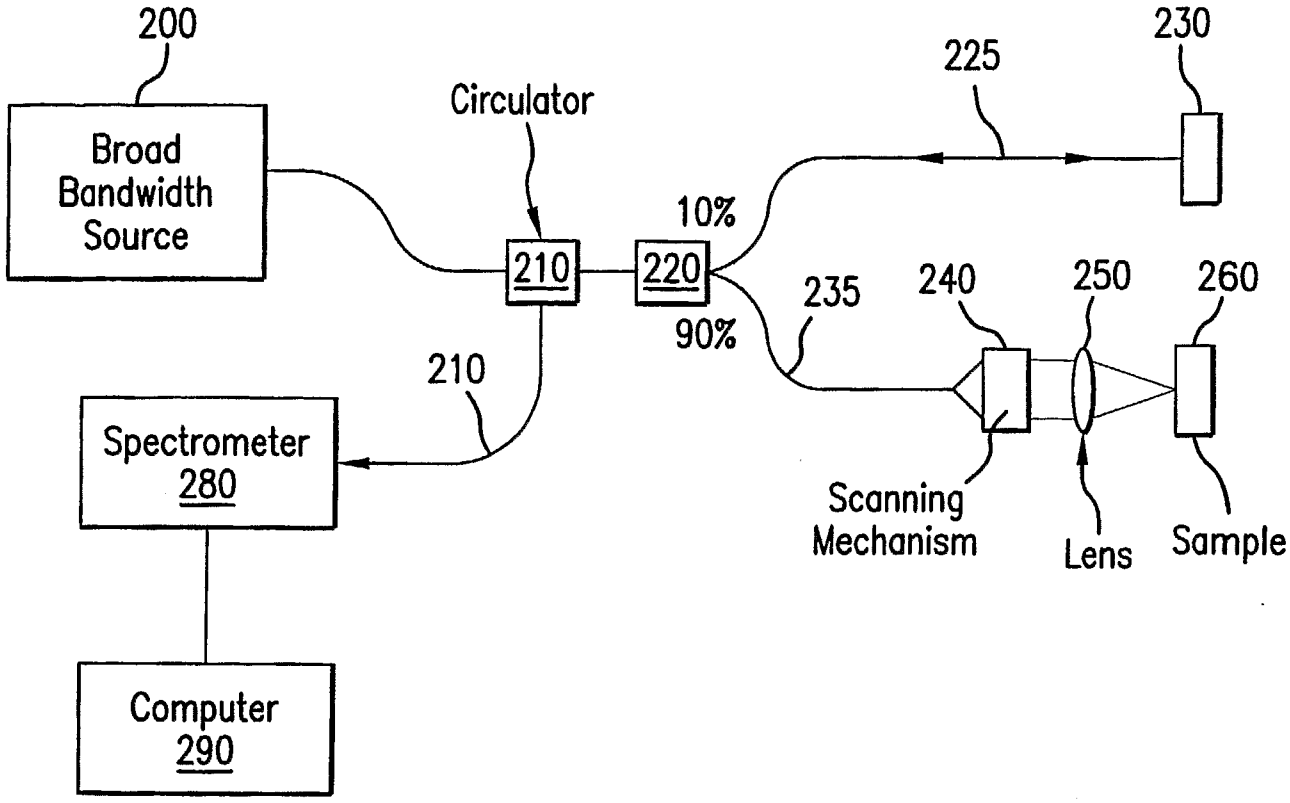
Grating
Lens

FIG. 1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Sample

FIG.2

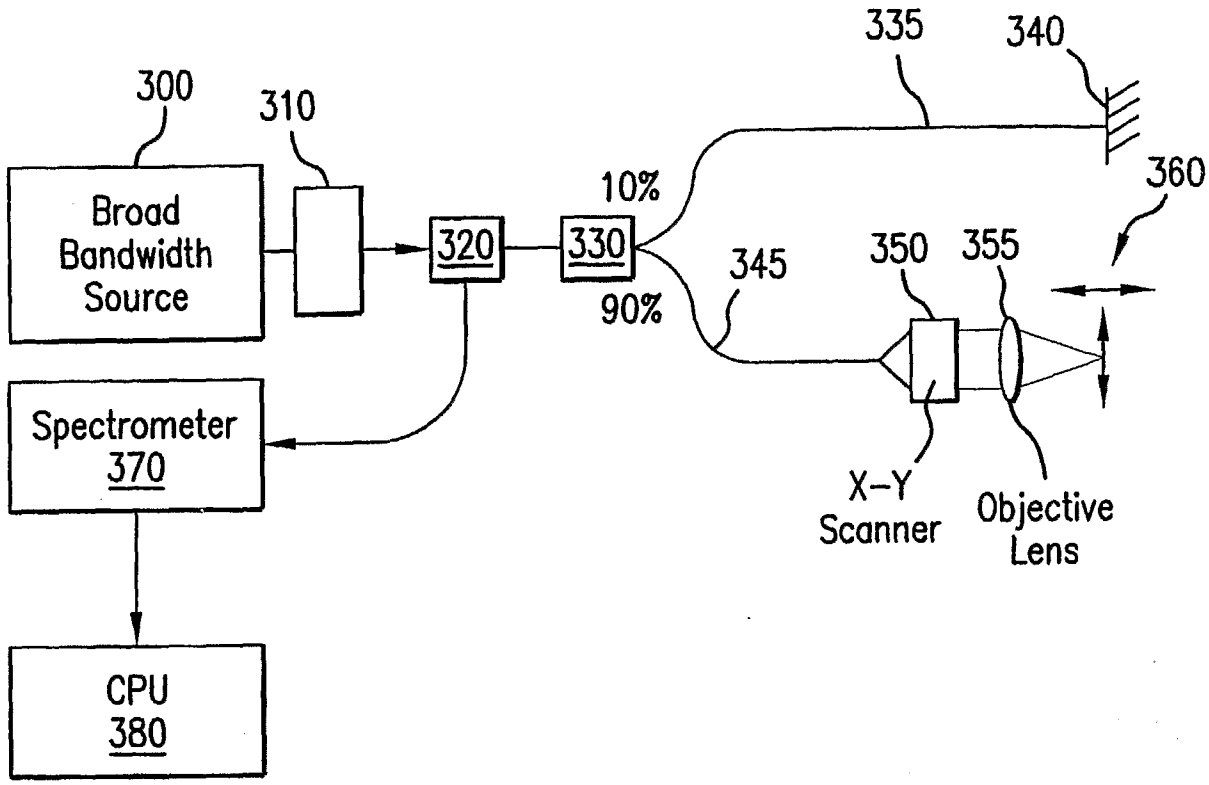
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FIG.3

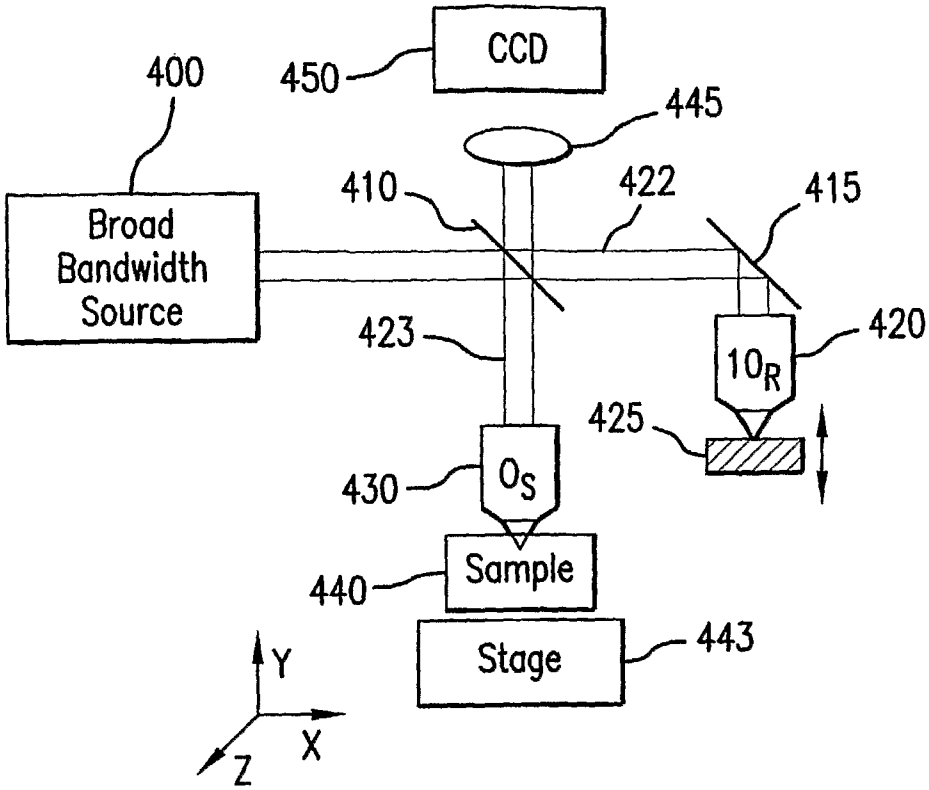
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FIG.4A

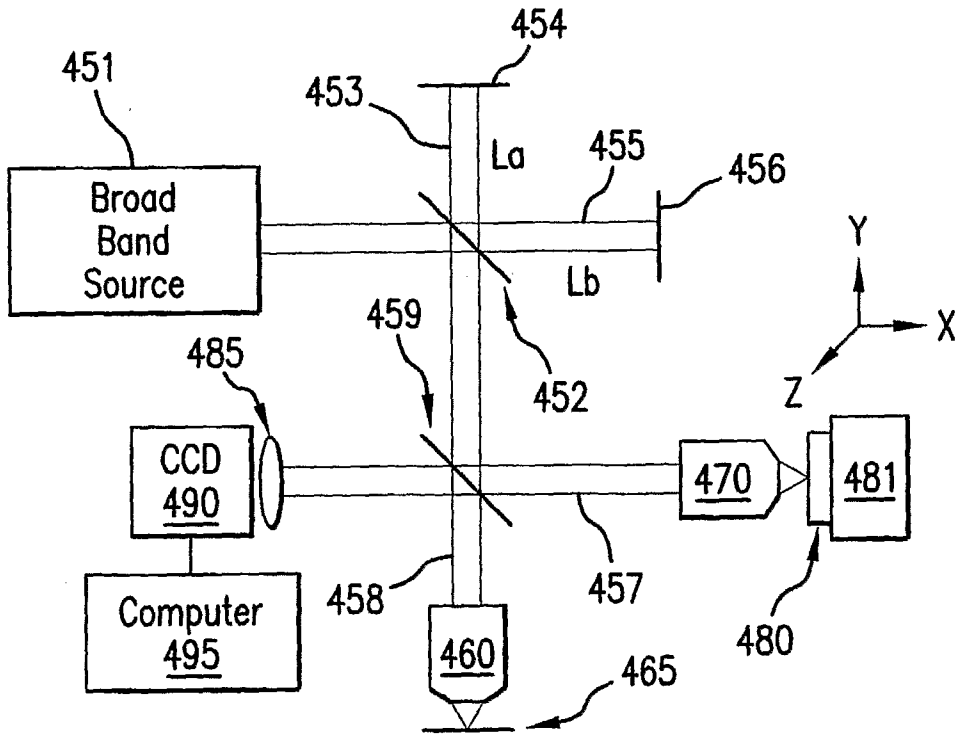
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FIG.4B

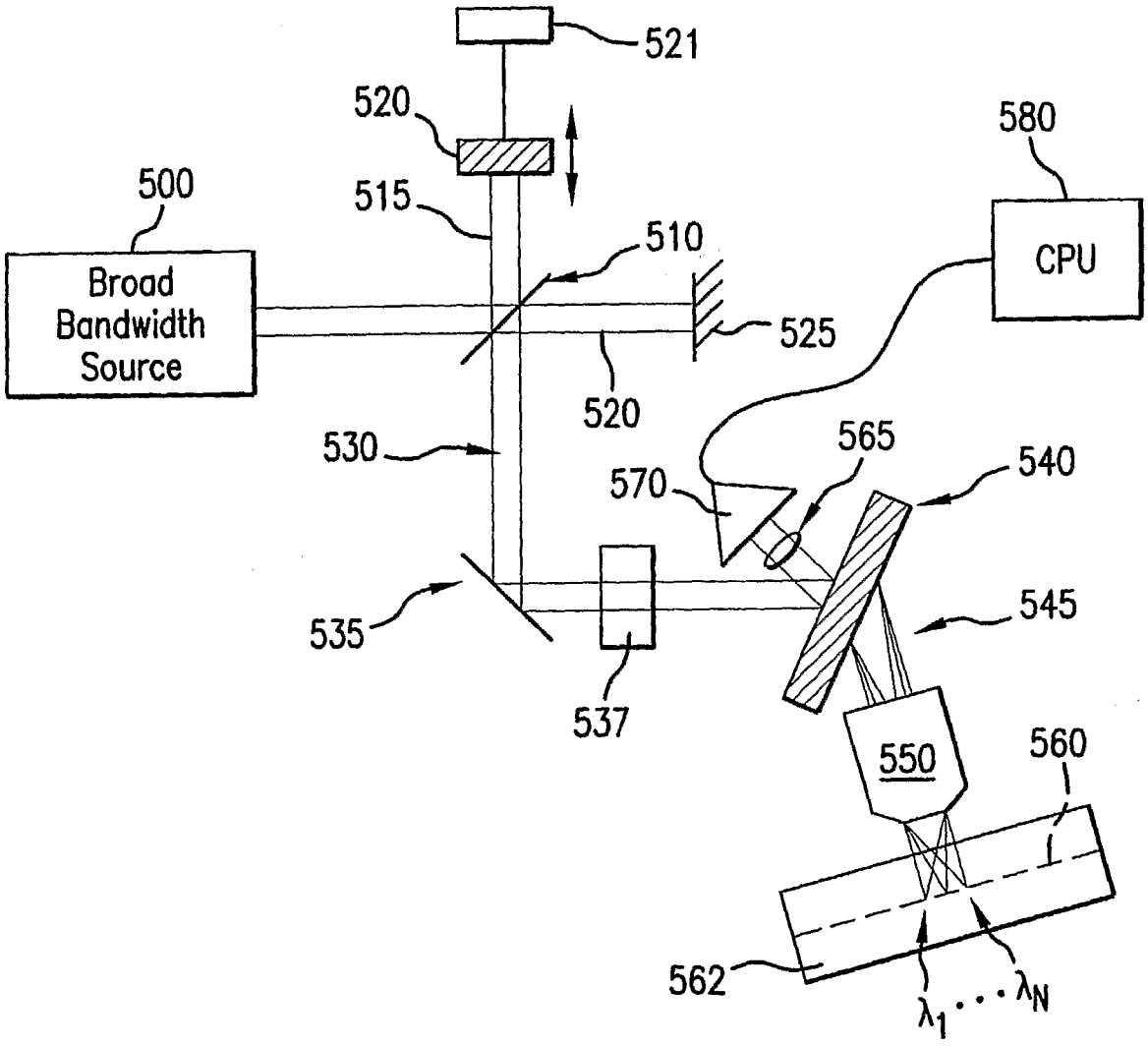
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FIG.5

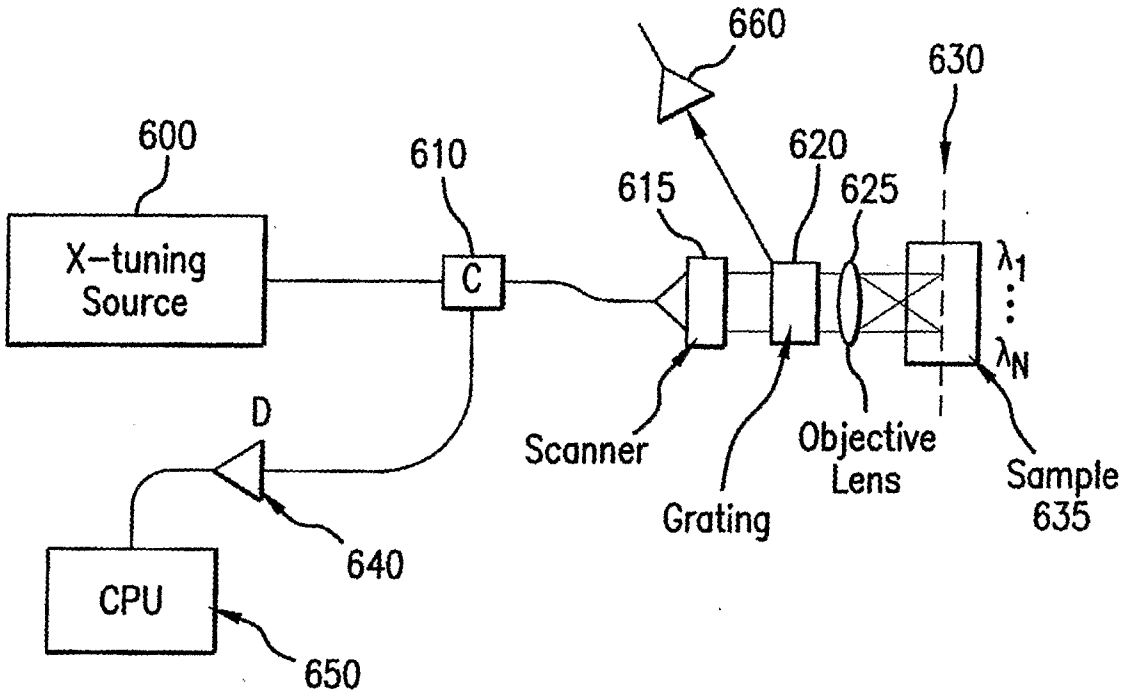
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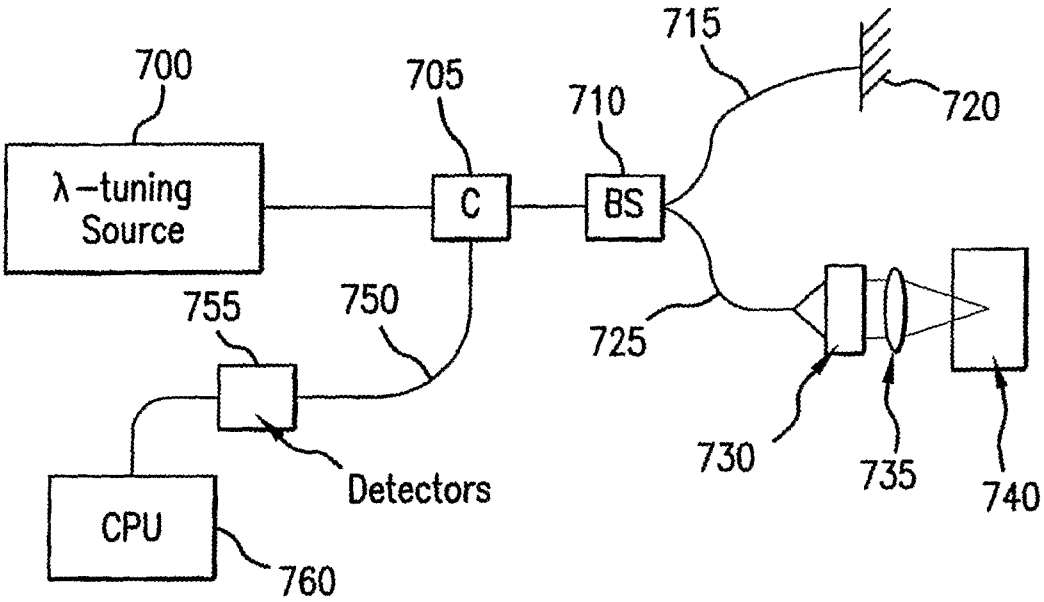
FIG.6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705
C
710
BS
715
720
725
730
735
740
750
755
Detectors
CPU
760

FIG. 7

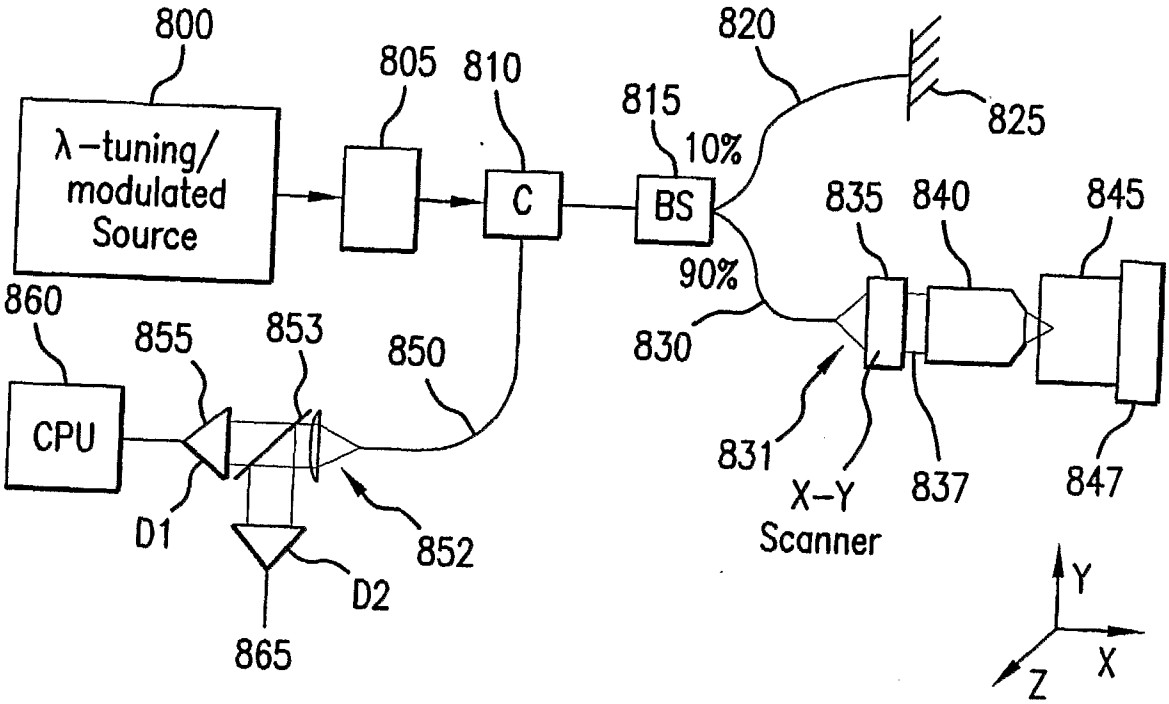
800
λ-tuning/
modulated
Source
805
810
C
815
10%
BS
820
825
90%
830
835
840
845
831
X-Y
Scanner
837
847
860
CPU
855
853
850
D1
852
D2
865
Y
X
Z

FIG.8

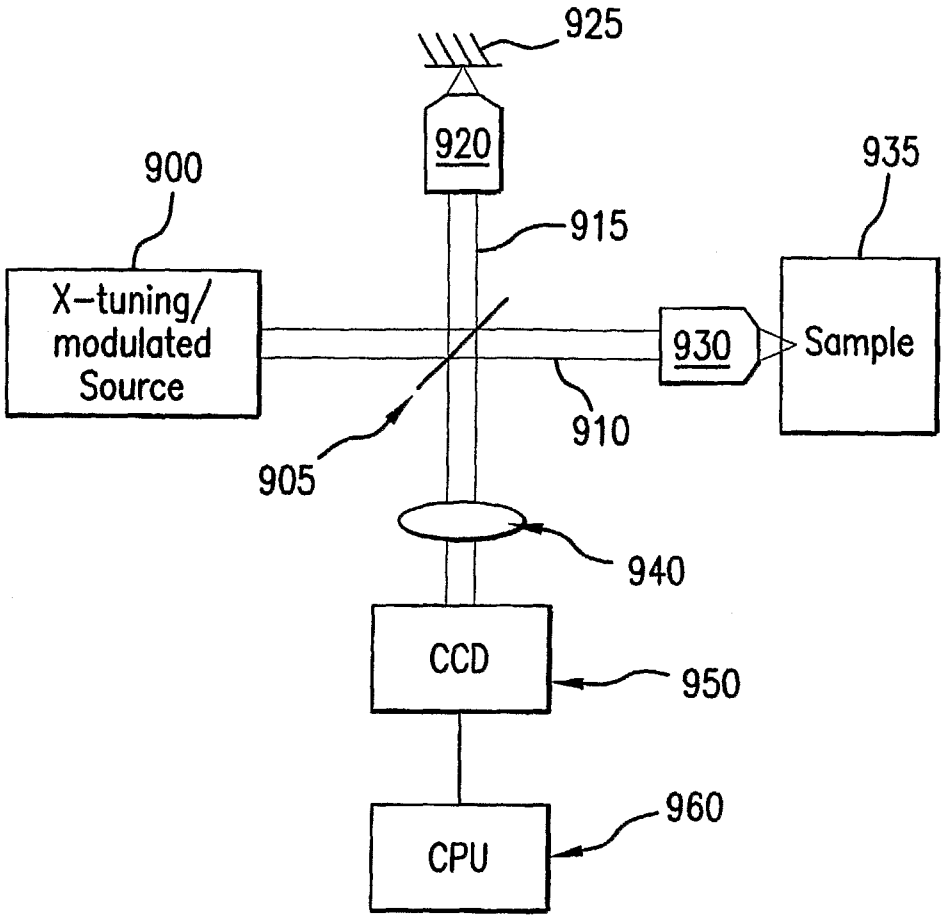
925
920
900
935
915
X-tuning/
modulated
Source
930
Sample
910
905
940
CCD
950
960
CPU

FIG.9

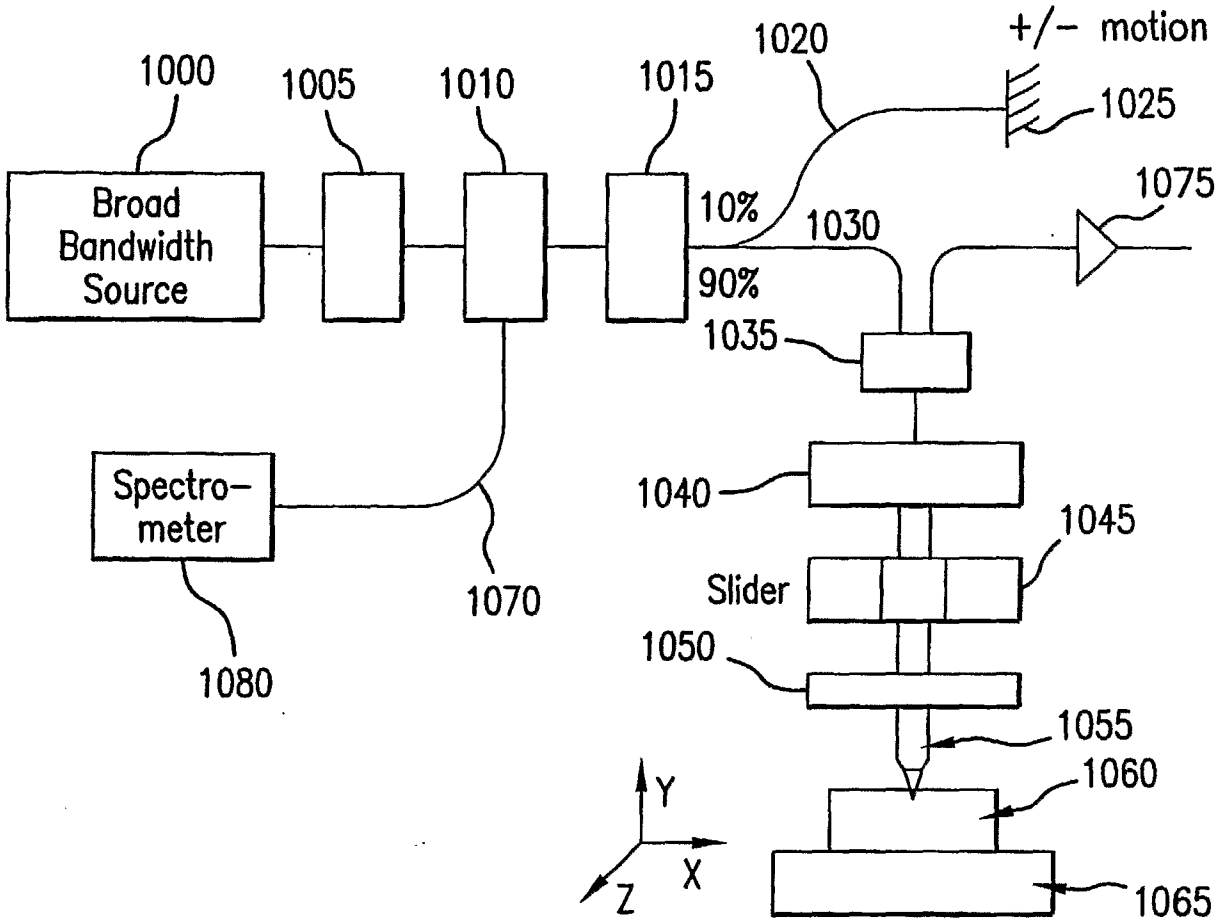
1000
1005
1010
1015
1020
+/- motion
1025
Broad Bandwidth Source
10%
90%
1030
1075
1035
Spectro-meter
1040
1045
Slider
1070
1050
1080
1055
1060
Y
X
Z
1065

FIG.10

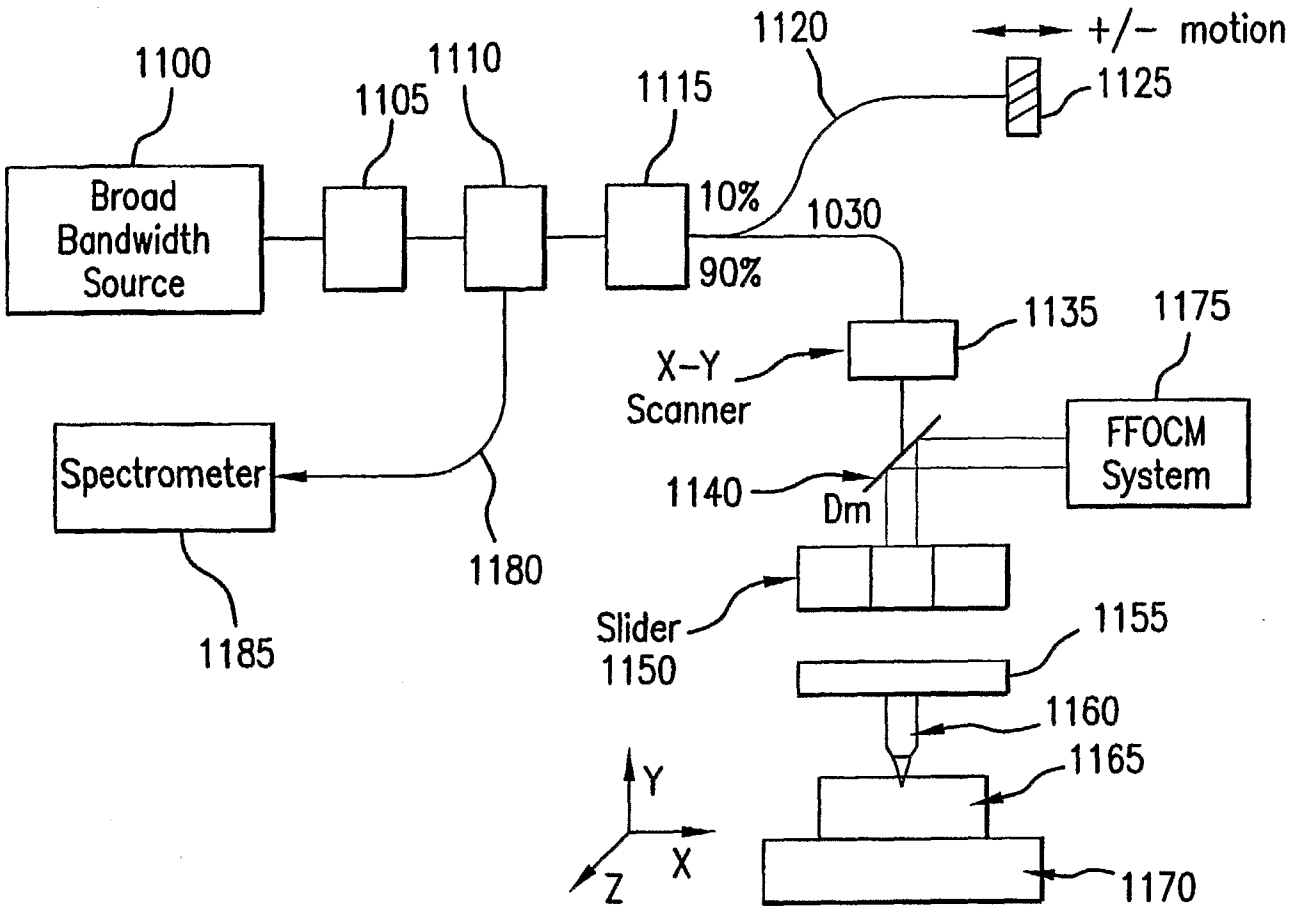
1100
1105
1110
1115
1120
+/- motion
1125
Broad Bandwidth Source
10%
90%
1030
1135
1175
X-Y Scanner
FFOCM System
Spectrometer
1140
Dm
1180
1185
Slider 1150
1155
1160
1165
Y
X
Z
1170

FIG.11

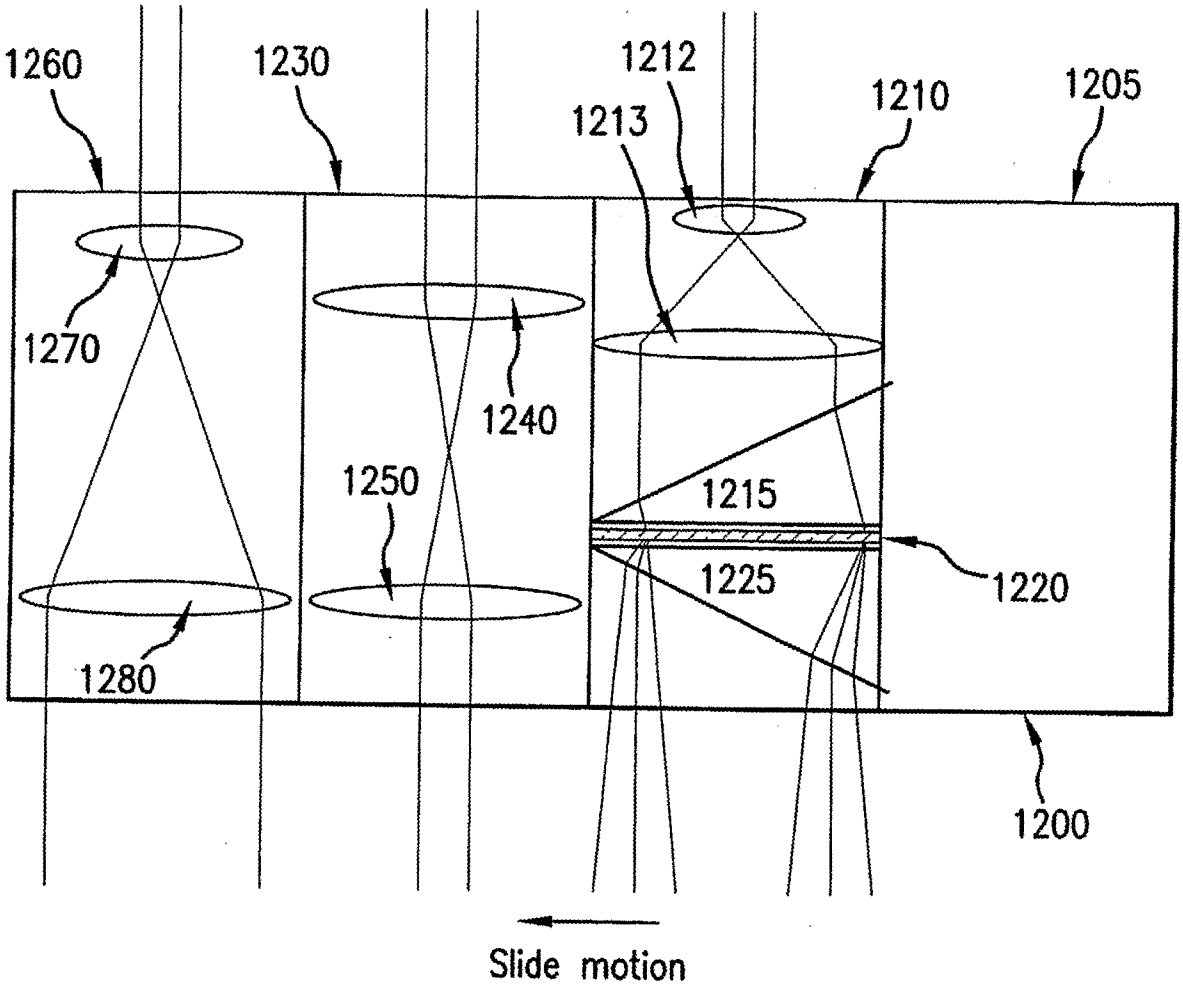
1260
1230
1212
1213
1210
1205
1270
1240
1250
1215
1225
1220
1280
1200
Slide motion

FIG. 12

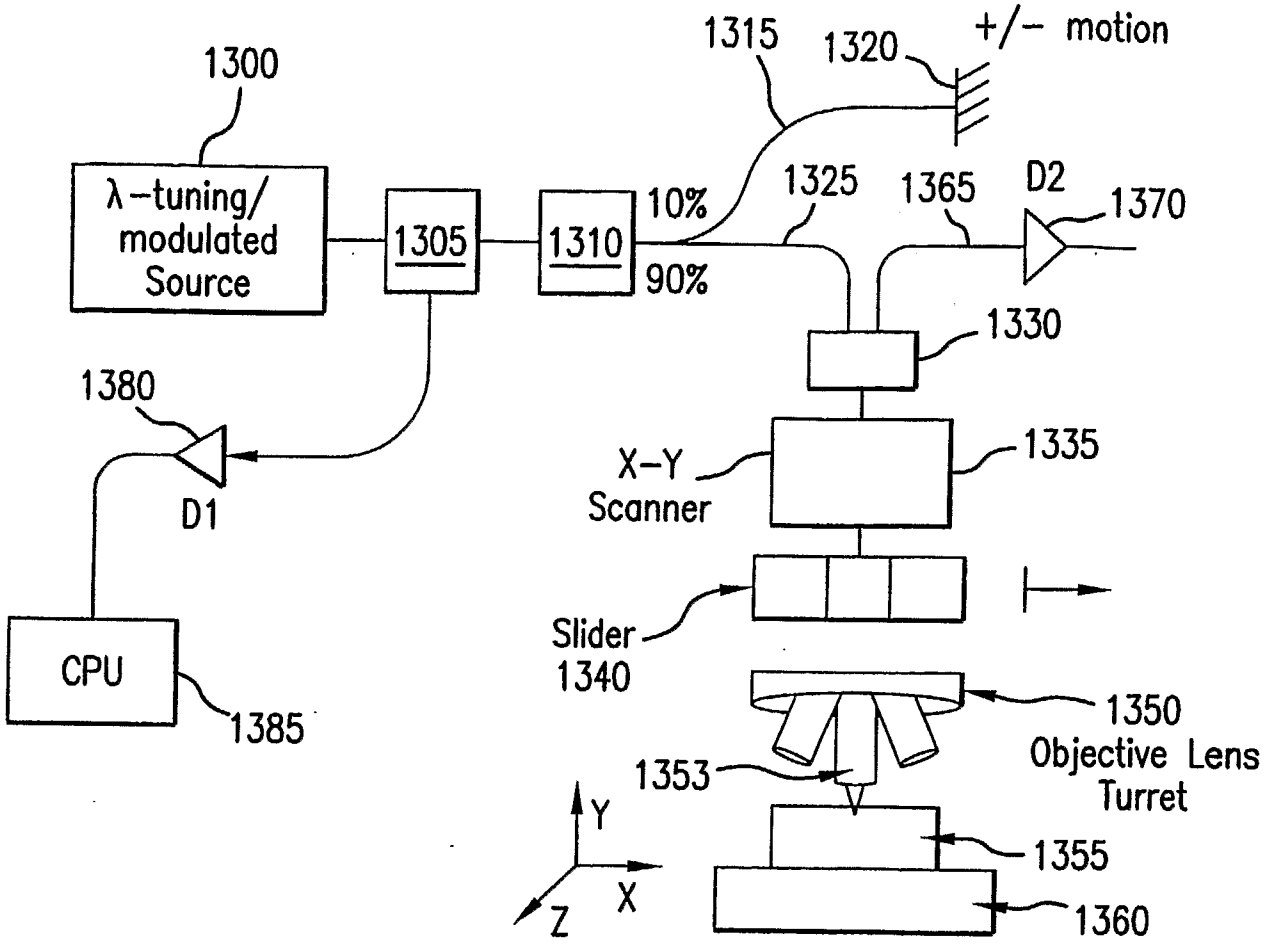
1300
λ-tuning/ modulated Source
1305
1310
10%
90%
1315
1320
+/- motion
1325
1365
D2
1370
1330
1380
D1
X-Y Scanner
1335
Slider 1340
CPU
1385
1350
Objective Lens Turret
1353
Y
X
Z
1355
1360

FIG.13

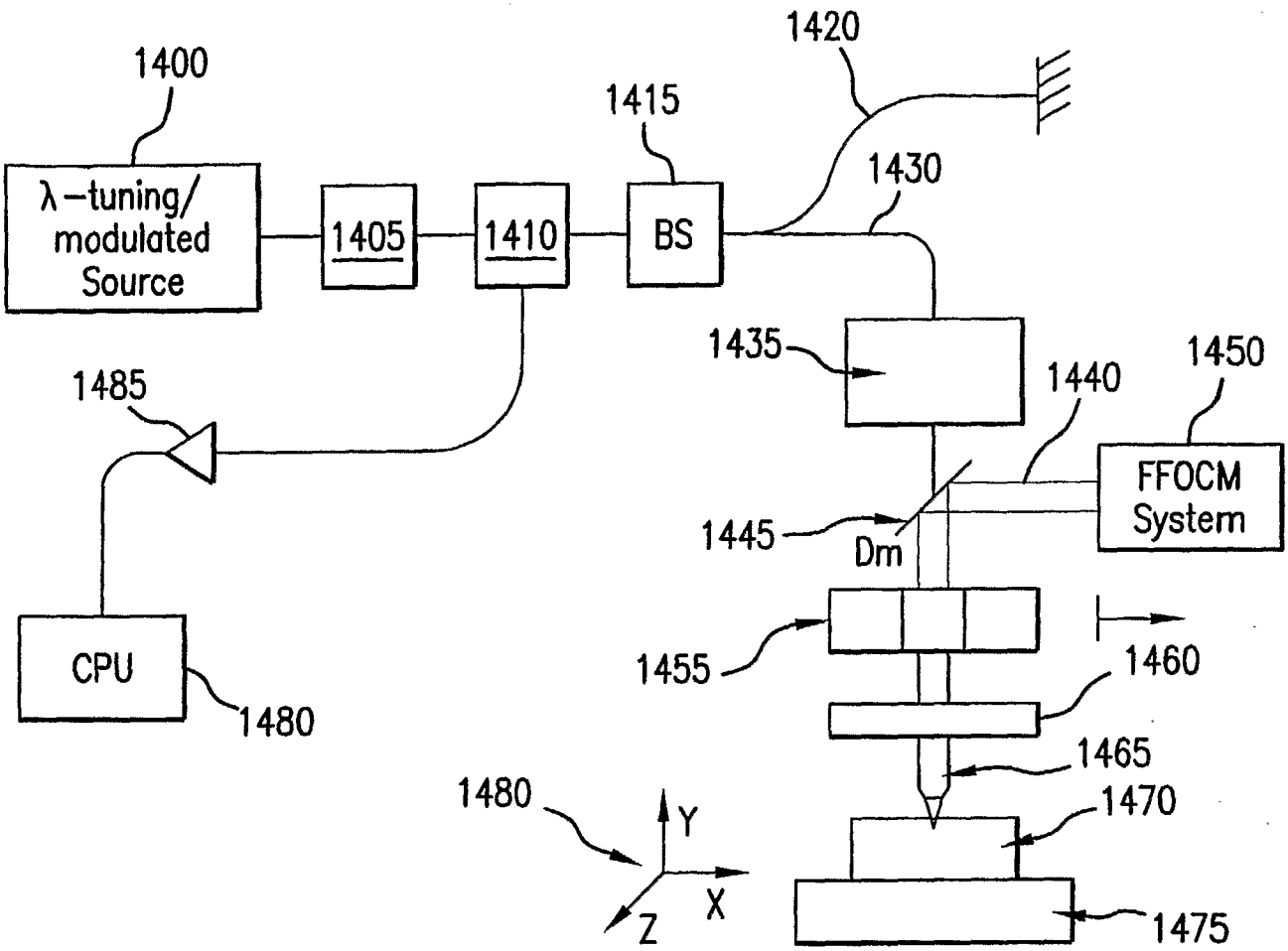
1400
λ–tuning/ modulated Source
1405
1410
1415
BS
1420
1430
1435
1440
1450
FFOCM System
1445
Dm
1455
1460
1465
1470
1475
1480
1485
CPU
1480
Y
X
Z

FIG.14

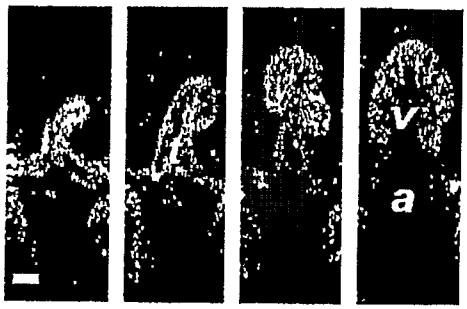

FIG.15a

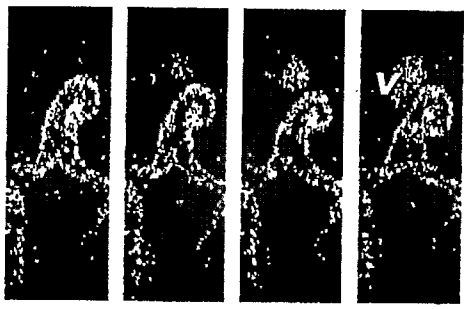

FIG.15b

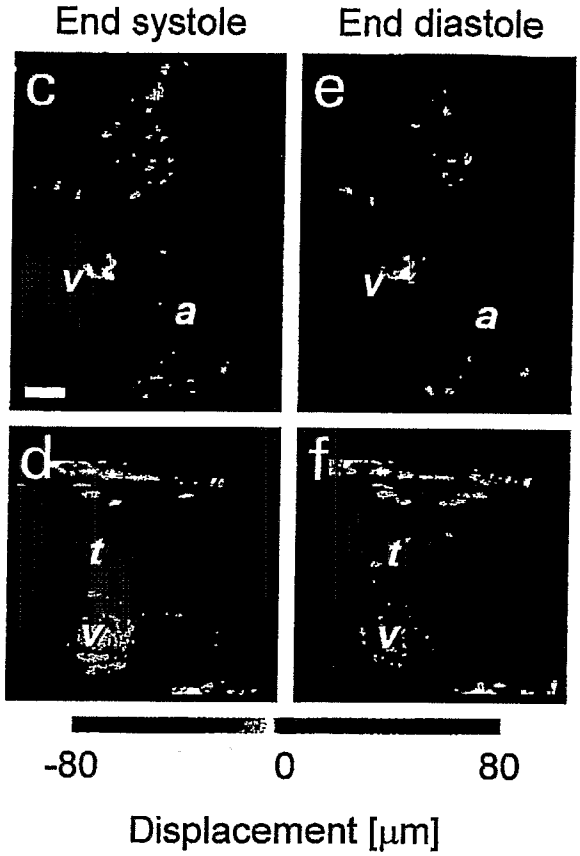

FIG.15

FIG.15g

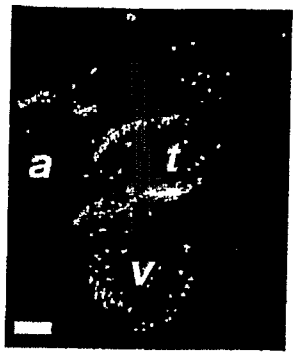

FIG.15h

FIG.15i

FIG.15j

FIG.15k

FIG.15l

FIG.15m

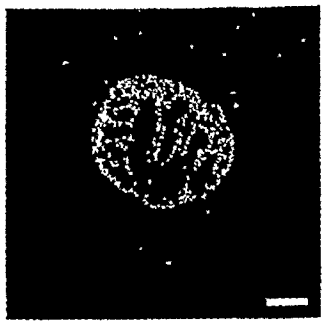

FIG.16a

FIG.16b

FIG.16c

FIG.16d

FIG.16e

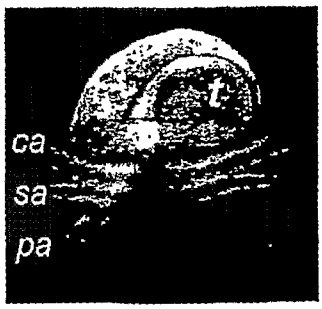

FIG.16f

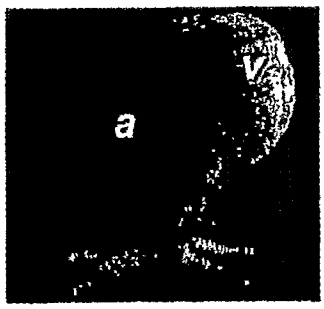

FIG.16g

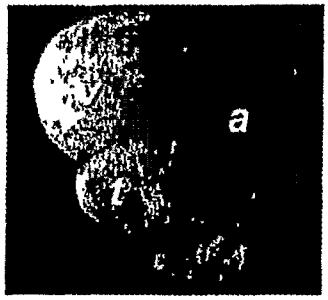

FIG. 16h

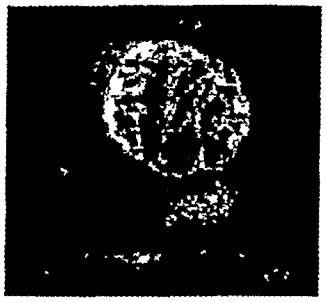

FIG. 16i

FIG. 16j

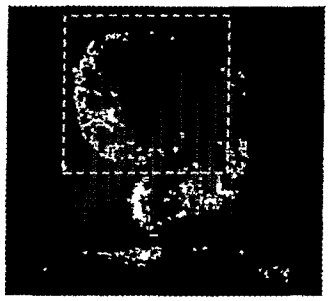

FIG. 16k

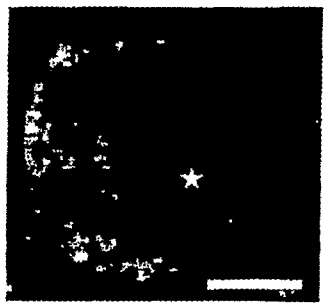

FIG. 16l

FIG. 16m

FIG.17a

FIG.17b

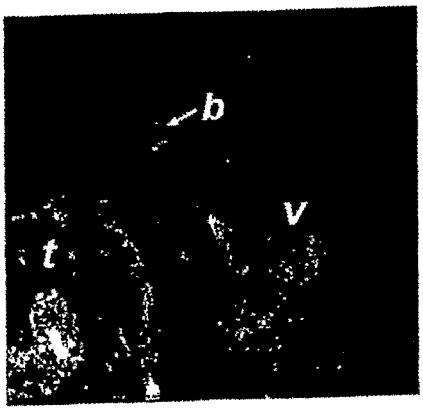

FIG.17c

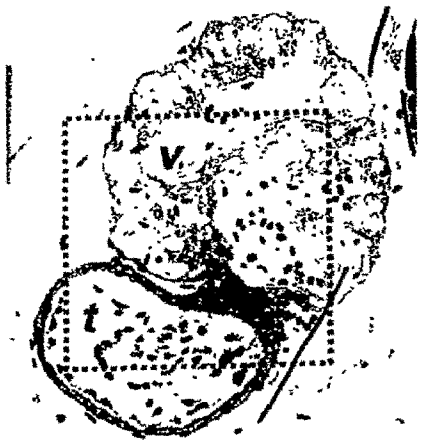

FIG.17d

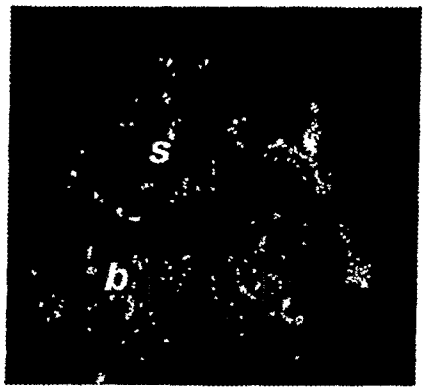

FIG.17e

FIG.17f

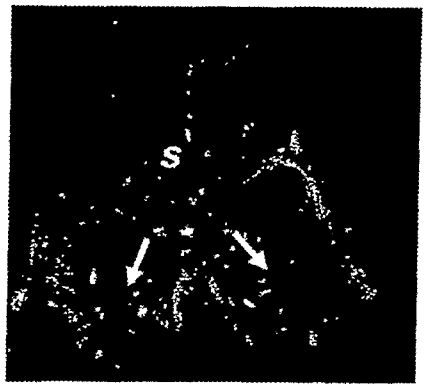

FIG.17g

FIG.17h

FIG.18a

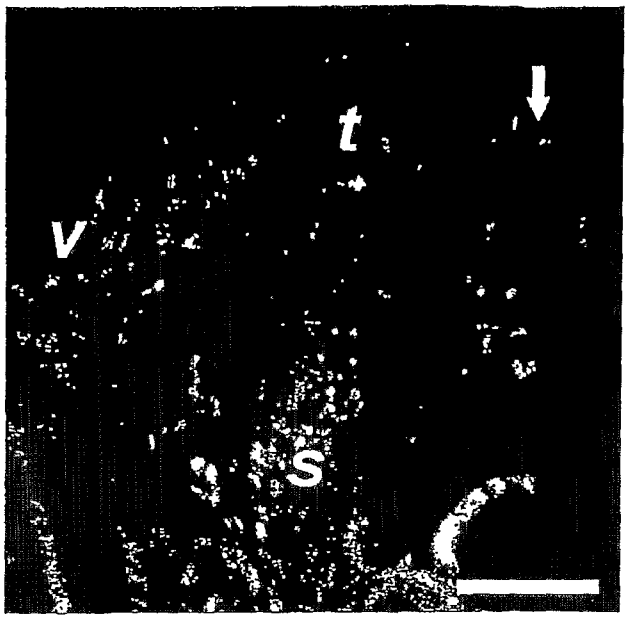

FIG.18b

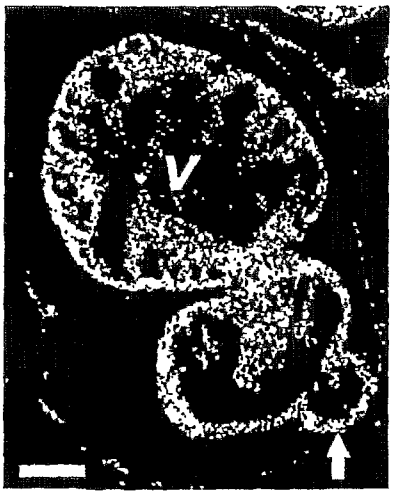

FIG.18c

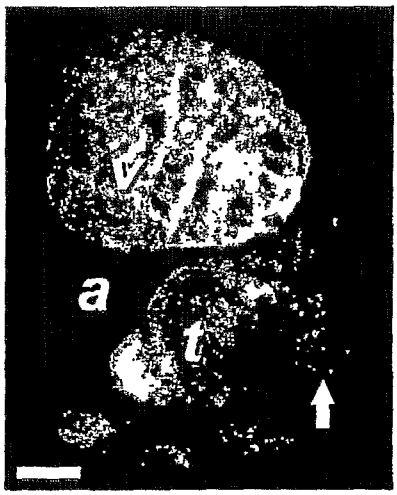

FIG.18d

FIG.18e

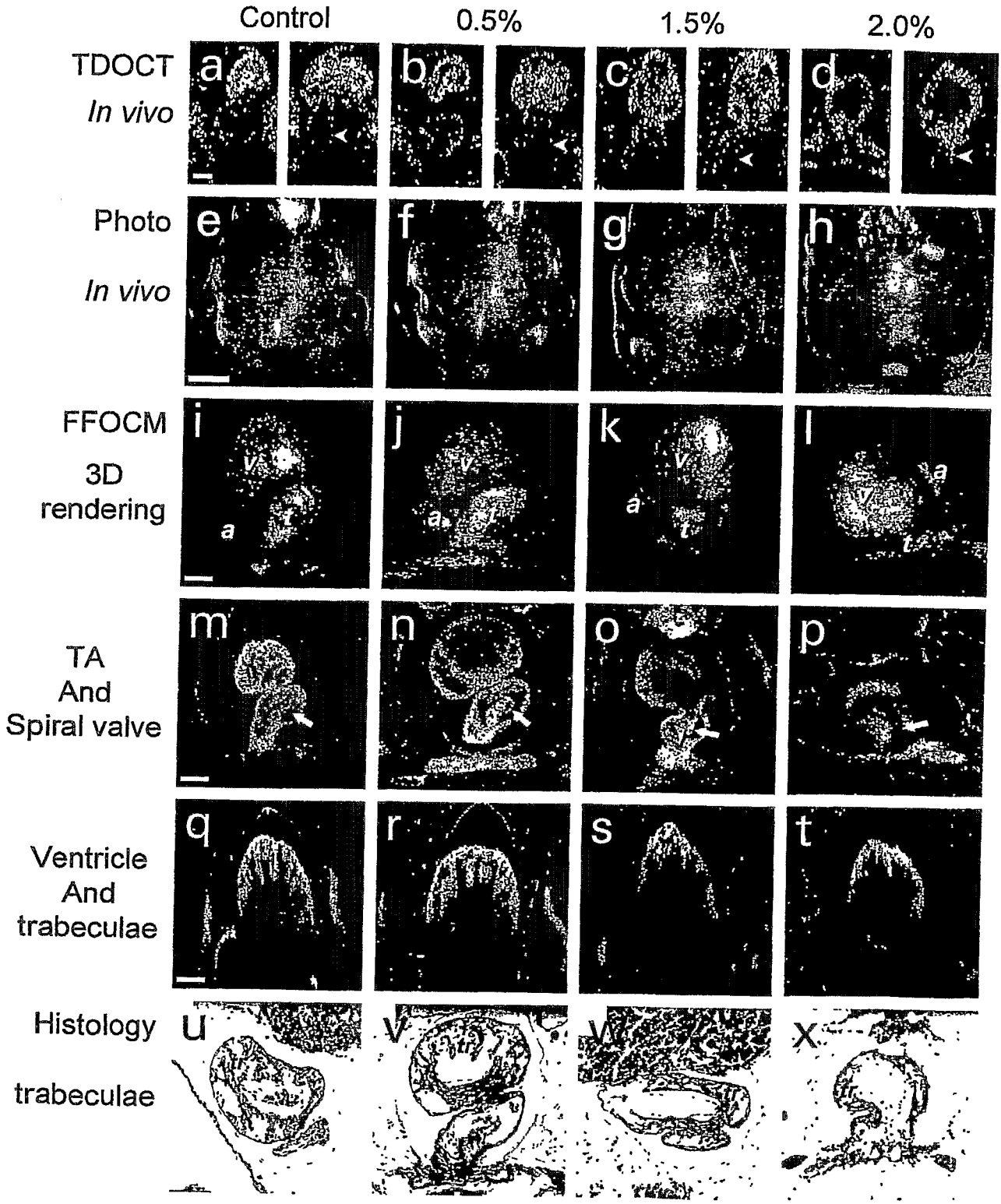
Control
0.5%
1.5%
2.0%
TDOCT
In vivo
Photo
In vivo
FFOCM
3D
rendering
TA
And
Spiral valve
Ventricle
And
trabeculae
Histology
trabeculae
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
u
v
w
x

FIG. 19

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/038223

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61B5/00 G02B21/00 G01B9/02 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N G01B A61B G02B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEAUREPAIRE E ET AL: "COMBINED SCANNING OPTICAL COHERENCE AND TWO-PHOTON-EXCITED FLUORESCENCE MICROSCOPY" OPTICS LETTERS, OSA, OPTICAL SOCIETY OF AMERICA, WASHINGTON, DC, US, vol. 24, no. 14, 15 July 1999 (1999-07-15), pages 969-971, XP000860575 ISSN: 0146-9592 figure 1	1,3-12, 14,15, 18-27, 29,30
Y	*Idem*	2,13,16, 17,28,31
Y	US 6 341 036 B1 (TEARNEY GUILLERMO J [US] ET AL) 22 January 2002 (2002-01-22) figures 3,4,5a	2,13,16, 17,28,31

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 December 2006	05/01/2007

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/038223

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/102111 A (UNIV BRUXELLES [BE]; DUBOIS FRANK [BE]; YOURASSOWSKY CATHERINE [BE]) 25 November 2004 (2004-11-25) figures 1,2	1
X	US 2003/220749 A1 (CHEN ZHONGPING [US] ET AL) 27 November 2003 (2003-11-27) figure 1	1
A	US 2002/016533 A1 (MARCHITTO KEVIN S [AU] ET AL) 7 February 2002 (2002-02-07) figures 1-5	1-31
X	US 2003/020922 A1 (CROWLEY ROBERT J [US] ET AL) 30 January 2003 (2003-01-30) figure 4a	1
A	US 6 790 175 B1 (FURUSAWA KOICHI [JP] ET AL) 14 September 2004 (2004-09-14) paragraph [0027]	1
X	WO 2004/002298 A (OTI OPHTHALMIC TECHNOLOGIES IN [CA]; PODOLEANU ADRIAN GH [GB]; JACKSON) 8 January 2004 (2004-01-08) figures 2-4	1
X	WO 03/055383 A (SCIMED LIFE SYSTEMS INC [BB]; CROWLEY ROBERT J [US]; BARBATO LOUIS J [) 10 July 2003 (2003-07-10) paragraph [0063]	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2006/038223

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		EP 1435835 A1	14-07-2004
		JP 2005514601 T	19-05-2005