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Method and apparatus for method for viewing and analyzing of one or more biological samples with progressively increasing resolutions

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ABSTRACT

Method, apparatus and arrangement according an exemplary embodiment of the present invention can be provided for analyzing and/or illustrating at least one portion of an anatomical structure. For example, light can be forwarded to such portion so as to generate first information which is related to the portion. For example, the light can be provided on or within a subject. The first information can be received, and at least one section of the portion may be selected based on the first information so as to generate second information. A magnification of a display of the portion may be progressively modified as a function of the second information.

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COMPLETE SPECIFICATION
STANDARD PATENT

Invention Title: **Method and apparatus for method for viewing and analyzing of one or more biological samples with progressively increasing resolutions**

The following statement is a full description of this invention, including the best method of performing it known to us:

METHOD AND APPARATUS FOR METHOD FOR VIEWING AND ANALYZING OF ONE OR MORE BIOLOGICAL SAMPLES WITH PROGRESSIVELY INCREASING RESOLUTIONS

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 relates to relates to methods and arrangements for viewing and analyzing of one or more biological samples and anatomic structures with changing magnifications, preferably progressively increasing resolutions.

BACKGROUND OF THE INVENTION

[0003] Radiological techniques such as X-ray computed tomography ("CT"), magnetic resonance imaging ("MRI"), and ultrasound can enable noninvasive visualization of human pathology at the organ level. Although these modalities may be capable of identifying large-scale pathology, the diagnosis of cancer can require the evaluation of microscopic structures that is beyond the resolution of conventional imaging techniques. Consequently, biopsy and histopathologic examination may be required for diagnosis. Because precancerous growth and early stage cancers often arise on a microscopic scale, they can present significant challenges for identification and diagnosis. Conventional screening and surveillance of these pathologies relies on unguided biopsy and morphological analysis of Hematoxylin and Eosin ("H&E") stained slides. Although this approach may be regarded as a current standard for microscopic diagnosis, it requires the removal of tissue from the patient and significant processing time to generate slides. More importantly, histopathology is inherently a point sampling technique; frequently only a very small fraction of the diseased tissue can be excised and often less than 1% of a biopsy sample may be examined by a pathologist.

[0004] It may be preferable to obtain microscopic diagnoses from an entire organ or biological system in a living human patient. However, the lack of an appropriate imaging technology can greatly limits options for screening for pre-neoplastic conditions (e.g. metaplasia) and dysplasia. In addition, an inability to identify areas of dysplasia and carcinoma in situ has led to screening procedures such as, e.g., random biopsy of the prostate, colon, esophagus, and bladder, etc., which can be highly undesirable and indiscriminate. Many diagnostic tasks presently referred to a frozen section laboratory, such as the delineation of surgical tumor margins, could be improved by a diagnostic modality capable of rapidly imaging large tissue volumes on a microscopic scale. A technology that could fill this gap between pathology and radiology would be of great benefit to patient management and health care.

[0005] Technical advances have been made to increase the resolution of non-invasive imaging techniques such as, e.g., micro-CT, micro-PET, and magnetic resonance imaging ("MRI") microscopy. Resolutions approaching 20 μm have been achieved by these technologies, but fundamental physical limitations can still prevent their application in patients. Microscopic optical biopsy techniques, performed in situ, have recently been advanced for non-excisional histopathologic diagnosis. Reflectance confocal microscopy ("RCM") may be particularly well-suited for non-invasive microscopy in patients, as it is capable of measuring microscopic structure without tissue contact and does not require the administration of extrinsic contrast agents. RCM can reject out of focus light and detects backscattered photons selectively originating from a single plane within the tissue. RCM can be implemented, e.g., by rapidly scanning a focused beam of electromagnetic radiation in a plane parallel to a tissue surface, yielding transverse or en face images of tissue. The large numerical aperture (NA) that may be used in RCM can yield a very high spatial resolution (1-2 μm), enabling visualization of subcellular structures. High NA imaging, however, can be particularly sensitive to aberrations that arise as light propagates through inhomogeneous tissue. Also, high-resolution imaging with RCM is typically limited to a depth of about 100-400 μm.

[0006] RCM has been extensively demonstrated as a viable imaging technique for skin tissue. Development of endoscopic confocal microscopy systems has been more

difficult, owing at least in part to the substantial technical challenges involved in miniaturizing a scanning microscope. One major obstacle to direct application of the concepts of confocal microscopy to endoscopy is the engineering of a mechanism for rapidly rastering a focused beam at the distal end of a small-diameter, flexible probe. A variety of approaches have been proposed to address this problem, including the use of distal micro-electromechanical systems ("MEMS") beam scanning devices and proximal scanning of single-mode fiber bundles. Also, RCM may provide microscopic images only at discrete locations - a "point sampling" technique. As currently implemented, point sampling can be inherent to RCM because it has a limited field of view, which may be comparable to or less than that of an excisional biopsy, and the imaging rate can be too slow for comprehensive large field microscopy.

[0007] Another challenge in adapting confocal microscopy to endoscopic applications can include miniaturization of high NA objectives that may be used for optical sectioning. Such miniaturization may be achieved by providing, e.g., a gradient index lens system, dual-axis objectives, or custom designs of miniature objectives. For example, detailed images of the morphology of cervical epithelium may be obtained in vivo using a fiber optic bundle coupled to a miniature objective lens, and fluorescencebased images of colorectal lesions may be achieved using commercial instruments such as those which may be obtained, e.g., from Olympus Corp. and Pentax/Optiscan.

[0008] Despite these advances, there may be a need to provide methods and arrangements that can parse data (e.g., provided either at the cellular level, architectural level or both that can be obtained from large surface areas or even possibly entire organs) so that it may be appropriately interpreted in a timely, accurate manner. Indeed, the amount of this data can be large and difficult to view at one time such data, and thus such methods and arrangements would be beneficial for viewing and analysis thereof.

[0008a] It is not admitted that any background information presented or referred to in this specification forms part of the common general knowledge in Australia or any other jurisdiction.

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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 methods and arrangements for viewing and analyzing of one or more biological samples and anatomic structures with changing magnifications, preferably at progressively increasing resolutions. Such exemplary methods and arrangements can be used along with a visual inspection of the data or by automatic processing procedures of the data to guide the visualization of areas that are most likely to contain abnormal and/or unhealthy tissue.

[0010] Accordingly, method, apparatus and arrangement according to exemplary embodiments of the present invention can be provided and which may analyze and/or illustrate at least one portion of an anatomical structure.

[0010a] In a first aspect, the invention provides an apparatus comprising:

at least one first arrangement configured to forward an electromagnetic radiation to an anatomical structure, and continuously scan an entire region of at least one portion of the anatomical structure using the electromagnetic radiation to generate at least one signal, wherein the entire region has an area that is greater than $1mm^2$; and

at least one second arrangement configured to review the signal and to generate at least one image based on the signal that has a transverse resolution that is better than 10μm.

[0010b] in a second aspect, the invention provides a method for imaging a region of an anatomical structure comprising:

forwarding at least one electromagnetic radiation to an anatomical structure;

continuously scanning an entire region of at least one portion of the anatomical structure using the electromagnetic radiation, wherein the entire region has an area that is greater than 1 mm^2;

obtaining at least one signal based on the electromagnetic radiation; and

generating at least one image based on the signal, wherein the image has a transverse resolution that is better than 10μm.

[0010c] Also disclosed herein but not claimed is a method for at least one of analyzing or illustrating at least one portion of an anatomical structure, comprising:

forwarding light to the at least one portion so as to generate first information which is related to the at least one portion, wherein a generating arrangement is situated within a subject containing the anatomical structure and provides the light is provided on or within the subject, the generating arrangement generating the first information, which first information is associated with an image;

receiving the first information, and selecting at least one section of the at least one portion based on the first information so as to generate second information; and

progressively modifying a magnification of a display of the at least one portion as a function of the second information.

[0010d] Further disclosed herein but not claimed is an apparatus for at least one of analyzing or illustrating at least one portion of an anatomical structure, comprising:

at least one first arrangement which is situated within a subject containing the anatomical structure and configured to forward light to the at least one portion so as to generate first information which is related to the at least one portion, the first information being associated with an image;

at least one second arrangement which is configured to receive the first information, and select at least one section of the at least one portion based on the first information so as to generate second information; and

at least one third arrangement which is configured to progressively modify a magnification of a display of the at least one portion as a function of the second information.

[0010e] Still further disclosed herein but not claimed is an arrangement for at least one of analyzing or illustrating at least one portion of an anatomical structure, comprising:

a tangible computer-accessible medium which stores software thereon comprising:

a first set of instructions which, when executed by a computer processing arrangement, configures the computer processing arrangement to receive first information which is related to the at least one portion, the first information being generated by a generating arrangement in response to light being forwarded by the generating arrangement to the at least one portion, the generating arrangement being situated within a subject containing the anatomical structure, the first information being associated with an image; a second set of instructions which, when executed by the computer processing arrangement, configures the

computer processing arrangement to receive the first information, and allows at least one of the computer processing arrangement or a user to select at least one section of the at least one portion based on the first information so as to generate second information; and

a third set of instructions which, when executed by the computer processing arrangement, configures the computer processing arrangement to progressively modify a magnification of display of the at least one portion as a function of the second information.

[0011] In an exemplary embodiment of the present invention, display of position and/or depth of the at least one portion can be modified (e.g., within the anatomical structure). The second information can be associated with a region provided within such portion, and/or may be obtained by user-selecting the region. The selction can be automatically performed by a processing arrangement without an input from a user. An area of an abnormality within the at least one portion can be determined, and the processing arrangement may perform the selection and modification so as to display at least one section of the abnormality. An area of an abnormality can be determined within the portion using the processing arrangement so as to generate third information, and the selection can be performed by the user and/or the processing arrangement as a function of the third information.

[0012] According to yet another exemplary embodiment of the present invention, the first information can be associated with two-, three- or four or more-dimensional, representation of the portion. Further, the portion can have an area that is greater than 1 mm^2 and/or 10 mm^2. A displayed section of the portion can have an area is less than 1 cm^2, 1 mm^2 and/or 100 μm^2. The first information can be associated with a confocal

microscopy procedure, a spectrally-encoded confocal microscopy procedure, an optical coherence tomography procedure, and/or an optical frequency domain interferometry procedure. An arrangement can be situated within the anatomical structure so as to provide the light to the portion.

[0013] 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

[0014] 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:

[0015] FIG. 1 is a schematic illustration of an exemplary spectrally encoded confocal microscopy (SECM) system;

[0016] FIG. 2A is an exemplary SECM image of a swine intestinal epithelium, obtained ex vivo, 100 μm from the tissue surface using a single mode source and single-mode detection (SM-MM) configuration;

[0017] FIG. 2B is another exemplary SECM image of a swine intestinal epithelium, obtained using a single-mode source and multi-mode detection (SM-MM) configuration;

[0018] FIG. 2C is a magnified view of an SECM image of a swine intestinal epithelium;

[0019] FIG. 3A is an exemplary SECM image of a swine intestinal epithelium, obtained ex vivo, after compression of the bowel wall at an imaging depth of 50 μm;

[0020] FIG. 3B is an exemplary SECM image of a swine intestinal epithelium, obtained ex vivo, after compression of the bowel wall at an imaging depth of 100 μm;

[0021] FIG. 4 is a schematic illustration of an exemplary SECM apparatus;

[0022] FIG. 5 is an exemplary SECM image of a USAF chart;

[0023] FIG. 6A is an exemplary SECM image based on data taken from a lens paper sample, displayed at a magnification of 1x;

[0024] FIG. 6B is an exemplary SECM image based on data taken from a lens paper sample, displayed at a magnification of 4.5x;

[0025] FIG. 6C is an exemplary SECM image based on data taken from a lens paper sample, displayed at a magnification of 16.7x;

[0026] FIG. 6D is an exemplary SECM image based on data taken from a lens paper sample, displayed at a magnification of 50x;

[0027] FIG. 6E is an exemplary SECM image based on data taken from a lens paper sample, displayed at a magnification of 125x;

[0028] FIG. 7 is a series of exemplary SECM data obtained from a lens paper sample at five different focal positions, together with a combine image that was generated by combining the data in the five individual images;

[0029] FIG. 8A is an exemplary SECM image based on data taken from a swine intestinal tissue fragment, displayed at a magnification of 1x;

[0030] FIG. 8B is an exemplary SECM image based on data taken from a swine intestinal tissue fragment, displayed at a magnification of 4x;

[0031] FIG. 8C is an exemplary SECM image based on data taken from a swine intestinal tissue fragment, displayed at a magnification of 20x;

[0032] FIG. 8D is an exemplary SECM image based on data taken from a swine intestinal tissue fragment, displayed at a magnification of 40x;

[0033] FIG. 9A are front and elevation side views of microscopic images of a porcine esophagus in vivo which shows a vascular network within the submucosa without image enhancement or exogenous contrast agents using an exemplary embodiment of a method and an arrangement according to the present invention;

[0034] FIG. 9B is a side view of the microscopic image of a longitudinal cross-section through a wall of the esophageal at a location illustrated in FIG. 9A;

[0035] FIG. 9C is a side view of an unwrapped transverse section at the location illustrated in A;

[0036] FIG. 9D is a side view of an expanded view of a selected section of the image illustrated in FIG. 9C;

[0037] FIG. 9E is an exemplary image of a representative histology section obtained from the anatomical region corresponding to the image illustrated in FIG. 9D;

[0038] FIG. 10 is a flow diagram of an exemplary embodiment of the method for progressively zooming into a microscopy dataset of an anatomical structure of the present invention;

[0039] FIG. 11 is a series of exemplary images of esophageal mucosa obtained using optical coherence tomography ("OCT") techniques, demonstrating an implementation of an exemplary embodiment of an automatic processing procedure for identifying normal squamous mucosa as compared to Barrett's esophagus and adenocarcinoma;

[0040] FIG. 12 is a set of exemplary images of atherosclerotic plaque obtained using the OCT techniques, which have been processed to identify a macrophage density; and

[0041] FIG. 13 is a flow diagram of another exemplary embodiment of the method according to the present invention for progressively zooming to a microscopy dataset of an anatomical structure based on the results obtained via a signal processing technique to automatically identify regions of interest that may be viewed at a high magnification.

[0042] 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

[0043] In accordance with exemplary embodiments of the present invention, methods and arrangements according to exemplary embodiments of the present invention can be provided for viewing and analyzing of one or more biological samples and anatomic structures with progressively increasing resolutions. Such exemplary methods and arrangements can be used along with a visual inspection of the data or by automatic processing procedures of the data to guide the visualization of areas that are most likely to contain abnormal and/or unhealthy tissue.

[0044] An exemplary SECM technique is shown in FIG. 1. The output from a single-mode optical fiber 100, which may be located at a distal end of a probe, can be collimated by a collimating lens 110, and then illuminate a dispersive optical element (such as, e.g., a transmission diffraction grating 120). An objective lens 130 can then focus each diffracted wavelength to a distinct spatial location within the specimen, resulting in a transverse line focus 140 where each point on the line may be characterized by a distinct wavelength. After reflection from the specimen, which may be, e.g., biological tissue, the optical signal can be recombined by the diffraction element 120 and collected by the single-mode fiber 100. The core aperture of the single-mode fiber 100 can provide a spatial filtering mechanism that is capable of rejecting out-of-focus light. Outside the probe (and optionally within a system console) the spectrum of the returned light can be measured and converted into confocal reflectance as a function of transverse displacement within the specimen. The spectral decoding can be performed rapidly.

Thus an image created by scanning the beam in a direction orthogonal to the line focus can be accomplished by relatively slow and straightforward mechanical actuation.

[0045] SECM techniques may allow the use of endoscopic RCM, and it can be capable of providing image data at extremely high rates using high-speed linear CCD cameras. Commercially available linear CCD arrays can obtain data at a rate greater than about 60 million pixels per second. When incorporated into an SECM spectrometer, these arrays can produce confocal images at speeds that are about 10 times faster than a typical video rate and up to 100 times faster than some endoscopic RCM techniques. The rapid imaging rate and fiber-optic design of typical SECM systems can permit comprehensive, large area microscopy through an endoscopic probe.

[0046] Techniques using optical coherence tomography ("OCT") and variations thereof may be used for comprehensive architectural screening. Acquiring an OCT signal in the wavelength domain, rather than in the time domain, can provide orders of magnitude improvement in imaging speed while maintaining excellent image quality. Using spectral domain OCT ("SD-OCT") techniques, high-resolution ranging can be conducted in biological tissue by detecting spectrally resolved interference between a tissue sample and a reference. Because SD-OCT systems can utilize the same high-speed linear CCD's as SECM systems, they can also be capable of capturing images at 60 million pixels/s, which is approximately two orders of magnitude faster than conventional time-domain OCT ("TD-OCT") systems. With this acquisition rate and resolution, SD-OCT systems can provide comprehensive volumetric microscopy at the architectural level in a clinical environment.

[0047] The information provided by SD-OCT and SECM systems can be complementary, and a hybrid platform utilizing both techniques can provide information on the architectural and cellular structure of tissue that may be essential to accurate diagnosis. Although a combination of disparate technologies typically requires extensive engineering and may compromises performance, SECM and SD-OCT systems can share key components, and a high-performance multi-modality system can be provided without substantially increasing complexity or cost of the individual systems.

[0048] An SECM system in accordance with certain exemplary embodiments of the present invention can utilize a wavelength-swept 1300 nm source and a single-element photodetector to obtain spectrally encoded information as a function of time. With this system, images can be acquired at rates of up to about 30 frames/second having high lateral (1.4 μm) and axial (6 μm) resolutions, over a 400 μm field of view ("FOV"). Images of freshly excised swine duodenum segments were imaged ex vivo with a high speed system to illustrate the capability of an SECM system to identify subcellular structures that may be found in specialized intestinal metaplasia ("SIM"), the metaplastic change of BE.

[0049] FIGS. 2A-2C depict exemplary SECM images of a swine intestinal epithelium obtained ex vivo using two imaging modes and corresponding fiber configurations: a single-mode illumination with single-mode detection ("SM-SM"), and a single-mode illumination with multi-mode detection ("SM-MM"). The SM-SM image in FIG. 2A shows the epithelium structure 100 μm from the tissue surface using a single mode source and single-mode detection. The image of the same tissue region shown in FIG. 2B, obtained using a using a single mode source and multi-mode detection (SM-MM) with a core:aperture ratio of 1:4, appears smoother and may be more easily interpreted because of the reduction in speckle noise. FIG. 2C is a magnified view of the image shown in FIG. 2B that shows evidence of villi containing a poorly reflecting core (e.g., lamina propria or "lp") and a more highly scattering columnar epithelium. Bright image densities visible at the base of the columnar cells, consistent with nuclei (indicated by arrows) are evident in FIG. 2C.

[0050] The thickness of an esophageal wall being imaged in vivo using OCT techniques can be decreased, e.g., by about a factor of two using an inflated balloon. The swine intestinal sample shown in FIGS. 2A-2C was decreased by the same amount, and the subcellular features observed using SECM techniques were well preserved. FIGS. 3A and 3B show images of this thinned sample obtained at a depth of 50 μm and 100 μm, respectively.

[0051] The penetration depth of a commercial 800 nm laser scanning confocal microscope was observed to be reduced by about 20% as compared to that obtained with a 1300 nm SECM system. This reduced penetration may be a result of increased scattering of the shorter wavelength source. Thus an SECM system using an 840 nm source may provide sufficient penetration to identify subcellular structure of, e.g., an intestinal epithelium.

[0052] An apparatus in accordance with certain exemplary embodiments of the present invention that is configured to provide comprehensive SECM images is illustrated schematically in FIG. 4. This exemplary apparatus can be configured to obtain images from a cylindrical sample having a length of 2.5 cm and a diameter of 2.0 cm, which are approximately the dimensions of the distal esophagus. A fiber-coupled 2.0 mW superluminescent diode 200, having a wavelength centered at 800 nm and a bandwidth of 45 nm (QSSL-790-2, qPhotonics, Chesapeake, VA) was configured to illuminate a 50/50 single-mode fiber optic beam splitter 405. Light transmitted through one port of the splitter was collimated by a collimator 410 and transmitted through a fiber 412 to a focusing apparatus 415 and to a grating-lens pair that includes a grating 420 (1780 lpmm, Holographix, LLC, Hudson, MA) and a 350230-B asphere lens 425 (Thor Labs, Inc., Newton, NJ) having a focal length, f, of 4.5 mm, a clear aperture of 5.0 mm, and a NA of 0.55. This arrangement was capable of producing a 500 μm longitudinal linear array, or line, of focused, spectrally-encoded spots 430 on an interior surface of the cylindrical sample. The grating-lens pair was affixed to the shaft of a motor 435 (1516SR, 15 mm diameter, MicroMo Electronics, Inc., Clearwater, FL) by a housing 440. As the motor 435 rotated, the spectrally encoded line was scanned across the inner circumference of the cylindrical sample. The motor 435, housing 440, and grating-lens pair were translated along a longitudinal axis of the cylindrical sample during rotation of the motor 435 using a computer-controlled linear stage 445 (Nanomotion II, 2.5 cm range, Melles Griot, Rochester, NY). This procedure produced a helical scan of the entire interior surface of the cylindrical sample.

[0053] Light reflected from the sample was transmitted back through the optical system into the single-mode fiber 412 and provided by the fiber 412 to a spectrometer

450 and linear CCD 455 that includes 2048 pixels and has a 30 kHz line rate (Basler L104K, Basler Vision Technologies, Exton, PA). A computer 460 was used to store, analyze and display image data provided by the spectrometer 450 and CCD 455. Approximately 60,000 points per motor rotation (at 0.5 Hz, or 30 rpm) were digitized. to achieve a 1.0 μm circumferential sampling density. The longitudinal velocity of the motor was 0.25 mm/s and the time required for one complete scan of the cylindrical sample was 100 seconds.

[0054] The $1/e^2$ diameter of the collimated beam on the grating-lens pair was 4.0 mm. As a result, the effective NA of this exemplary apparatus was approximately 0.4, which corresponds to a theoretical spot diameter of approximately 1.2 μm and a confocal parameter of approximately 2.5 μm. In a system that is free of optical aberrations, the theoretical spectral resolution on the sample may be 0.8 Å, which can yield up to approximately 630 resolvable points across the spectrally encoded line 430. The spectrometer 450 in the detection arm was designed to exceed the predicted spectral resolution of the probe.

[0055] An SECM scan of a 1951 USAF resolution chart obtained using this apparatus is shown in FIG. 5. The smallest bars in this Figure, which are separated by 2.2 μm, were resolved. A transverse line spread function full-width-half-maximum ("FWHM") and an axial FWHM function obtained using a mirror scanned through the focus were measured as 2.1 μm and 5.5 μm, respectively. The field of view was observed to be about 500 μm. These measurements were slightly lower than corresponding theoretical values, which may be attributed to aberrations in the optical path. These actual parameters indicate that the exemplary apparatus described herein is capable of providing sufficient resolution to be used for confocal microscopy in biological tissue.

[0056] SECM image data for a complete pullback image of a 2.5 cm phantom specimen are shown in FIG. 6. Polar coordinates were converted to rectangular coordinates prior to generating these displayed images. The phantom specimen was made using lens paper affixed to the inner surface of a 2.1 cm inner diameter Teflon tube. In a low magnification image shown in FIG. 6A, macroscopic structure of the paper,

including folds and voids, can be observed. Circumferential stripes that are visible may have resulted from the lower spectral power and lens aberrations that may be present at or near the ends of the spectrally-encoded line. Individual fibers and fiber microstructure can be clearly resolved in regions of this data set that are presented at higher magnifications, as shown in FIGS. 6B-6E.

[0057] By adjusting the focusing apparatus 415 in FIG. 4A, cylindrical two-dimensional ("2D") images of the phantom sample were acquired at five discrete focal depths over a range of 120 μm. These five images 710-750 shown in FIG. 7 were then summed to create an integrated image 760, which demonstrates a nearly complete coverage of the surface of the phantom sample.

[0058] Imaging biological samples using an SECM apparatus such as that described herein can be complicated by the lack of a centering apparatus for the optical scan head. In order to provide further improvements for generating wide-field microscopy images and data, a sample of swine intestine was placed on top of a 2.0 cm diameter transparent cylinder. A 360° scan of this sample, which was acquired in 1 second, is shown in FIG. 8A. Imaged tissue appears in only one sector of the cylindrical scan because the probe was not centered and the sample did not wrap completely around the cylinder. FIGS. 8B-8D show a sequence of magnified regions of this tissue sample. The image shown in FIG. 8B is an expansion of a 1.5 cm sector outlined by a dotted rectangle in FIG. 8A. Similarly, the image in FIG. 8C represents an expansion of the rectangle outlined in FIG. 8B, and the image in FIG. 8D represents an expansion of the rectangle outlined in FIG. 8C. Magnified images of the tissue in the image FIG. 8B are suggestive of a glandular structure. The magnified images in FIGS. 8C-8D exhibit villi and nuclear features that are similar to those observed using a 1300 nm SECM system, as shown in FIGS. 2 and 3. Other areas of the SECM scan in FIG. 8A show artifacts, including specular reflectance from the transparent cylinder and complete signal dropout, both of which may result from improper positioning of a focused SECM beam.

[0059] Conducting comprehensive confocal microscopy in patients can present a variety of technical challenges. Such challenges may include, e.g., increasing the

imaging rate, miniaturizing the probe optics and mechanical components, incorporating a centering mechanism, and implementing a technique for dynamically changing the focal plane.

[0060] The image acquisition speed of an SECM system can be improved by, e.g., a factor of about 2-4 as compared with the exemplary system described hereinabove. Such an improvement can be realized by providing certain modifications. For example, a higher power semiconductor light source (such as, e.g., a Superlum Diode, T-840 HP: 25 mW, 840 nm, 100 nm spectral bandwidth) can provide 1000 spectrally resolvable points. The increase in optical power can improve sensitivity and the larger bandwidth may widen the field of view, making it possible to scan the SECM beam approximately two times faster. Also, using an optical circulator such as, e.g., an OC-3-850 (Optics for Research, Caldwell, NJ) can increase the efficiency of light delivered to the probe and collected from the probe. Using a faster, more sensitive linear CCD such as, for example, an AVIIVA M4-2048 having 2048 pixels and a 60 kHz readout rate (Atmel Corporation,) can provide a twofold increase in data acquisition speed and an improved spectral response over the wavelength range used to generate image data. Performance may also be improved by using, e.g., a Camera Link interface that can be capable of transferring data at a rate of approximately 120 MB/s from a camera to a hard-drive array for storage.

[0061] Sensitivity, which can be understood to refer to a minimum detectable reflectance, is a system parameter that can affect confocal image quality and penetration depth. A fraction of the incident light, which may be approximately 10^{-4} to 10^{-7}, can be reflected from skin at depths up to approximately 300 μm when using a near-infrared RCM technique. Based on the NA of the objective lens used in the exemplary system in accordance with certain exemplary embodiments of the present invention described herein and the observation that skin may attenuate light more significantly than non-keratinized epithelial mucosa, the exemplary SECM probe objective described herein may collect approximately $3x10^{-4}$ to $3x10^{-7}$ of the illuminating light reflected from deep within tissue. The 25 mW light source may be separated into, e.g., approximately 1000 independent beams. A maximum double pass insertion loss can be estimated to be approximately 10 dB (6 dB from the probe, and 4 dB from the fiber optics and

spectrometer). Each pixel in an array may thus be illuminated by approximately 50 to 50,000 photons/pixel for each line integration period based on these estimated parameters.

[0062] Using a multi-mode detection technique, a factor of 10 signal gain may be achieved, resulting in approximately 500 to 500,000 photons/pixel per scan for such a configuration. A single pixel on an Atmel AVIIVA M4 camera, e.g., can reliably detect light if a signal is above the dark current fluctuation that occurs at approximately 240 photons. If this device has approximately a 50% quantum efficiency at these wavelengths, a minimum detectable signal can be produced at approximately 480 photons/pixel per scan. Based on these approximations, an Atmel camera may have sufficient sensitivity to allow SECM imaging at deeper tissue depths. Quantum noise-limited detection of a predicted minimum reflectance can be achieved by using a multi-mode fiber for collection or by increasing the source power.

[0063] According to one exemplary embodiment of the present invention, methods and arrangements can be provided for navigating, analyze and display large microscopic datasets from anatomical structures.

[0064] FIGS. 9A-9E illustrate various images of a porcine esophagus in vivo obtained using comprehensive microscopy and the exemplary embodiments of the methods and arrangements of the present invention. These exemplary images can be generated by a computer 460 (e.g., personal computer, mini computer, etc.) shown in FIG. 4 or another processing arrangement which may be configured (e.g., by software) to forward such images to a display 470 of FIG. 4 or another output arrangement. In addition, the computer 460 can control various component of the exemplary system of FIG. 4 (e.g., motor 435, line translator 445, focusing apparatus 415, etc.) to focus on various areas of anatomical structures automatically and/or under a manual control which would enable the navigation, analysis and display of the large microscopic datasets associated with the anatomical structures.

[0065] For example, FIG. 9A shows front and elevation side views 900, 905, respectively, of microscopic images of a porcine esophagus in vivo which provides a

vascular network within the submucosa without image enhancement or exogenous contrast agents using such exemplary embodiment of the method and arrangement. Indeed, e.g., 14 GB volumetric data set of FIG. 9A can be rendered and downsampled for a presentation in arbitrary orientations and perspectives. The vascular network within the submucosa is shown without such image enhancement or exogenous contrast agents. Cross-sectional images can be located on the volume image for higher resolution viewing using the computer 460 configured for such exemplary task(s) and other components of the system of FIG. 4.

[0066] FIG. 9B shows a side view 910 of the microscopic image of a longitudinal cross-section through a wall of the esophageal at a location illustrated in FIG. 9A. For example, this image 910 is inverted with epithelium at the top; dimensions: 45 mm horizontal, 2.6 mm vertical. In the raw data, a periodic vertical offset corresponding to the motion of the beating heart can be observed. An exemplary embodiment of a surface-aligning procedure can be used to reduce this artifact but a residual vertical banding, that may still be observed with a period of 300 microns corresponding to a heart rate of 90 beats/min. The exemplary longitudinal pitch between adjacent A-lines is shown as 32 μm.

[0067] FIG. 9C shows a side view 920 of an unwrapped transverse section (e.g., cylindrical coordinates r & θ are mapped to vertical and horizontal) at the location illustrated in FIG. 9A. For example, the exemplary dimensions of the illustration are as follows: 57 mm - horizontal, 2.6 mm - vertical. Both FIGS. 9B and 9C illustrate the imaging through the entire esophageal wall, and can enable an identification of the squamous epithelium (e), lamina propria (lp), muscularis mucosa (mm), submucosa (s), and muscularis propria (mp). FIG. 9D shows a side view 930 of an expanded view of a selected section of the image illustrated in FIG. 9C which can be used to assist with such identification. FIG. 9E shows an exemplary image of a representative histology section (H&E stain) obtained from the anatomical region corresponding to the image illustrated in FIG. 9D.

[0068] For example, FIG. 10 depicts a flow diagram describing an exemplary embodiment of a method or a procedure according to the present invention for analyzing and/or viewing the data set at progressively higher resolutions, which can be executed using the computer 460 shown in FIG. 4. Particularly, in step 1000, a microscopic dataset, which can have a resolution of less than 10 μm, may be acquired over a large area of tissue or from a volume of tissue, or organ therein. The data can then be formatted (in step 1010) in a representation that may illustrate a low magnification or low power view of the entire data set or a portion of the data set. In step 1020, the user may view the data set, and using a computer interface, can select (a) a rectangular region, (b) a point, (c) an arbitrary shaped region, and/or (d) a depth in which to visualize a higher magnification view. The new region can be viewed in step 1020, and the user can (a) select another region, (b) zoom in at a point, (c) zoom out, (d) translate the current view in three dimensions, and/or (e) change the depth location of viewing within the dataset.

[0069] The entire exemplary process illustrated in FIG. 10 can be repeated until the area or areas of interest can be identified for visualization. The user can select different images at any magnification or view to store for later inspection. Labeling of each individual view can also be conducted during the exemplary navigation procedure. Various regions/images at different magnifications/locations can be bookmarked so that the user can return to the same region/image during a subsequent navigation session. FIGS. 6A-6E, 7 and 9A-9E illustrate examples of the progressive magnification while viewing a large area microscopic dataset.

[0070] The exemplary embodiment of the navigation procedure described herein can be implemented by the computer 460, and also utilize various processing techniques to assist the user in determining various areas to magnify and view the sample, and different portions and regions thereof. For example, FIG. 11 depicts a series of exemplary images of esophageal mucosa obtained using optical coherence tomography ("OCT") techniques, demonstrating an implementation of an exemplary embodiment of an automatic processing procedure for identifying normal or benign squamous mucosa as compared to Barrett's esophagus and adenocarcinoma via an analysis of OCT image spatial frequencies.

[0071] As shown in FIG. 11, OCT images 1100, 1110, and 1120 and spatial frequency distributions 1105, 1115, and 1125 of different disease states are shown. Squamous epithelium 1100 (SE) has vertical spatial frequencies (see arrows 1007 in panel 1105), corresponding to horizontal layers that may not be present in SIM. A widely varying spatial frequency distribution are shown in the exemplary OCT images of adenocarcinoma (CA) 1120 and its corresponding spatial frequencies 1125 compared with SIMND 1110 and 1115. FIG 12 depicts an illustration of a macrophage content 1210 obtained from OCT images of atherosclerotic plaques 1200 by determining the normalized standard deviation parameter (NSD). The density of macrophages can be obtained and displayed as an image using a color table 1220.

[0072] These and other exemplary image processing analysis procedures and steps can be applied to the microscopic data set and utilized to highlight regions of potential disease for subsequent directed navigation. FIG. 13 depicts a flow diagram of an exemplary embodiment of the method and procedure according to the present invention for navigating and evaluating the microscopic image data set. In this exemplary method/procedure, a microscopic dataset can be obtained in step 1300, which preferably has a resolution of less than 10 μm, possibly acquired over a large area of tissue or from a volume of tissue or organ therein. The data is then processed automatically by a processing arrangement (e.g., using the computer 460) in step 1310 to identify regions/locations that either contain areas suspect for disease or conversely areas that are suspected to contain no disease (i.e., healthy portions). In step 1320, the unhealthy areas can be represented using a color or other marking method, and then viewed at low magnification of the entire microscopic data volume in step 1330.

[0073] The user can then select a region to view in step 1340, guided by the processing data and the representation thereof. The user may then view the data set and, using a computer interface, select (a) a rectangular region, (a) a point, (c) an arbitrary shaped region, and/or (d) a depth in which to visualize a higher magnification view. The new region can be viewed, and the user (or the computer 460) can manually or automatically (a) select another region, (b) zoom in at a point/zoom out (step 1350), (d) translate the current view in three dimensions, and/or (e) change the depth location of

viewing within the dataset. Further, in step 1360, the user can view the newly illustrated region. The exemplary method/procedure may be repeated until the area(s) of interest is/are identified for visualization. The user or the computer 460 can select different images at any magnification or view to store for later inspection. Labeling of each individual view can also be conducted during the exemplary navigation process. The regions/images at different magnifications/locations can be bookmarked and stored so that the user can return to the same region/image during a subsequent navigation session.

[0074] 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.

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What is claimed is:

1. An apparatus comprising:

at least one first arrangement configured to forward an electromagnetic radiation to an anatomical structure, and continuously scan an entire region of at least one portion of the anatomical structure using the electromagnetic radiation to generate at least one signal, wherein the entire region has an area that is greater than $1mm^2$; and

at least one second arrangement configured to review the signal and to generate at least one image based on the signal that has a transverse resolution that is better than $10\mu m$.

2. The apparatus according to claim 1, wherein the entire region is a volume of the structure.

3. The apparatus according to claim 1, wherein the at least one image is contiguous without substantial gaps therein.

4. The apparatus according to claim 1, wherein the at least one portion is provided on a surface of the anatomical structure.

5. The apparatus according to claim1, wherein the at least one portion is provided below a surface of the anatomical structure.

6. The apparatus according to claim 1, wherein the electromagnetic radiation comprises a plurality of wavelengths.

7. The apparatus according to claim 1, wherein the electromagnetic radiation comprises one or more wavelengths that vary over time.

8. The apparatus according to claim 1, wherein at least one of the at least one first arrangement or the at least one second arrangement includes a microscope arrangement, and

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where the microscope arrangement, and where the microscope arrangement is at least one of a multiphoton microscope arrangement or a confocal microscope arrangement.

9. The apparatus according to claim 8, wherein the microscope arrangement includes a spectral encoding arrangement.

10. The apparatus according to claim 1, wherein the at least one anatomical structure is an internal organ.

11. The apparatus according to claim 1, wherein at least one of the at least one first arrangement forwards the electromagnetic radiation via an optical fiber arrangement, or at least one second arrangement receives the signal via an optical fiber arrangement.

12. The apparatus according to claim 11, wherein the optical fiber arrangement includes a plurality of electromagnetic radiation guiding arrangements.

13. The apparatus according to claim 1, wherein the signal is associated with at least one portion of an intensity of the electromagnetic radiation received from the anatomical structure.

14. The apparatus according to claim 1, wherein the at least one first arrangement is provided in a probe.

15. The apparatus according to claim 1, wherein the first arrangement includes at least one optical component which is configured to compensate for at least one optical aberration.

16. The apparatus according to claim 15, wherein the at least one optical component comprises a curved surface.

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17. The apparatus according to claim 16, wherein the at least one optical aberration is an astigmatism.

18. The apparatus of claim 1, further comprising a positioning arrangement configured to position at least one of the at least one first arrangement or the at least one second arrangement at a particular location relative to the anatomical structure.

19. The apparatus according to claim 1, wherein the at least one first arrangement is further configured to position a focus of the electromagnetic radiation at a plurality of depths within the anatomical structure.

20. The apparatus according to claim 1, further comprising at least one third arrangement configured to:

generate a further signal;

determine at least one location of a particular section associated with the anatomical structure based on the further signal; and

control at least one of a motion or a position of a focus of the at least one electromagnetic radiation to a further location within the anatomical structure based on the further signal.

21. The apparatus according to claim 20, wherein the further signal is the signal.

22. The apparatus according to claim 21, wherein the further signal is at least one of an interferometric signal, a time-of-flight signal, or an intensity of the electromagnetic radiation.

23. The apparatus according to claim 20, wherein the further signal is associated with at least one distance between at least one location within the region of the anatomical structure and at least one component of the at least one first arrangement.

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24. The apparatus according to claim 1, further comprising:

at least one third arrangement which is different from the first and second arrangements and configured to receive the signal and an additional signal from a reference to generate an interferometric signal.

25. The apparatus according to claim 1, further comprising at least one third arrangement configured to automatically control a position of a focus of the at least one first arrangement as a function of the signal to the predetermined location within the anatomical structure.

26. The apparatus according to claim 25, wherein the automatic control of the position of the focus by the at least one third arrangement is performed approximately parallel to an optical axis of the electromagnetic radiation that impinges on the anatomical structure.

27. The apparatus according to claim 25, wherein the predetermined location is determined based on an interferometric structure.

28. The apparatus according to claim 1, wherein the signal is at least one signal provided from the anatomical structure.

29. A method for imaging a region of an anatomical structure comprising:

forwarding at least one electromagnetic radiation to an anatomical structure;

continuously scanning an entire region of at least one portion of the anatomical structure using the electromagnetic radiation, wherein the entire region has an area that is greater than 1 mm^2;

obtaining at least one signal based on the electromagnetic radiation; and

generating at least one image based on the signal, wherein the image has a transverse resolution that is better than 10μm.

FIG. 1

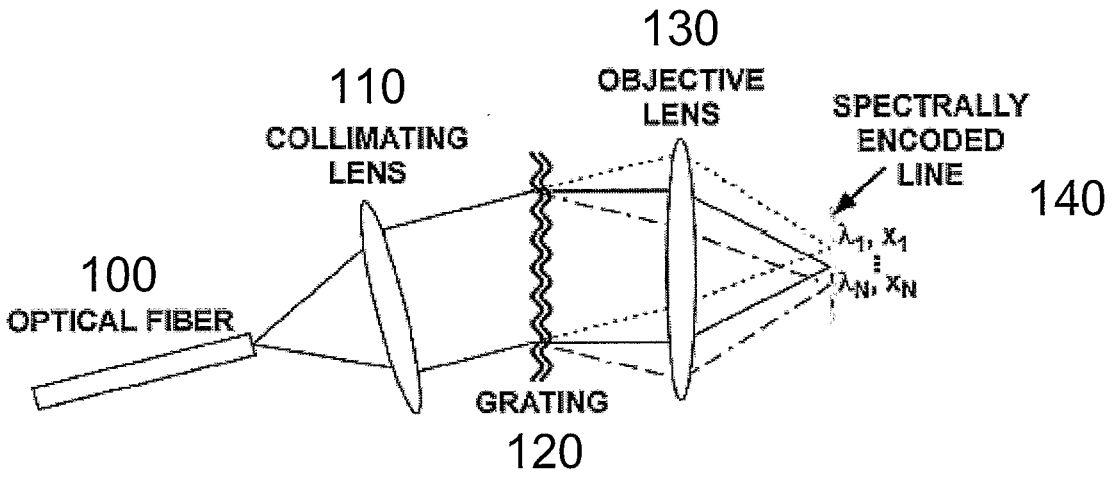

130
110
OBJECTIVE LENS
COLLIMATING LENS
SPECTRALLY ENCODED LINE
140
λ_1, x_1
λ_N, x_N
100
OPTICAL FIBER
GRATING
120

FIG. 2

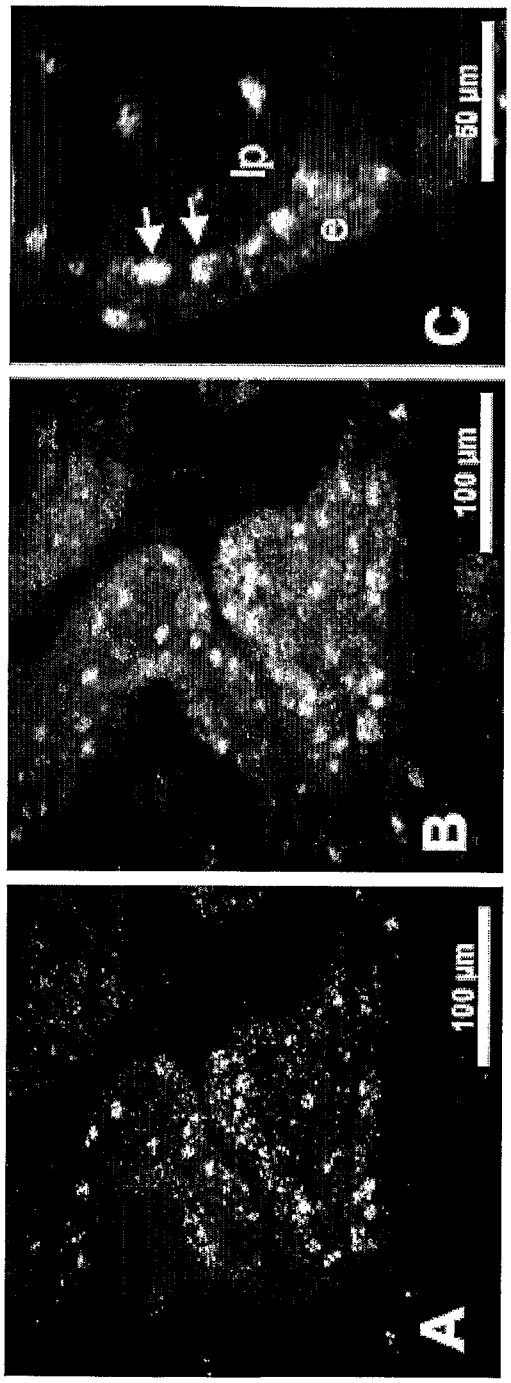


FIG. 3

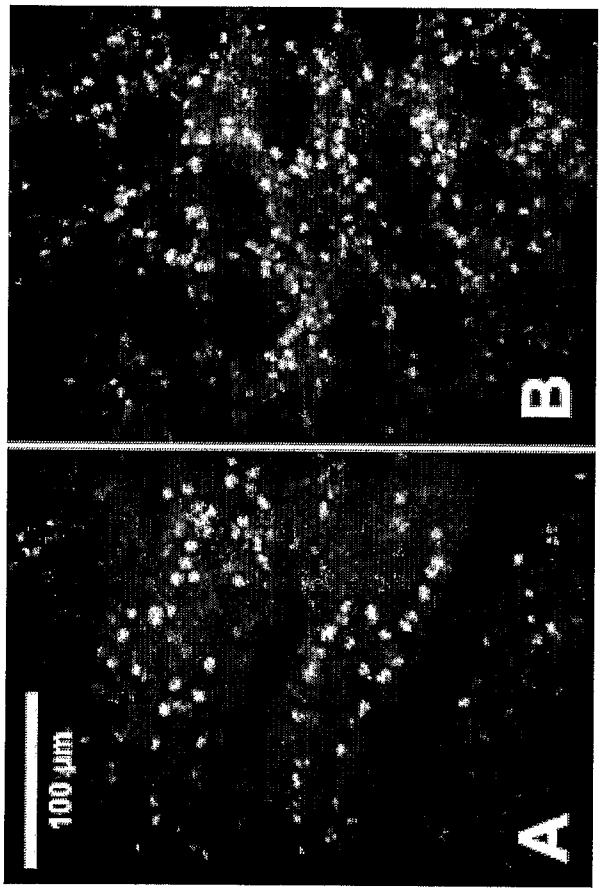


FIG. 4

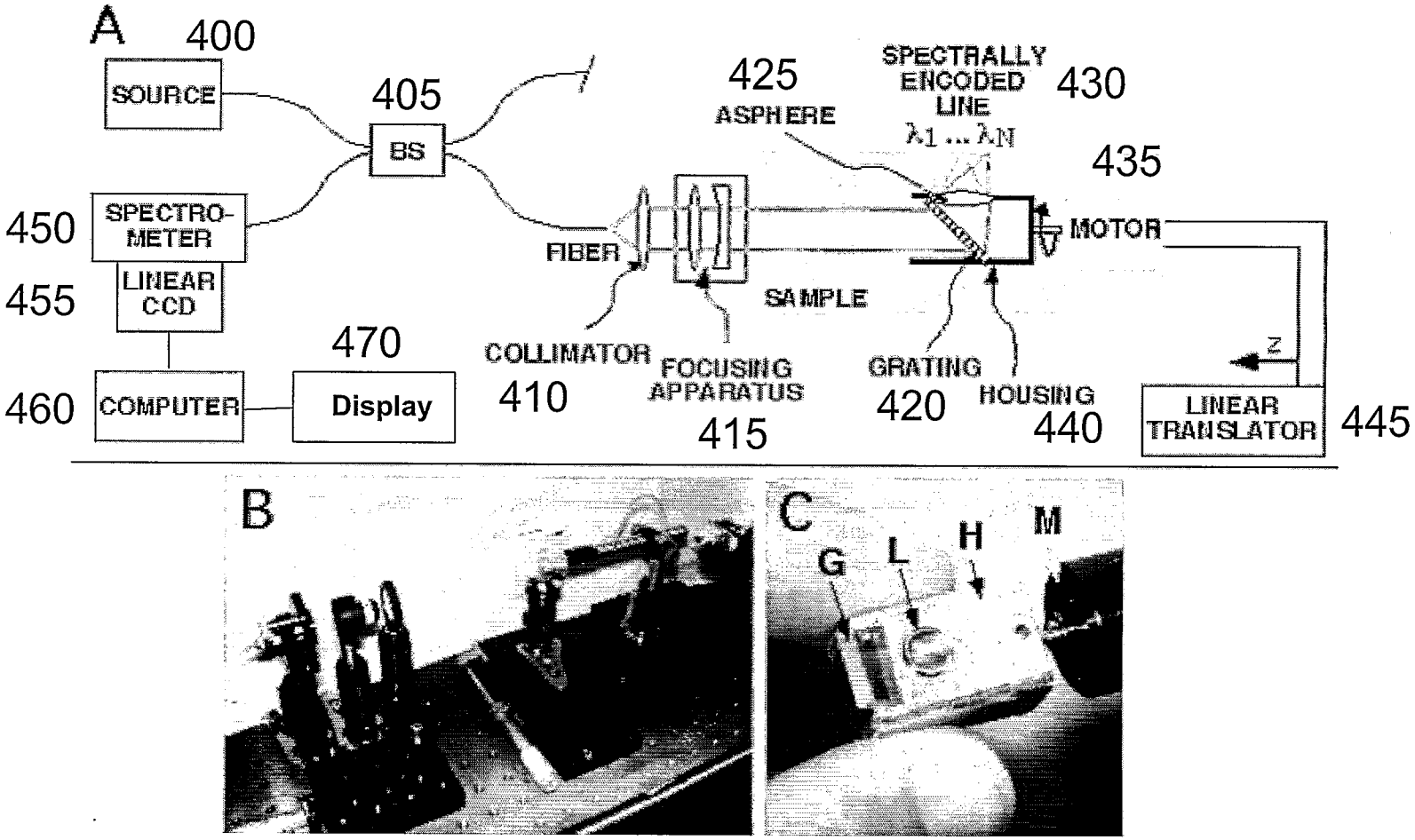

A
400
SOURCE
405
BS
425
ASPHERE
SPECTRALLY ENCODED LINE
λ1 ... λN
430
435
MOTOR
450
SPECTRO-METER
455
LINEAR CCD
460
COMPUTER
470
Display
FIBER
COLLIMATOR
410
FOCUSING APPARATUS
415
SAMPLE
GRATING
420
HOUSING
440
LINEAR TRANSLATOR
445
Z
B
C
G
L
H
M

FIG. 5

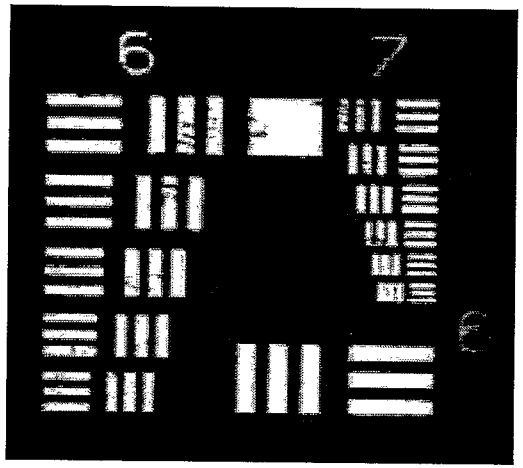


FIG. 6

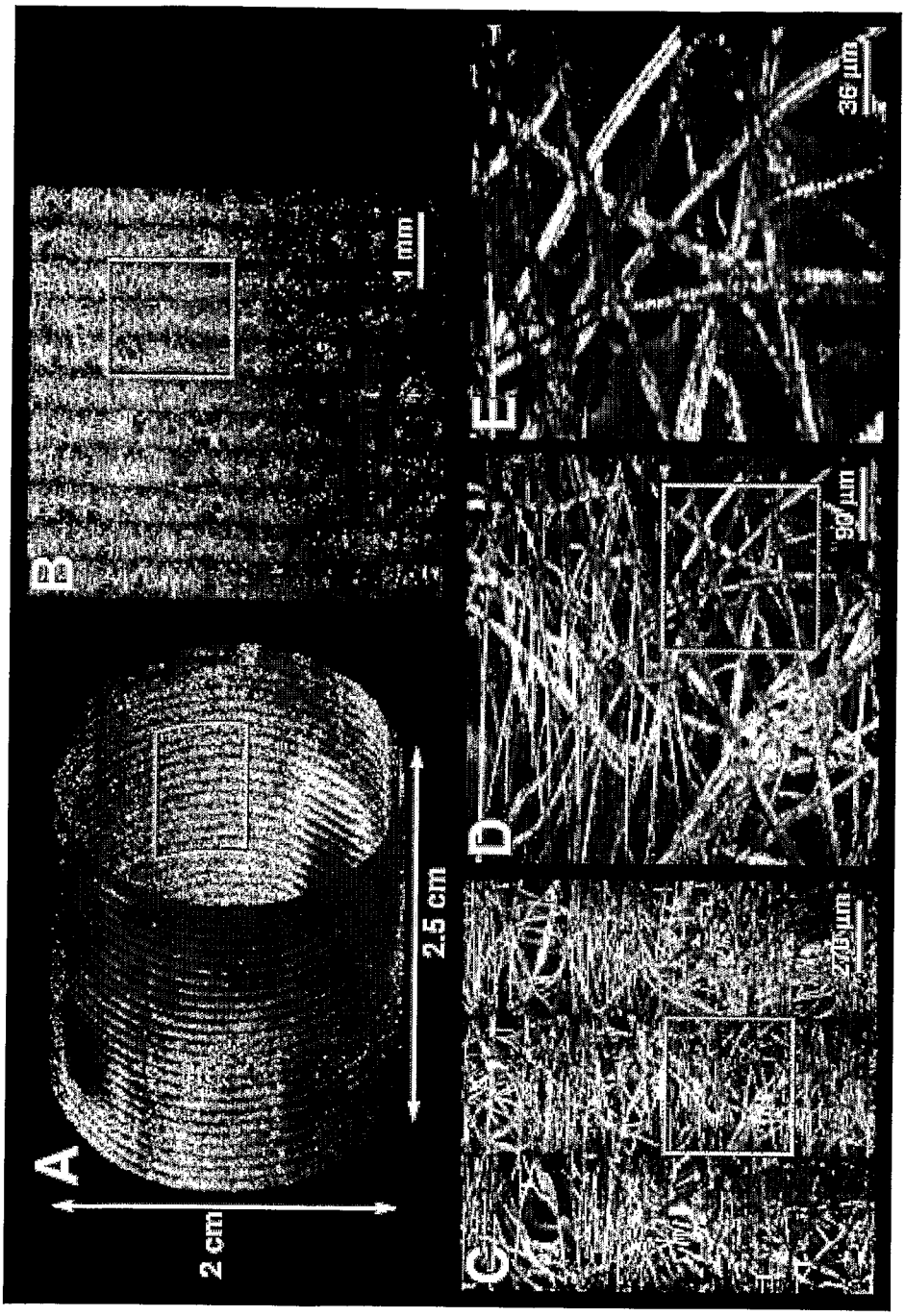


FIG. 7

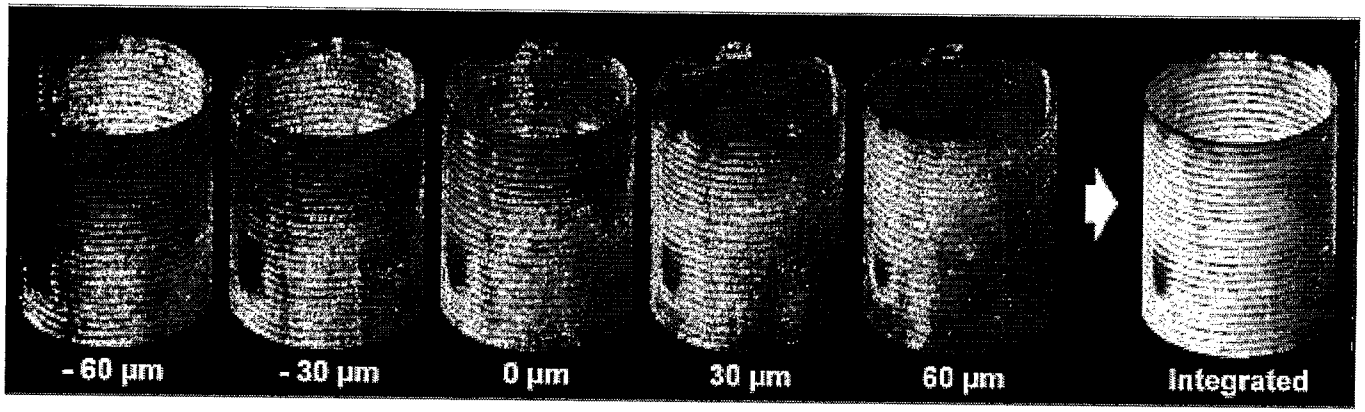


FIG. 8

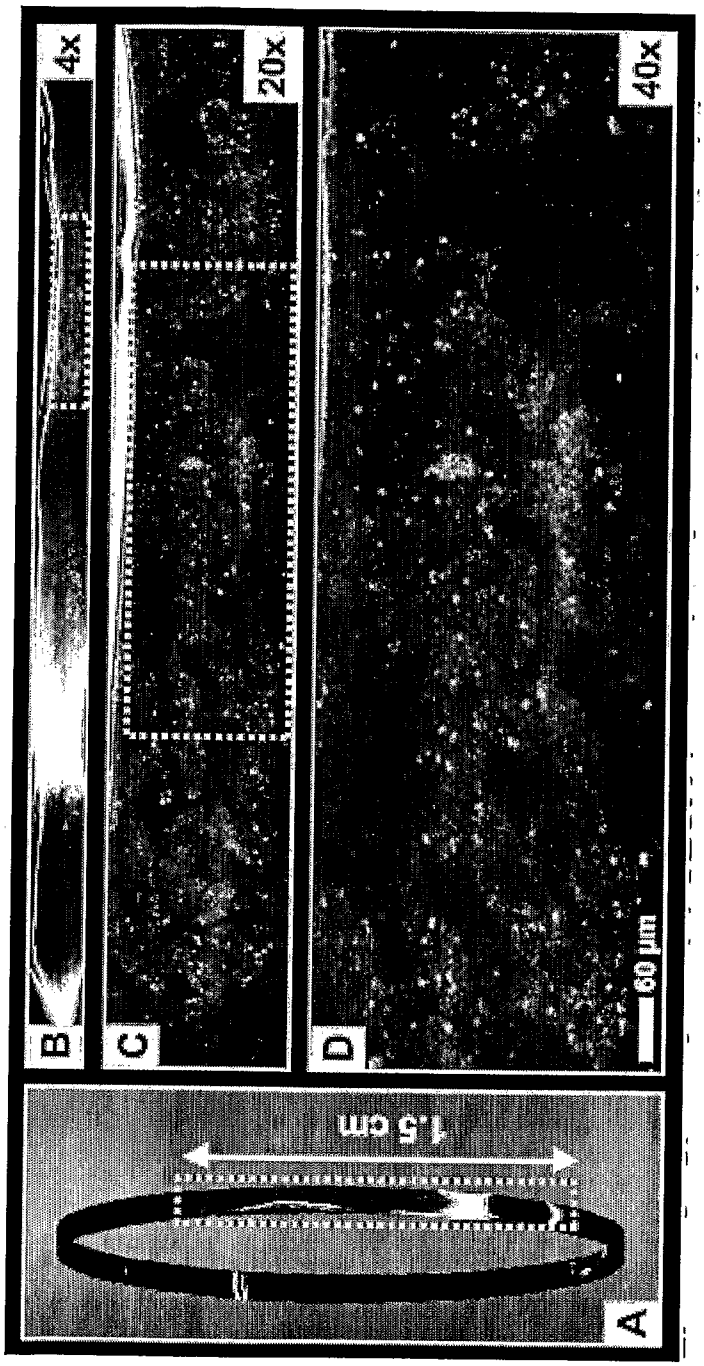


FIG. 9

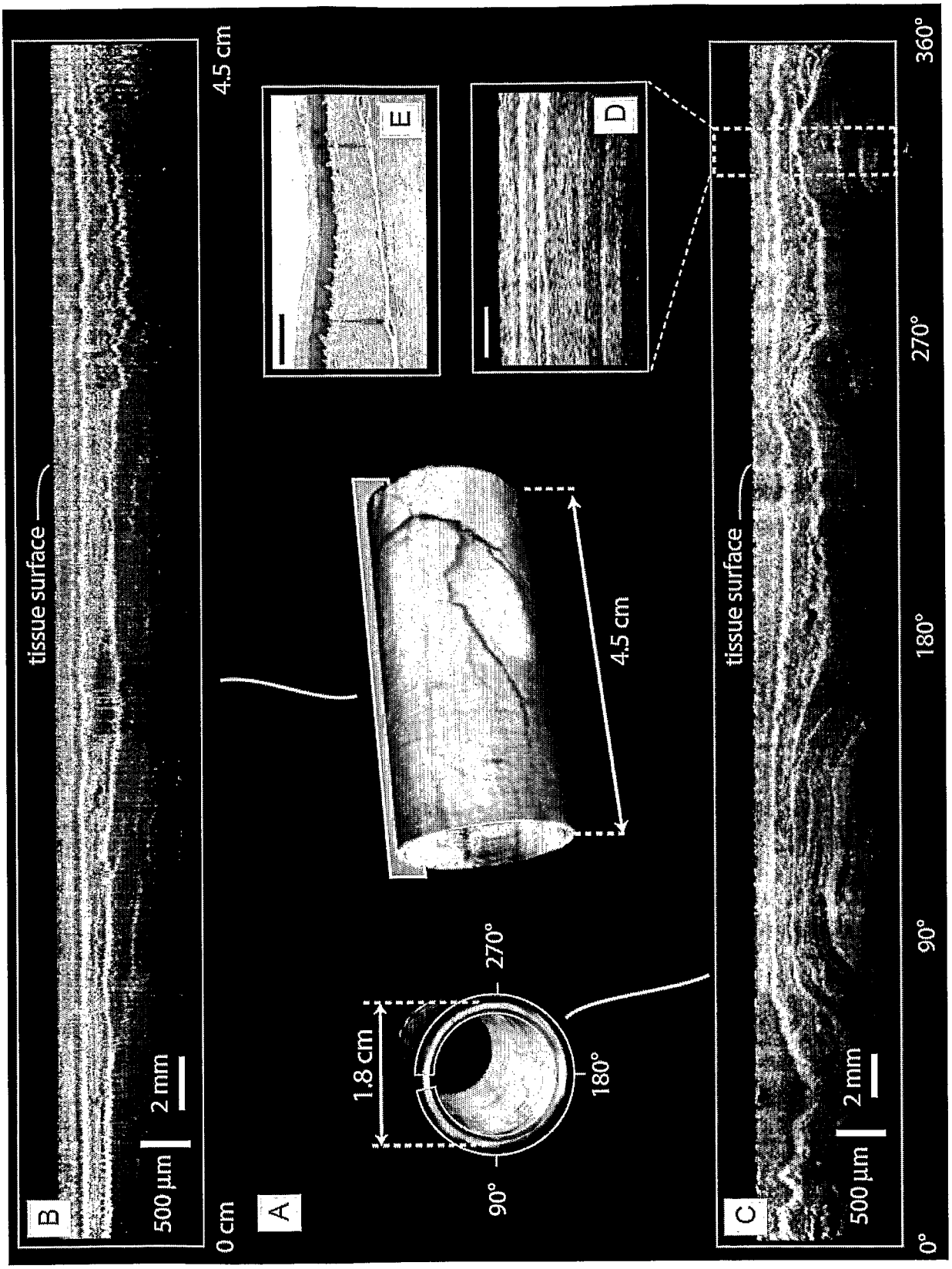

B
tissue surface
500 µm
2 mm
0 cm
4.5 cm
A
1.8 cm
270°
180°
90°
4.5 cm
E
D
C
tissue surface
500 µm
2 mm
0°
90°
180°
270°
360°

FIG. 10

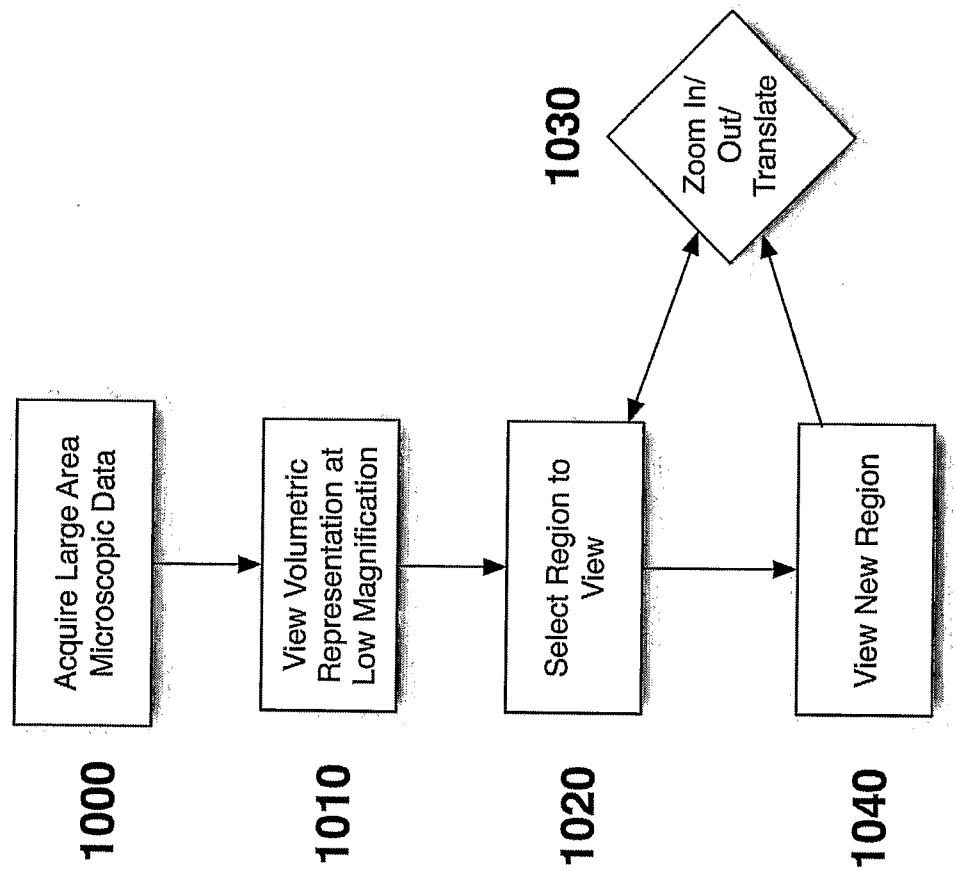

Acquire Large Area Microscopic Data
1000
View Volumetric Representation at Low Magnification
1010
Select Region to View
1020
Zoom In/ Out/ Translate
1030
View New Region
1040

FIG. 11

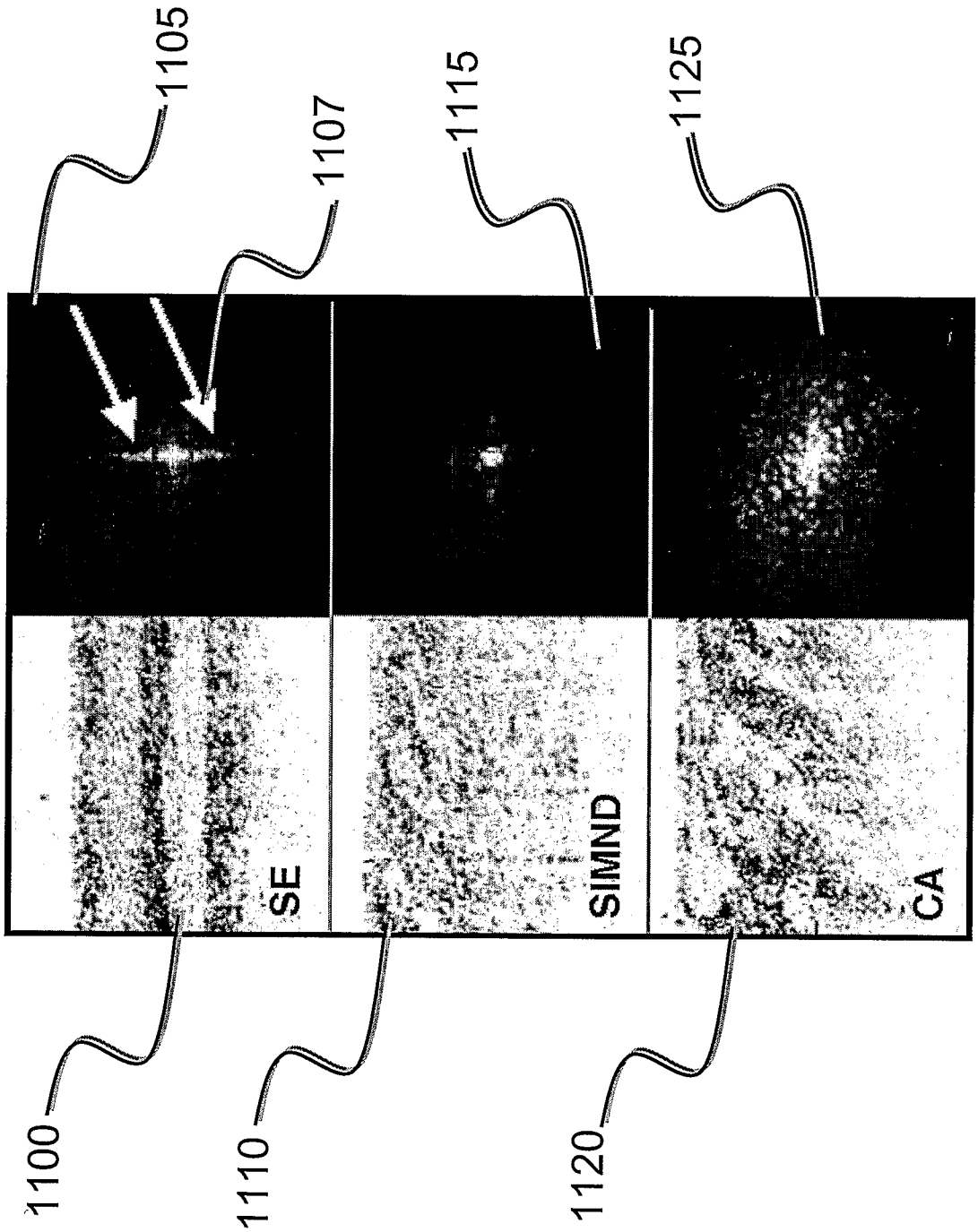


FIG. 12

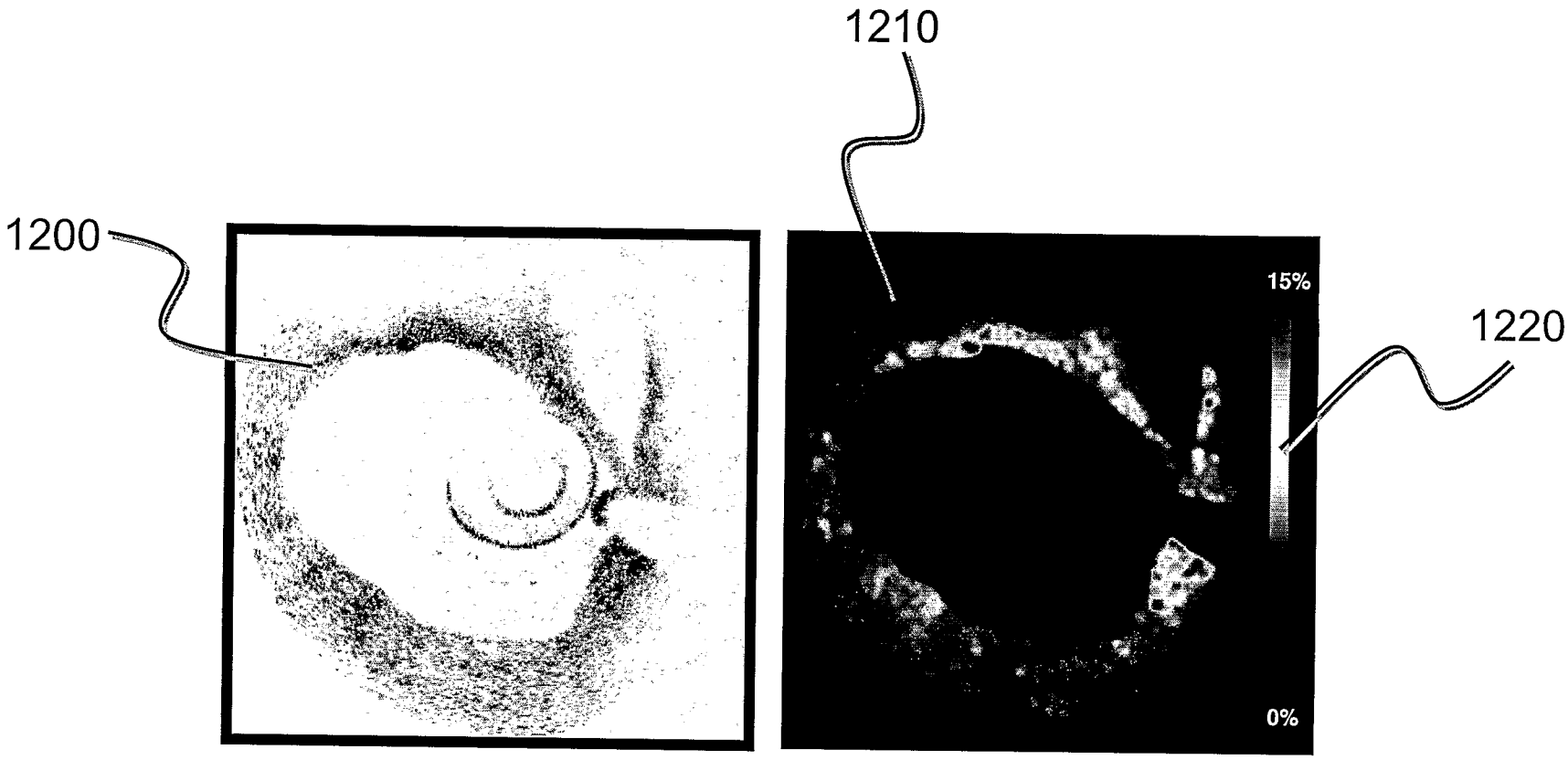
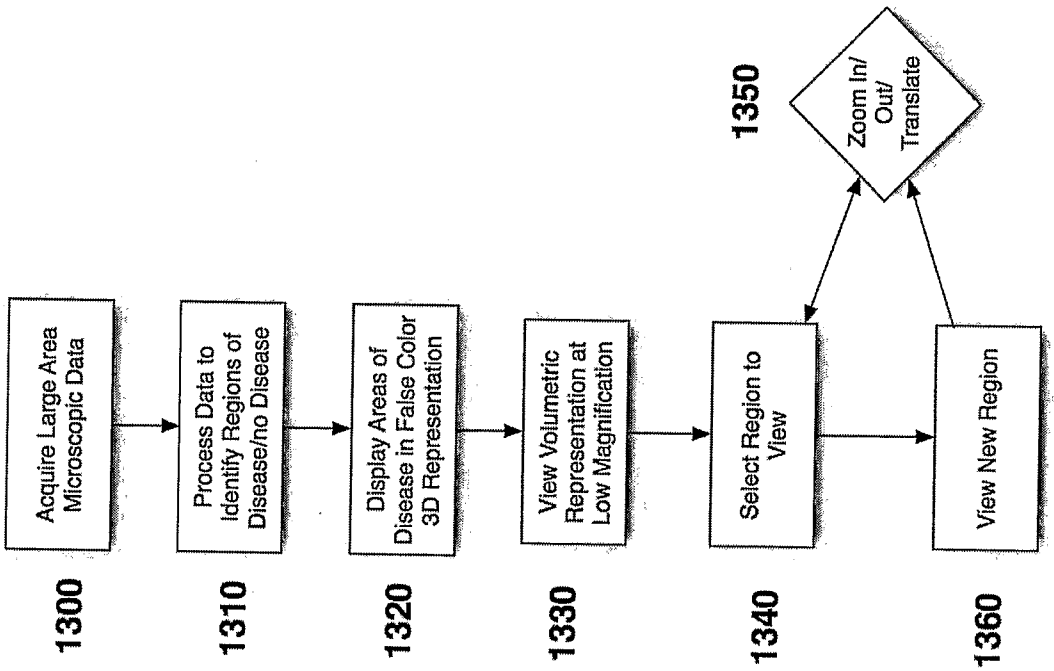


FIG. 13


Acquire Large Area Microscopic Data
1300
Process Data to Identify Regions of Disease/no Disease
1310
Display Areas of Disease in False Color 3D Representation
1320
View Volumetric Representation at Low Magnification
1330
Select Region to View
1340
1350
Zoom In/ Out/ Translate
View New Region
1360