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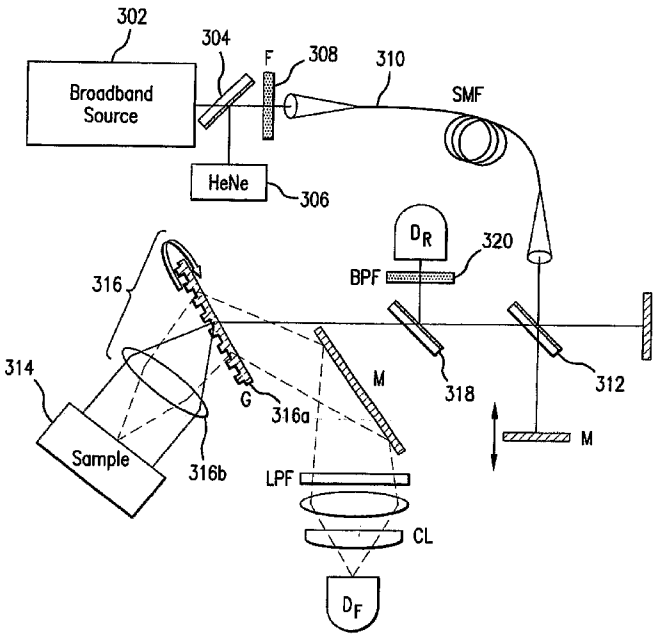
(54) **Title:** SPECTRAL- AND FREQUENCY- ENCODED FLUORESCENCE IMAGING



(57) **Abstract:** A system and method for obtaining fluorescence images from a sample are provided. Broadband excitation light (302) is encoded with a wavelength-dependent frequency modulation and dispersed onto a sample (314), e.g. with a grating (316a), to simultaneously illuminate an entire image line. The frequency-encoded fluorescence emission is measured to provide one line of the image. Mechanical scanning along a direction orthogonal to the wavelength-encoded axis allows creation of a two-dimensional fluorescence image. The system and method is especially useful for obtaining fluorescence images via endoscopes, catheters, or small-diameter probes.

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SPECTRAL AND FREQUENCY-ENCODED FLUORESCENCE IMAGING

CROSS-REFERENCE TO RELATED APPLICATION(S)

This application is based upon and claims the benefit of priority from U.S. Patent Application Serial No. 60/727,215, filed October 14, 2005, the entire disclosure of which is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

The invention was made with the U.S. Government support under Contract No. FA9550-04-1-0079 awarded by the Air Force Office of Scientific Research, Department of Defense, and Contract No. 5 T32 AR07098 awarded by the National Institute of Health. Thus, the U.S. Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention generally relates to arrangements and methods for facilitating photoluminescence imaging, and particularly for, e.g., obtaining fluorescence images via endoscopes, catheters, or small-diameter probes.

BACKGROUND OF THE INVENTION

In vivo fluorescence macro- and microscopic imaging is increasingly being used for clinical disease diagnosis and small animal research. In order to extend fluorescence imaging for a wide range of basic and clinical applications, it may be preferable to utilize flexible, miniaturized endoscopes. The performance of high quality fluorescent imaging procedures through a miniature flexible probe may be difficult due to the inability to incorporate a rapid beam scanning mechanism at the

distal end of miniature probes and the limited number of optical fibers that can fit within the confines of small diameter fiber-optic imaging bundles.

Conventional procedures which apparently implemented fluorescence imaging through probes with a diameter of less than 2 mm have been performed using fiber optic bundles. For example, probes which vary in diameter from 600 μm to 1.8 mm have been used to obtain images of vessels in the mouse cremaster muscle, and which visualized labeled circulating cells. (See E. Laemmel et al., "Fibered confocal fluorescence microscopy (Cell-viZio (TM)) facilitates extended imaging in the field of microcirculation - A comparison with intravital microscopy," J. Vasc. Res., Vol. 41(5), 400 (2004)). As described in this publication, images of cells labeled with Fluorescein Isothiocyanate ("FITC") (e.g., excitation with 488 nm) were obtained at 12 Hz with a maximal field of view of 400 μm x 280 μm through probes with ~10,000 optical fibers.

An 800 μm diameter endoscope with 10,000 optical fibers which can be used with Cy5.5 and Cy7, excited at 673 nm can also be utilized. (See M. A. Funovics et al., "Miniaturized multichannel near infrared endoscope for mouse imaging," Molecular Imaging, Vol. 2(4), 350 (2003)). The imaging tip, which has a 56° field of view in water, can also facilitate white light reflectance imaging with a resolution of 7 line pairs per millimeter, as determined with an USAF 1951 resolution target. Exemplary images were presented from mouse vasculature and of protease activity in an ovarian tumor with rates ranging from 3 to 10 Hz. (See M. A. Funovics et al., "Catheter-based in vivo imaging of enzyme activity and gene expression: Feasibility study in mice," Radiology, Vol. 231(3), 659 (2004)). According to this publication, tumors expressing green fluorescent protein were also observed.

Spectral encoding has been previously demonstrated for reflectance imaging. (See G. J. Tearney et al., "Spectrally encoded confocal microscopy," Opt. Lett., Vol. 23(15), 1152 (1998); and G. J. Tearney et al., "Spectrally encoded miniature endoscopy," Optics Letters, Vol. 27(6), 412 (2002)). In this exemplary technique, broadband light from an optical fiber may be dispersed by a grating, and focused onto a line on the sample. In this matter, the image does not have to be scanned in this dimension. A reflected light returns through the lens, grating, and optical fiber and the spectrally encoded image is then decoded *via* heterodyne Fourier transform spectroscopy (see G. J. Tearney et al., "Spectrally encoded confocal microscopy," Opt. Lett., Vol. 23(15), 1152 (1998)) or with another grating in conjunction with a CCD detector (see G. J. Tearney et al., "Spectrally encoded miniature endoscopy," Optics Letters, Vol. 27(6), 412 (2002)).

The transverse dimension can then be scanned by, for example, rotating the fiber and distal optics, which can be implemented in small diameter probes. (See G. J. Tearney et al., "Scanning single-mode fiber optic catheter-endoscope for optical coherence tomography," Opt. Lett., Vol. 21(7), 543 (1996)). Using this conventional technique, the number of resolvable points (n) along one spectrally encoded line can be determined by the spectral bandwidth ($\Delta\lambda$), center wavelength (λ_0), beam diameter (d), and grating:

$$n \cong \frac{\Delta\lambda d G}{\lambda_0 \cos(\theta_i)}, \tag{1}$$

where G and θ_i are the grating groove density and incidence angle, respectively. (See G. J. Tearney et al., "Spectrally encoded miniature endoscopy," Optics Letters, Vol. 27(6), 412 (2002)).

The spectrally encoded photoluminescient techniques are generally based on a similar concept. In this exemplary embodiment, the fluorescence emission may be Stokes shifted, and the spatial locations are generally no longer uniquely related to the detected wavelengths. As a result, spectroscopic methods and arrangements implementing the same may not be effective for decoding the image. In order to recapture the spatial information, a spectral-and-frequency-encoded ("SFE") imaging techniques can utilize a wavelength-dependent frequency modulation of the excitation light before it is dispersed onto the sample *via* the grating. The fluorescence emission at each location can therefore be modulated in concert with the frequency of the excitation light, thereby producing an additional level of encoding.

Accordingly, it may be beneficial to address and/or overcome at least some of the deficiencies described herein above. For example, the reference interferometer signal could be used for active feedback control to correct non-linear movement of the scanning mirrors, thereby eliminating the need for post-acquisition processing.

OBJECTS AND SUMMARY OF THE INVENTION

One of the objectives of the present invention is to overcome certain deficiencies and shortcomings of the prior art arrangements and methods (including those described herein above), and provide exemplary embodiments of arrangements and methods for facilitating photoluminescence imaging, e.g., to obtain fluorescence images via endoscopes, catheters, or small-diameter probes

According to certain exemplary embodiments of the present invention, the arrangements and methods for fluorescent imaging, e.g., spectrally and frequency

encoded ("SFE") fluorescence imaging, can be provided, which can be performed in a sub-millimeter diameter endoscope with a high number of resolvable points.

A high number of resolvable points may be obtained within a small diameter probe, since the excitation bandwidth and the grating groove density govern the number of points in the image. For a given beam diameter, the number of resolvable points attained by SFE is affected by the excitation spectra of the fluorophore. Table 1 depicts the predicted number of resolvable points for several common fluorescent labels, assuming beam diameters of 1.0 and 0.5 mm and a grating groove density of 1500 lines/mm. For each case, the theoretical number of resolvable points either equals or exceeds that of fiber bundles of comparable diameter.

Table 1. Theoretical Number of Resolvable Points for Typical Fluorophores

Fluorophore	**Excitation Bandwidth (nm)**	**SFE # resolvable points (1.0 mm)**	**SFE # resolvable points (0.5 mm)**
GFP	145 (375-520)	266,000	66,000
FITC	90 (430-520)	92,000	23,000
Cy5.5	110 (570-680)	89,000	22,000
ICG (plasma)	184 (670-854)	195,000	49,000

Table 1. Theoretical SFE number of resolvable points for 1.0 mm and 0.5 mm beam diameters (rounded to nearest 4th digit). SFE parameters: 1500 lines/mm grating, incident illumination at Littrow's angle. Excitation bandwidth is defined as the full width at 10% maximum.

Since exemplary SFE procedures may be conducted using a single optical fiber, images obtained by these exemplary techniques may not contain pixilation artifacts that are commonly observed in fiber bundles. (See E. Laemmel et al., "Fibered confocal fluorescence microscopy (Cell-viZio (TM)) facilitates extended imaging in the field of microcirculation - A comparison with intravital microscopy," J. Vasc. Res., Vol. 41(5), 400 (2004)). Furthermore, flexibility of the SFE miniature probe will likely be greatly increased, as the bend radius for a single fiber is significantly less than that of imaging bundles. These exemplary advantages of SFE could be of significant benefit for applications where image quality and maneuverability are of concern.

In addition, an exemplary spectral encoding technique according to an exemplary embodiment of the present invention can be utilized for fluorescence imaging using a swept source laser. In this exemplary case the laser wavelengths can be rapidly tuned over the absorption band of the fluorophore. Each wavelength can be dispersed to a different location on the sample. The collected sample fluorescence can then be decoded as a function of time to reconstruct the image.

For example, according to one exemplary embodiment of the present invention, a reference interferometer signal can be used for active feedback control to correct non-linear movement of the scanning mirrors, thereby eliminating the need for post-acquisition processing.

Indeed, according to one exemplary embodiment of the present invention, systems and methods can be provided for obtaining a photoluminescence radiation from at least one portion of a sample. For example, using at least one arrangement, it is possible to receive a first radiation and disperse said first radiation into at least one

second radiation and at least one third radiation. The second and third radiations can be provided to different locations of the portion. In addition, the photoluminescence radiation can be received from the portion based on the first, second, or third radiations.

Such arrangement can include a grating, a prism, a grism, a dual prism-grism and/or a lens. For confocal applications, the lens may have a numerical aperture that is greater 0.5. The arrangement may also include at least one optical fiber, which can have multiple claddings. The arrangement can include a plurality of optical fibers and/or at least one of at least one pin hole arrangement or at least one slit arrangement. At least one of the optical fiber(s) can be a multimode fiber.

According to another exemplary embodiment of the present invention, a wavelength tuning light source can be provided which may be configured to provide the first radiation. Further, a light source can be included and configured to provide the first radiation that has multiple wavelengths. In addition, a further arrangement can be provided which may be configured to modulate the wavelengths at different frequencies. The further arrangement can include an interferometric arrangement which may include at least one translatable component. The further arrangement may include a further interferometric arrangement configured to correct for non-linearities in the translatable component. A further arrangement may include an acousto-optical, or electro-optical modulator to provide the frequency encoding.

In yet another exemplary embodiment of the present invention, the arrangement can be configured to generate information associated with the different locations as a function of the photoluminescence radiation. A processing arrangement can be provided configured to generate at least one image based on the information.

For example, the processing arrangement can be configured to receive the signal, and Fourier transform the signal to generate the image. The image can include a microscopic image and/or an endoscopic image.

According to a further exemplary embodiment of the present invention, the arrangement can include a detecting arrangement which may be configured to receive the photoluminescence radiation and generate at least one signal which can be associated with the photoluminescence radiation. The arrangement can also be configured to be able to control a position of the second and third radiations on the different locations on the portion of the sample.

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

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:

Figure 1 is a block diagram of portions of a spectrally and frequency encoded ("SFE") system;

Figure 2 is a block diagram of an exemplary embodiment of a Michelson interferometer arrangement, which can be used for spectral modulation, containing a

stationary and scanning mirror in accordance with an exemplary embodiment of the present invention;

Figure 3 is a schematic diagram of an exemplary embodiment of an apparatus according to the present invention which can be used to demonstrate an exemplary SFE procedure;

Figure 4A is a first operational and schematic diagram providing details on the operation of the exemplary embodiment of the SFE procedure according to the present invention;

Figure 4B is a first operational and schematic diagram providing details on the operation of the exemplary embodiment of the SFE procedure according to the present invention;

Figure 5A is an exemplary SFE fluorescence image of microfluidic channels filled with Indocyanine Green ("ICG" - a fluorescent compound);

Figure 5B is an exemplary reflectance image of microfluidic channels filled with ICG taken with an epi-illuminated microscope;

Figure 6 is a detailed diagram of an exemplary SFE system utilizing a single fiber probe in accordance with an exemplary embodiment of the present invention;

Figure 7 is a detailed diagram of an exemplary embodiment of the SFE probe according to the present invention which uses and/or includes a single dual-clad fiber;

Figure 8 is a detailed diagram of an exemplary embodiment of the SFE probe according to the present invention which uses a single-mode fiber for illumination and a large-core multimode fiber for collection of fluorescence;

Figure 9 is a detailed diagram of an exemplary embodiment of the SFE probe according to the present invention which uses multiple multimode fibers for collection;

Figure 10 is a detailed diagram of an exemplary embodiment of the SFE probe according to the present invention which has another tip, where an orientation of lens and fibers have been modified;

Figure 11 is a block diagram of an exemplary embodiment of a system according to the present invention for correcting artifacts induced by non-linear motions in an interferometer thereof;

Figure 12 is a detailed diagram of an exemplary embodiment of the SFE probe according to the present invention for microscopic applications which uses a high numerical aperture lens and/or objective placed after a grating;

Figure 13 is a detailed diagram of an exemplary embodiment of the SFE probe according to the present invention for microscopic applications in which an inline optic axis can be maintained via one or more prisms before and after the grating;

Figure 14 is a detailed diagram of an exemplary embodiment of a confocal microscopic SFE probe according to the present invention which uses a slit and/or pinholes at a distal probe tip to increase a confocal sectioning;

Figure 15 is a detailed diagram of a further exemplary embodiment of a confocal microscopic SFE probe according to the present invention which uses the slit and/or pinholes at a proximal end of collection fibers to increase the confocal sectioning; and

Figure 16 is a set of graphs of results of exemplary spectral procedures used to correct signals for non-linear motion of the scanning mirror in the Michelson interferometers.

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

Figure 1 shows a schematic diagram of -portions of a spectrally and frequency encoded ("SFE") system. For example, light 100 can be filtered by filter 105 to match the absorption band of the fluorophore, and provided into an interferometer 110, which can be a Michelson interferometer, as well as a Sagnac, Mach-Zehnder, Twyman-Green interferometers, etc. The input light is affected by the interferometer 110 so as to produce a spectral modulation on the input light. Light 130 from the interferometer 110 then can illuminate a dispersive element 135 which can precede or follow a lens. Each wavelength component with its unique modulation frequency can

be focused at a distinct location on a fluorescent sample 140. Fluorescence in the sample 140 is excited, a fluorescent light 145 returns through the grating lens pair 135, and can be collected and directed to a detector 150.

The detected light can be processed via a Fourier transformation or the like to recover a fluorescence intensity as a function of one-dimensional location from the sample 140. Additional detectors may be utilized to measure the excitation spectra and/or absorption and/or diffuse reflectance spectra of the sample as a means for correcting for the excitation spectrum shape and/or the absorption and/or scattering artifacts in turbid samples. A second reference light 115, is directed to one component of the interferometer and utilized to compensate the nonlinearity of a moving component of the interferometer.

According to the exemplary embodiment illustrated in Figure 1 and discussed above, broadband excitation light, which is possibly filtered to match the fluorophore's absorption spectrum, can be directed through a scanning Michelson interferometer. The scanning interferometer introduced a wavelength-dependent frequency modulation whose intensity was:

$$I_{ex}\left(\lambda_{ex,i},t\right)=\frac{1}{2}I_{ex}\left(\lambda_{ex,i}\right)\left[1+\cos\left(\frac{4\pi}{\lambda_{ex,i}}vt\right)\right], \qquad (2)$$

where $I_{ex}(\lambda_{ex,i})$ is the spectral intensity corresponding to the i^{th} wavelength ($\lambda_{ex,i}$) of the excitation light, and v is the velocity of the interferometer scanning mirror. As long as the interferometer scans a large enough distance to provide sufficient spectral resolution, the number of resolvable points in SFE fluorescence imaging is also governed by Eq. 1. After being dispersed and focused onto the sample the modulated

fluorescence light generates fluorescence emission. Reconstruction of a single line in the SFE image is performed by taking the Fourier transform of the detected fluorescent signal, following correction by the reference interferometer signal. Multiple SFE lines are acquired as the probe is slowly scanned to create an SFE image.

Michelson Interferometer

A block diagram of an exemplary embodiment of an interferometer, e.g., the Michelson interferometer, is shown in Figure 2. As shown in Figure 2, light 200 is incident on a beam splitter 215, which directs one portion of the light 200 to a stationary mirror 225 and the other portion of the light 200 to a scanning mirror 230. The reflected beams return to the beam splitter 215, and are combined to exit the interferometer as spectrally modulated light 235 which is incident upon the sample in the case of the interferometer, or a detector for a reference measurement. The beam splitter 215 may be preceded by a filter 205 so as to filter out wavelengths not contained within the fluorophore's excitation spectrum. A compensator 220 may be inserted into one arm of the interferometer to correct for a dispersion differences in the mirror arms 225, 230.

Exemplary embodiment of a procedure according to the present invention

Figure 3 depicts an exemplary embodiment of an apparatus according to the present invention which can be used to indicate the exemplary SFE procedure, and Figure 4 depicts an exemplary implementation of such procedure in more detail. For example, light from a broadband source 302 can be delivered through a filter (F) 308 and single-mode fiber (SMF) 310 to a Michelson interferometer containing a beam-splitter 312 and two mirrors (M), at least one of which may be scanned. The

interferometer may generate a wavelength dependent frequency modulation on a broadband light 400. For example, a pair of a compact grating 316a and a lens 316b as a pair 316 can be used to illuminate a sample 314, 420, thus simulating, e.g., a ~1 mm diameter miniature endoscope.

The lens 316b, 415 (e.g., f=12.5 mm) can focus each of uniquely modulated excitation wavelengths 410 onto a different location of the sample 314 after they had been dispersed by the holographic transmission grating 316a, 405 (being impacted by light 400 having 1200 lines/mm in Figure 4) , which can be any diffractive element. The fluorescence emission 425 at each location can consequently be modulated at the frequency $f_{ex,i}=2v/\lambda_{ex,i}$ as shown in Figure 4, where I_{em} is the entire fluorescence emission spectrum. Light from a Helium-Neon laser 306 was also directed into the interferometer through beam-pickoff 304 and single-mode fiber 310, and then deflected by beam-pickoff 318 through a 633 nm bandpass filter (BPF) 320 for detection by an avalanche photodiode (D_R) to obtain a reference signal for correction of scanning mirror nonlinearities.

The emitted light was transmitted back through the same lens 316b, 415 and the grating 316a, 405. The fluorescence 430 (also shown in Figure 3 as dashed lines), which can be diffracted at a different angle than the reflected light, can then be deflected by a mirror M shown in Figure 3 through an 830 nm long-pass filter LPF, and focused onto a second avalanche photodiode D_F by a combination of spherical and cylindrical lenses CL. In this exemplary configuration, the grating 316a, 405 can be mounted to a galvanometer, and rotated to provide the slow-axis scanning. A reconstruction of the exemplary image can be accomplished by taking the Fourier-

transform of the modulated fluorescence for each galvanometer scan angle after using the reference signal to correct the interferogram.

Indocyanine green ("ICG") can be used, which is a near-infrared fluorophore (e.g., having an excitation 650-850 nm, and an emission 805-950 nm) that has been FDA approved for several clinical indications (see C. H. Tung, "Fluorescent peptide probes for *in vivo* diagnostic imaging," Biopolymers, Vol. 76(5), 391, (2004)), and modified for targeted antibody labeling (see S. Ito et al., "Detection of human gastric cancer in resected specimens using a novel infrared fluorescent anti-human carcinoembryonic antigen antibody with an infrared fluorescence endoscope in vitro," Endoscopy, Vol. 33(10), 849, (2001), and T. Bando et al., "Basic studies on a labeled anti-mucin antibody detectable by infrared-fluorescence endoscopy," Journal of Gastroenterology, Vol. 37(4), 260 (2002)).

Figure 5A shows a fluorescence image 500 of microfluidic channels (as described in M. Shin et al., "Endothelialized networks with a vascular geometry in microfabricated poly(dimethyl siloxane)," Biomedical Microdevices, Vol. 6(4), 269 (2004)) filled with ICG (2 mg ICG/mL DMSO) obtained using the SFE technique. A corresponding epi-illuminated reflectance micrograph image 505 is shown in Fig. 5B which demarcates the smallest, 35 μm wide channels. The exemplary SFE image can be collected at 2 Hz with a 1.4 mm x 1.4 mm field of view. The center wavelength and spectral range (full-width at 15% maximum) of illumination after low-pass filtering can be 780 nm and 50 nm, respectively. The Michelson interferometer scanning mirror can be translated ±0.25 mm around the zero-path-length difference position to provide a spectral resolution of ~0.6 nm, corresponding to a theoretical 83 resolvable points. (See J. Kauppinen et al., *Fourier Transforms in Spectroscopy*,

Wiley-VCH, New York, p. 271, (2001)). The probe input beam can be 1.1 mm in diameter, and may be incident on the grating at 22°, also likely resulting in a spectral resolution of 0.6 nm. Since the usable excitation bandwidth of ICG can be 125 nm in DMSO, this probe configuration could theoretically enable >200 resolvable points per frequency-encoded line. However, the limited bandwidth of the current source can reduce this number to, e.g., 84.

Exemplary lateral resolution measurements along the spectrally encoded line can be estimated by measuring the edge response function of, e.g., 15 lines in the image along the wavelength-encoded axis at vertical edges in the microfluidic channels. The exemplary measurements can demonstrate a spatial resolution of 15.9±4.9 μm (mean±stdev), corresponding to a total of approximately 88 resolvable points across the field of view. This is in approximate agreement with the expected calculations. The resolution along the transverse axis can be limited by the imaging optics.

The total number of resolvable points obtained by the exemplary embodiment of the SFE fluorescence imaging apparatus according to the present invention, which approximates the dimensions of a 1 mm endoscope, can be, e.g., $n^2 = 7{,}744$. This exemplary value can be comparable to state-of-the-art fiber-bundle based technologies of similar diameter, and may be improved by increasing the excitation bandwidth or utilizing a higher density grating while simultaneously increasing the scanning range of the interferometer.

Fiber-optic exemplary embodiment: Single dual-clad fiber

Exemplary SFE techniques can be advantageous in that high quality imaging may be obtained using a single optical fiber. In order to minimize size in the

development of future SFE endoscopes, it may be advantageous to collect the fluorescent emission through the probe grating. Stokes-shifted fluorescent light, however, may not couple back to the core of a single-mode illumination fiber. An exemplary solution to this challenge may be the use of a dual-clad fiber (as described in D. Yelin et al., "Double-clad fiber for endoscopy," Opt. Lett., Vol. 29(20), 2408 (2004)) such that excitation light can be transmitted through a central core, and the fluorescence may be obtained through the inner cladding. Using exemplary ray-tracing models, this exemplary approach is effective without significantly increasing the probe diameter or compromising resolution.

Figure 6 shows a detailed diagram of an exemplary SFE system utilizing a single dual clad fiber probe in accordance with an exemplary embodiment of the present invention for collecting fluorescent images. For example, illumination light generated by a source 600 can be filtered by a filter 605. Such filtered light, and light from a reference 625 can be directed through a Michelson interferometer including a beam splitter 610, a stationary mirror 615, and a moving mirror 620. The light provided from the reference 625 can be deflected by a notch filter or a reference beam splitter 622 to a reference detector 630. The illumination light can pass through the reference beam splitter 622 and a dichroic filter or beam splitter 635, and can be coupled via a coupler to a central single-mode core of a dual clad fiber 645. Light 655 exiting the core of the fiber 645 can be dispersed and focused onto a sample 660 by a dispersive element and lens 650. The emitted fluorescence can return through a lens 650 and the dispersive element 650. Since such light is Stokes shifted, it may not easily couple back into the single-mode core, but would likely couple to the inner cladding of the dual-clad fiber 645. The emitted fluorescence emerging from the

proximal end of the fiber can be deflected by the dichroic filter or beam splitter 635 to a fluorescence detector 640. On the other hand, the reflected light can couple directly back into the single-mode core. After passing through the dichroic filter or beam splitter 635, this signal can be directed to an additional detector for a reconstruction of a spectrally encoded reflectance image.

Figure 7 shows an exemplary embodiment of a distal end of a probe according to the present invention which is configured to utilize the exemplary apparatus shown in Figure 6 and using a dual clad-fiber. For example, spectrally modulated light 700 from the interferometer can be provided to a central core 715 of a dual-clad fiber 710 via coupling optics 705. Illumination light 720 emerging from the core 715 can be divergent, and collimated by a lens 725, which can be a micro-lens, GRIN lens, etc. Collimated light 730 can be dispersed by a dispersive element 735, which may be a transmissive grating, a reflective grating, prism, hologram, or any other diffractive element. Dispersed light 745 can be focused onto a sample 750 by a lens 740, thus causing a fluorescence emission. The spectrally modulated emitted fluorescence 755 can be gathered by and collimated by the lens 740, transmitted through a grating 735, and focused by the lens 725 to an inner cladding 760 of the dual-clad fiber 710. The modulated fluorescence 755 can then be transmitted back down the inner cladding 760 for detection.

Multiple fiber exemplary embodiments: Two or more fibers

The exemplary SFE arrangements can also be configured to be provided in a multiple fiber configuration. In such exemplary embodiment of the present invention, a single-mode fiber can be used to send the illumination light to the sample, and one or multiple multi-mode fibers may be used to collect the emitted fluorescence. The

reflectance image can be reconstructed because the reflected light would couple back to the illumination fiber.

Figure 8 an exemplary embodiment of a distal end of a probe according to the present invention which is configured to utilize the exemplary apparatus shown in Figure 6 and using two optical fibers. The description of this exemplary arrangement is similar to that of Figure 7 provided herein, and elements thereof have the same description, except that elements 700, 705, etc. have been replaced with elements 800, 805, etc., respectively, except as indicated below. In the exemplary embodiment of Figure 8, the spectrally modulated illumination light can be transmitted to the distal end of the probe via single-mode fiber 815. The emitted fluorescence can be coupled back to the core of a multimode fiber 860. In another exemplary embodiment of the present invention shown in Figure 9, a multimode core 860 of Figure 8 can be replaced by a linear array of multimode fibers 960.

Alternate exemplary probe tip embodiment

Another exemplary embodiment of a distal end of the probe according to the present invention is shown in Figure 10. Although an exemplary multiple-fiber embodiment is shown in Figure 10, this exemplary embodiment of the probe tip can be used in all fiber configurations. In this exemplary embodiment, the ordering of the optics can be changed. For example, illumination light 1020 diverging from the illumination fiber can be passed through a lens 1025, which can be a GRIN lens, and/or other types of lenses or objectives. The fiber-lens separation can be selected such that the light begins to converge to a focus beyond a grating 1035 and on a sample 1050. The emitted fluorescence can then pass through the grating, and may be focused onto collection fiber(s) by the lens 1025.

Fluorescence microscopy and fluorescence confocal microscopy

Similar to exemplary spectrally encoded confocal imaging procedures, the exemplary SFE procedures can also be implemented in configurations that may enable endoscopic fluorescence microscopy. For these exemplary applications, it may be advantageous to utilize an imaging lens with a high magnification or numerical aperture. For certain exemplary endoscopic microscopy configurations, a numerical aperture can be greater than 0.3, and may preferably be greater than 0.5. Due to aberrations that may occur when large angles illuminate the grating, as shown in Figure 12, it may furthermore be advantageous to place a grating 1225 prior to a lens 1230 as is conducted in a technique termed spectrally-encoded confocal microscopy (SECM). (See G. J. Tearney et al., "Spectrally encoded confocal microscopy," Opt. Lett., Vol. 23(15), 1152 (1998); and C. Pitris et al., "A GRISM-based probe for spectrally encoded confocal microscopy," Optics Express, Vol. 11(2), 120 (2003)).

Figure 13 shows a further exemplary embodiment of the arrangement according to the present invention which can utilize one or more prisms 1325, 1335 in front or behind a grating 1330 in order keep spectrally dispersed light 1345 along the same axis as the optical probe. Similar to macroscopic imaging, exemplary multiple core/fiber combinations may be utilized to collect the fluorescent light. For implementing confocal fluorescence microscopy procedures in which the optical sectioning depth can be defined by an axial response function, as shown in Figure 14, an additional spatial filter comprising one or more fiber apertures 1465 (or a physical aperture such as a slit or pinhole 1460) may be placed at the distal fiber tip of the collection fiber(s) to reject out-of-focus light. Alternatively, as shown in Figure 15,

such exemplary spatial filter 1570 may be placed at the proximal end of the detection fibers in a fiber bundle or array of fibers.

Exemplary reference interferometer

Even slight (<1%) non-linearities in the translation of the scanning mirror of the Michelson interferometers can cause incorrect spectral information, both in line-shape and frequency (wavelength), therefore distorting or ruining the image. This can be corrected as using the exemplary embodiment of the arrangement according to the present invention which is depicted as a block diagram in Figure 11. For example, as shown in Figure 11, normal or broadband illumination light 1100 can pass through a Michelson interferometer 1105, and may emerge as spectrally modulated light 1110. This modulated light 1110 can be transmitted to a fluorescent sample or a reference illumination detector 1115. Single-frequency light 1120 (e.g., a laser, such as a Helium-Neon laser, etc., or provided from another source, etc.) may also pass through the Michelson interferometer 1105. A resultant spectrally modulated light 1125 can be detected by a reference correction detector 1130. The spectrally modulated light 1125 can have a single modulation frequency with equally spaced zero-crossings. However, non-linearities in the motion of the scanning mirror can change the spacing of the zero-crossings, and may result in an incorrect reconstruction of spectra and image lines when the Fourier transform is performed.

Exemplary embodiments of a correction procedure according to the present invention as described below can result in a re-interpolation of the data signals, based on the a priori knowledge that the reference signal should have equally-spaced zero-crossings. Figure 16 shows exemplary graphs associated with representative data with respect to this point. For example, the original reference signal detected by the

reference correction detector 1130 is illustrated in a first graph 1600. The correction factor of the upper middle panel can be used to re-interpolate the original data to obtain the trace in a second graph 1610. A further graph 1605 illustrates a Hilbert transform of a reference signal used to correct the data. The Fourier transform of the uncorrected data is shown in a fourth graph 1615 (e.g., spectrum of monochromatic reference signal prior to correction, obtained as a Fourier transform of the first graph 1600) and a spectrum of monochromatic reference signal after correction, obtained as a Fourier transform of the second graph 1610 is shown in a fourth graph 1620. The graphs 1615 and 1620 demonstrate the recovery of a single-frequency signal when the correction factor is applied to the reference data itself. Exemplary applications to other detected signals can be equally effective in a spectral correction procedure.

Exemplary excitation spectra measurement

Because the illumination light can be dispersed onto the sample to provide spectral encoding, each spot on the sample can be illuminated with a different wavelength. For example, by scanning the spectrally encoded line along the sample, approximately parallel to the line of dispersion, each point can be sequentially illuminated by the full bandwidth of the illumination light. By monitoring the intensity at each point as the wavelengths are scanned, the excitation spectrum can be recovered for each location on the sample.

Emission spectra measurement

An exemplary embodiment of the SFE procedure according to the present invention can allow for a recovery of the emission spectrum. Each point on the sample can be illuminated by a different wavelength, each of which may be encoded with a different modulation frequency. For example, if some or all of the emitted light is coupled into a spectrometer, the emission spectrum can be recovered by conventional procedures and/or methods. The spectrometer can be dispersive and/or Fourier transform type. The Fourier transform-type spectrometer may be a second interferometer added to the exemplary system. By scanning the spectrally encoded line in both directions (e.g., one to form the image, and the other to collect the excitation spectra), the excitation-emission matrix can be reconstructed for each point in the image.

Lifetime measurement

According to further exemplary embodiments of the present invention, it is also possible to determine the fluorescence lifetime at each location in the image. As indicated in Eq. 2, the illumination light oscillates sinusoidally, forcing the fluorescence emission to oscillate in the same manner. However, the fluorescence may be emitted with a slight phase shift (φ) and a decreased amplitude:

$$I_{em,j}\left(\lambda_{ex,i},\lambda_{em,j},t\right)=m\left(\lambda_{ex,i},\lambda_{em,j}\right)\frac{1}{2}I_{ex}\left(\lambda_{ex,i}\right)\left[1+\cos\left(\frac{4\pi}{\lambda_{ex,i}}vt-\varphi\left(\lambda_{ex,i},\lambda_{em,j}\right)\right)\right], \quad (3)$$

where m is the demodulation factor which depends on both the excitation and emission wavelengths. The fluorescence lifetime can be measured as:

$$\tau\left(\lambda_{ex},\lambda_{em}\right)=\frac{\tan\left[\varphi\left(\lambda_{ex},\lambda_{em}\right)\right]}{2\pi f\left(\lambda_{ex}\right)}, \quad (4)$$

where $\varphi(\lambda_{ex},\lambda_{em})$ is the phase difference between the illumination light and the emitted fluorescence. Lifetime can also be calculated as

$$\tau = \frac{\sqrt{1-\left[m(\lambda_{ex},\lambda_{em})\right]^2}}{2\pi f(\lambda_{ex})m(\lambda_{ex},\lambda_{em})}. \quad (5)$$

Exemplary light sources

In order to attain a high number of resolvable points shown in Table 1, it is preferable for the source to be capable of illuminating the entire excitation spectrum. This can be made possible through the use of, e.g., thermal lamps, arc lamps, solid-state lasers, and LEDs. Additionally, alternative sources such as supercontinuum generation with photonic crystal fiber technology can be utilized, the description of which is provided in G. McConnell, "Confocal laser scanning fluorescence microscopy with a visible continuum source," Opt. Express, Vol. 12(13), 2844 (2004). The use of broadband NIR lasers as a light source can facilitate SFE two-photon fluorescence imaging.

Alternatively, the fluorescence imaging can be accomplished with swept-source lasers. In this case the frequency encoding is not necessary and the technique reduces to traditional spectral encoding because the individual locations of the sample are sequentially illuminated. It is still possible to obtain excitation, emission, and lifetime spectra with this embodiment, as well as reconstruction of the EEM.

Exemplary Procedures for reconstructing a fluorescent image

An exemplary embodiment of the method according to the present invention for reconstructing the fluorescent image begins with the flow diagram of Figure 17. As shown in this figure, Eq. 2 is inverse Fourier transformed in step 1710. The resultant signal may likely be one line in the image. Then, intensity corrections can be accomplished by dividing by the illumination spectrum, as indicated in step 1720.

An exemplary excitation spectrum can be obtained using the exemplary procedure of Figure 18, in which, after scanning along the direction of the spectrally encoded line, Eq. 3. is inverse Fourier transformed in step 1810. Then, in step 1820, source cross-correlation spectra is divided (e.g., by an inverse Fourier transform of Eq. 2). An amplitude of resultant signal is an exemplary excitation spectra, and the phase of resultant signal can be related to time constants *via* Eq. 4, where $\varphi(\lambda_{em},\lambda_{ex})$ may be the phase difference between the and the source cross-correlation ($\varphi_I(\lambda_{ex})$).

The exemplary excitation lifetime can also be obtained. For example, the inverse Fourier transform of Eq. 3 for a phase of the resultant signal can be the phase of the fluorescence ($\varphi(\lambda_{em},\lambda_{ex})$). The inverse Fourier transform of Eq. 1 for the phase of the resultant signal can be the phase of the source cross-correlation ($\varphi_I(k_X)$). The phase difference may be related to the fluorescence lifetime *via* Eq. 4 where:

$$\varphi(\lambda_{em},\lambda_{ex}) = \varphi(\lambda_{em},\lambda_{ex}) - \varphi_I(\lambda_{ex}). \quad (6)$$

An exemplary embodiment of a spectral procedure for correcting non-linear mirror motion according to the present invention is shown in a flow diagram of Figure 19. Each full scan (e.g., back and forth) of the mirror can correspond to two lines in

the image. This exemplary correction procedure can utilize a simultaneous acquisition of two interferograms for each line in the image, e.g., (a) the signal of interest, and (b) a signal with a known spectrum, preferably a single-frequency source such as a He-Ne laser, which can be used as a reference to correct the time-traces for non-linear motion of the scanning mirror of the Michelson interferometer.

As shown in Figure 19, for example, data is truncated to linear region of scan, containing 2^n data points for efficient Fast Fourier transforms (usually symmetric about the center point, dictated by duty cycle of the driving waveform) in step 1910. In step 1920 the mean of the data is subtracted from each signal if acquisition was not AC-coupled (e.g., see the first graph 1600 of Figure 16 showing only a small portion of data). The signals are then time-reversed for most or all odd scans (e.g., to correct for the opposite direction of the scan) in step 1930. In step 1940, the Hilbert transform of the reference signal is taken. For example, the Fourier transform of a single-frequency laser (e.g., the reference) can be an infinite sine wave with equally spaced zero-crossings. Further, the Hilbert transform can be based on the Fast Fourier transform, and the imaginary part may correspond to the original data with a 90° phase shift.

Then, in step 1950, the unwrapped phase of the Hilbert transform of the reference signal is taken (as shown in the graph 1605 of Figure 16). This can correspond to the actual position of the mirror as a function of time, and may be monotonically increasing (e.g., not locally linear). Then, a new linear mirror position can be generated ranging from the minimum to maximum of the actual mirror position – step 1960. In step 1970, the signal of interest can be re-interpolated onto the new linear mirror position space, e.g., using the unwrapped phase of the reference

Hilbert transform. The second graph 1610 of Figure 16 shows the signal of interest which is the reference itself. The spectrum of interest can then be determined from the Fourier transform of the interpolated signal in step 1980. For example, see the third and fourth graphs 1615 and 1620 for uncorrected and corrected signals, respectively.

Alternatively, the time-trace of reference signal can be used to generate a clock signal which may be used to gate the data acquisition at equally spaced mirror locations, thereby automatically correcting the data, and obfuscating the post-processing algorithm described above.

Exemplary components

Various components can be used for the exemplary embodiments of the present invention. Provided below are merely samples of such components, and in no way limit the scope of the present invention.

For example, the broad bandwidth light source can include LED, filament lamp (e.g. Tungsten-halogen, Mercury, Xenon, Deuterium), array of diode lasers, continuum generation source, femtosecond solid-state source, semiconductor optical amplifier, rare-earth doped fiber, ASE source, dye fluorescence, SLED, swept-source laser, etc. The reference source can include a monochromatic light source, such as HeNe laser, gas laser, diode laser, filtered broad bandwidth light source, etc. The optical fiber can include Dual-clad fiber, single-mode fiber, multimode fiber(s), photonic crystal fiber, hollow-core fiber, hollow waveguide, etc.

Further, the dispersive element can include transmission grating, reflection grating, hologram, prism, etc. The compensator can include a neutral density filter. The dispersion compensator can include dual opposing prisms or optical glass, crystal

or other dispersion modifier, etc. The wavelength dependent frequency can be one or more of the following: Scanning mirror *via* galvanometer, piezoelectric transducer, or solenoid. Rapidly scanning optical delay line (RSOD) as described in G. J. Tearney et al, "High-speed phase- and group-delay scanning with a grating-based phase control delay line," Optics Letters, Vol. 22(23), 1811 (1997), phase control delay line, acousto-optic modulator, electro-optic modulator, spinning helical cam, rotating hologram, spinning mirror array, spinning cube, piezoelectric fiber stretcher, variable reflectance plate beam splitter (Fabry-Perot interferometer), etc.

The exemplary interferometer can include any arrangement for combining light returned from two arms, such as, e.g., Mach-Zehnder, Sagnac, Michelson, Fabry-Perot interferometers. It is noted that the reflection from these arms is not necessary, and such arrangements can operate in a transmission mode. It is also possible to incorporate polarization beam splitters, common path elements and/or circulators in such exemplary arrangements. The dichroic splitter can include an interference filter, diffraction grating, dichroic mirror, etc. The exemplary spectral dispersion can be accomplished using Grating spectrometer, Fourier Transform spectrometer, prism spectrometer, etc. The exemplary detectors can include photodiode, photomultiplier tube, avalanche photodiode, CCD, etc.

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. A system for obtaining a photoluminescence radiation from at least one portion of a sample, comprising:

at least one arrangement configured to:

i. receive a first radiation and disperses the first radiation into at least one second radiation and at least one third radiation, the second and third radiations being provided to different locations of the at least one portion, and

ii. receive the photoluminescence radiation from the at least one portion based on the second and third radiations.

2. The system according to claim 1, wherein the at least one arrangement comprises at least one of a grating, a prism, a grism, a dual prism-grism or a lens.

3. The system according to claim 1, wherein the at least one arrangement comprises a lens having a numerical aperture that is greater 0.5.

4. The system according to claim 1, wherein at least one first arrangement comprises at least one optical fiber.

5. The system according to claim 3, wherein the at least one optical fiber has multiple claddings.

6. The system according to claim 4, wherein the at least one optical fiber includes a plurality of optical fibers.

7. The system according to claim 4, wherein the at least one arrangement comprise at least one of at least one pin hole arrangement or at least one slit arrangement.

8. The system according to claim 4, wherein at least one of the at least one optical fiber is a multimode fiber.

9. The system according to claim 1, further comprising a wavelength tuning light source configured to provide the at least one first radiation.

10. The system according to claim 1, further comprising a light source configured to provide the at least one first radiation that has multiple wavelengths.

11. The system according to claim 7, further comprising a further arrangement configured to modulate the wavelengths at different frequencies.

12. The system according to claim 11, wherein the further arrangement comprises an interferometric arrangement.

13. The system according to claim 12, wherein the interferometric arrangement includes at least one translatable component.

14. The system according to claim 13, wherein the further arrangement comprises a further interferometric arrangement configured to correct for non-linearities in the at least one translatable component.

15. The system according to claim 11, wherein the further arrangement can include at least one of an acousto-optical modulator or an electro-optical modulator configured to provide frequency encoding capabilities.

16. The system according to claim 1, wherein the at least one arrangement is configured to generate information associated with the different locations as a function of the photoluminescence radiation, and further comprising a processing arrangement configured to generate at least one image based on the information.

17. The system according to claim 16, wherein the processing arrangement is configured to receive the at least one signal, and to Fourier transform the at least one signal to generate the image.

18. The system according claim 16, wherein the at least one image includes at least one of a microscopic image or an endoscopic image.

19. The system according to claim 14, wherein the at least one arrangement comprises a detecting arrangement which is configured to receive the photoluminescence radiation and generate at least one signal which is associated with the photoluminescence radiation.

20. The system according to claims 1, wherein the at least one arrangement is configured to be able to control a position of the second and third radiations on the different locations on the at least one portion of the sample.

21. A method for obtaining a photoluminescence radiation from at least one portion of a sample, comprising:

receiving a first radiation and disperses the first radiation into at least one second radiation and at least one third radiation, the second and third radiations being provided to different locations of the at least one portion, and

receiving the photoluminescence radiation from the at least one portion based on the second and third radiations.

22. The method according to claim 21, further comprising:

generating information associated with the different locations as a function of the photoluminescence radiation; and

generating at least one image based on the information.

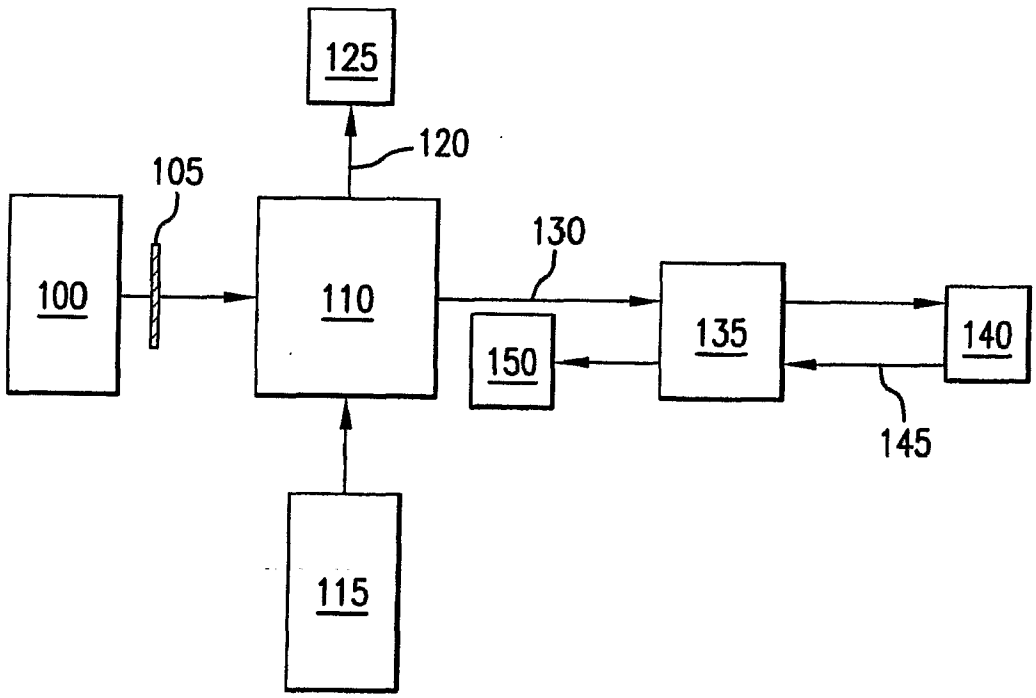

FIG. 1

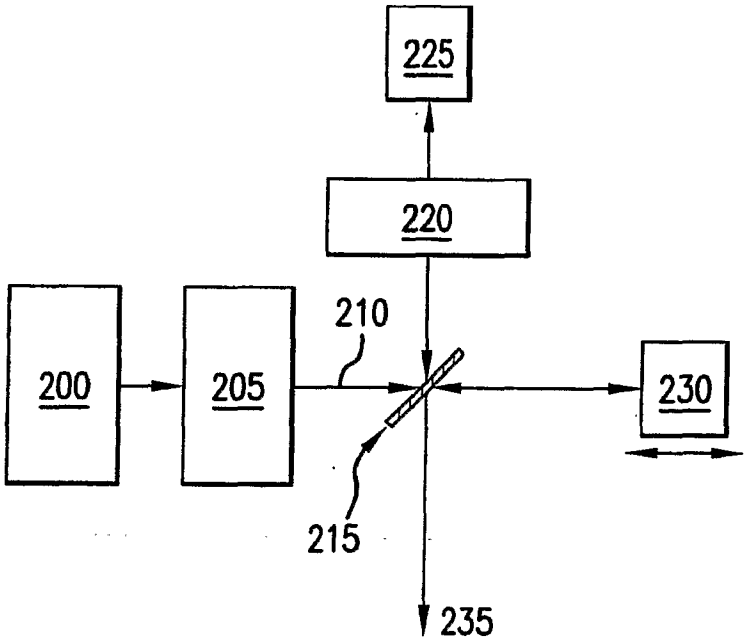

FIG.2

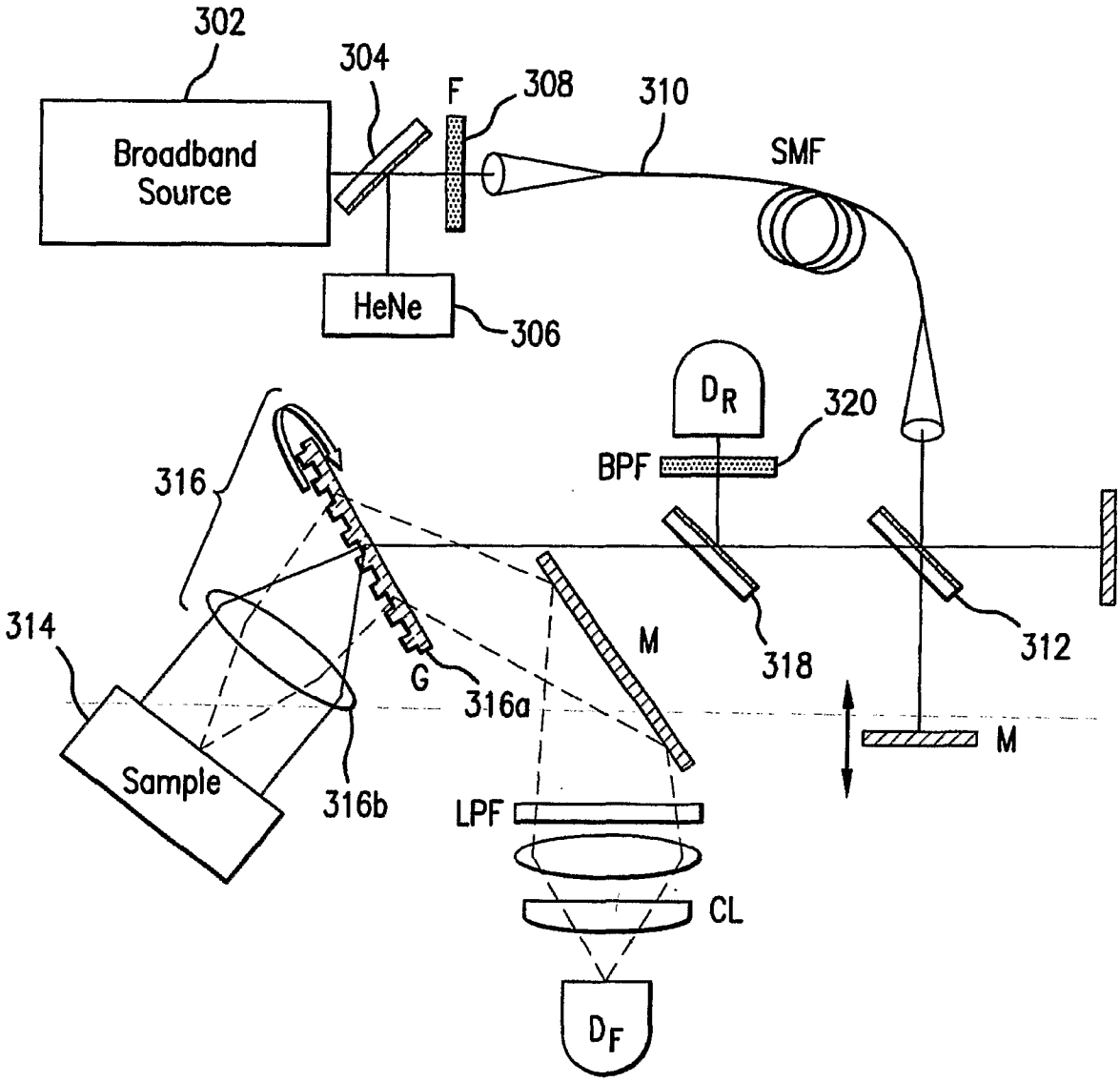
302
304
F
308
310
SMF
Broadband Source
HeNe
306
D_R
320
BPF
316
314
G
316a
M
318
312
M
Sample
316b
LPF
CL
D_F

FIG.3

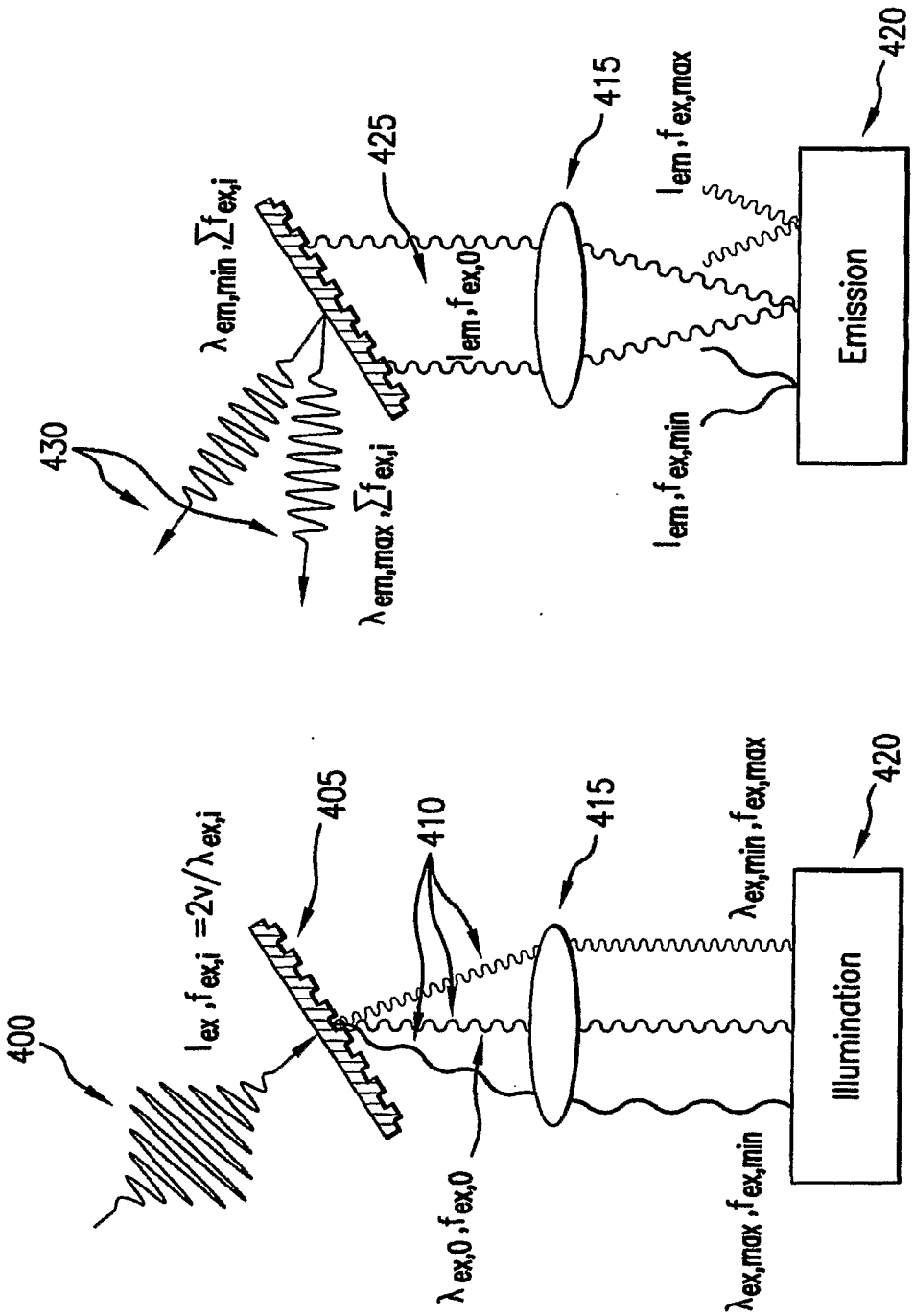
400
$l_{ex}, f_{ex,i} = 2v/\lambda_{ex,i}$
405
410
415
420
Illumination
$\lambda_{ex,0}, f_{ex,0}$
$\lambda_{ex,max}, f_{ex,min}$
$\lambda_{ex,min}, f_{ex,max}$
425
430
$\lambda_{em,min}, \sum f_{ex,i}$
$\lambda_{em,max}, \sum f_{ex,i}$
$l_{em}, f_{ex,0}$
$l_{em}, f_{ex,min}$
$l_{em}, f_{ex,max}$
Emission

FIG.4A

FIG.4B

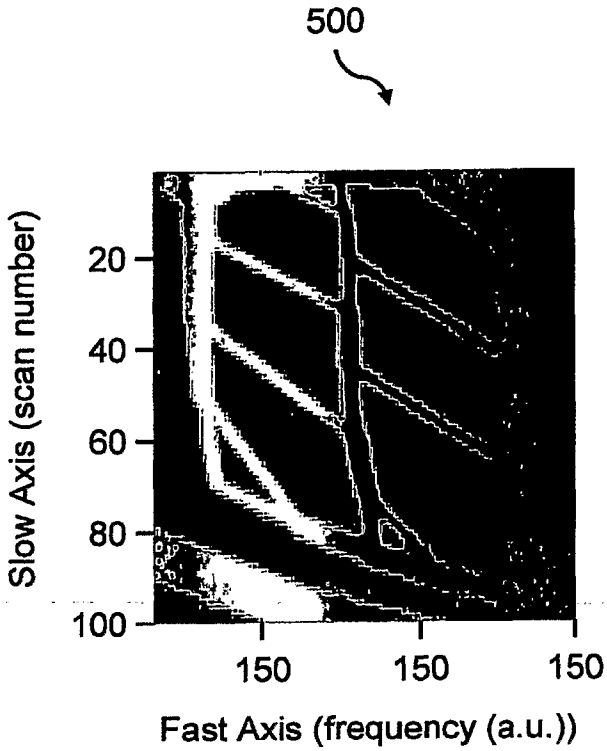
500
Slow Axis (scan number)
20
40
60
80
100
150
150
150
Fast Axis (frequency (a.u.))

FIG.5A


505
500 µm

FIG.5B

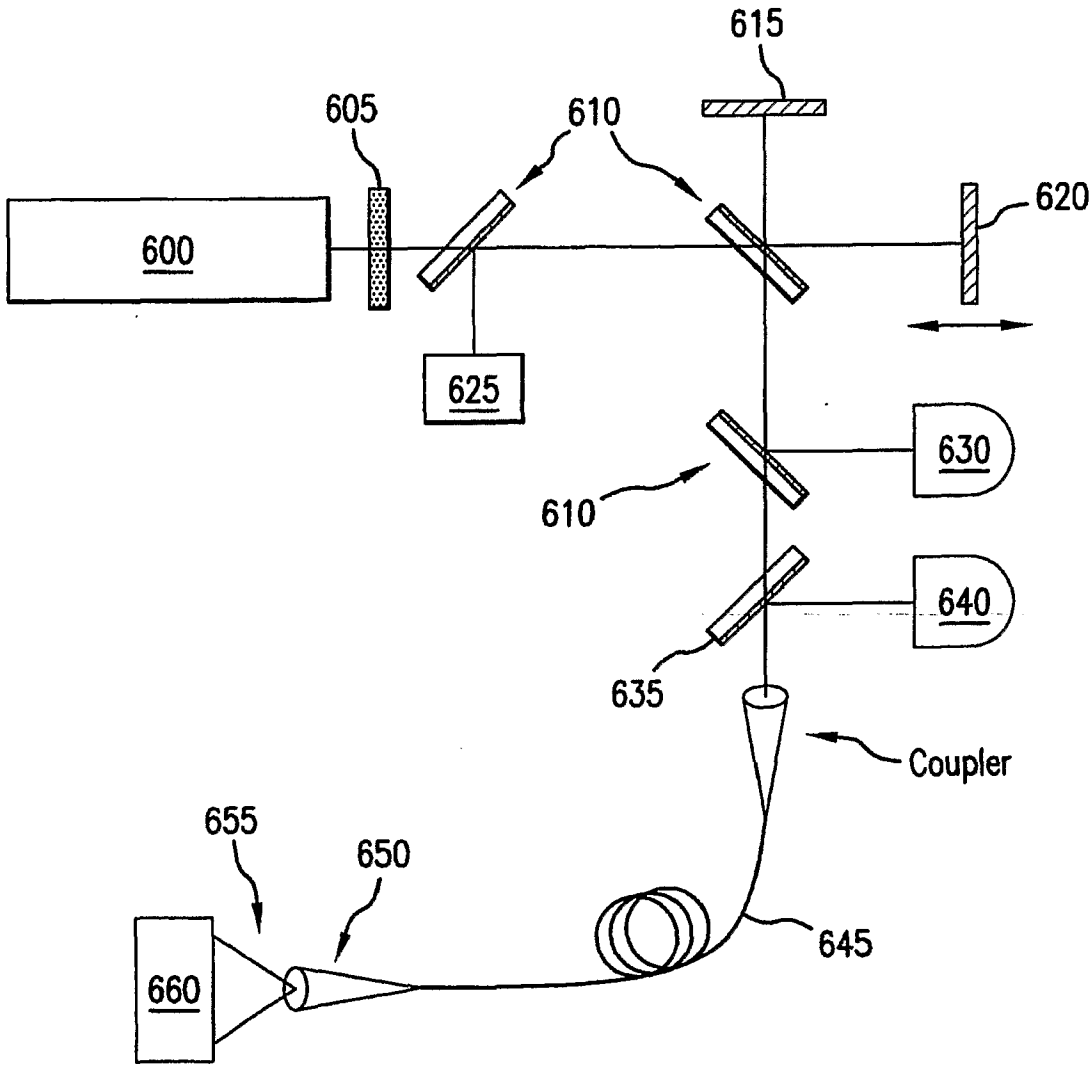

FIG.6

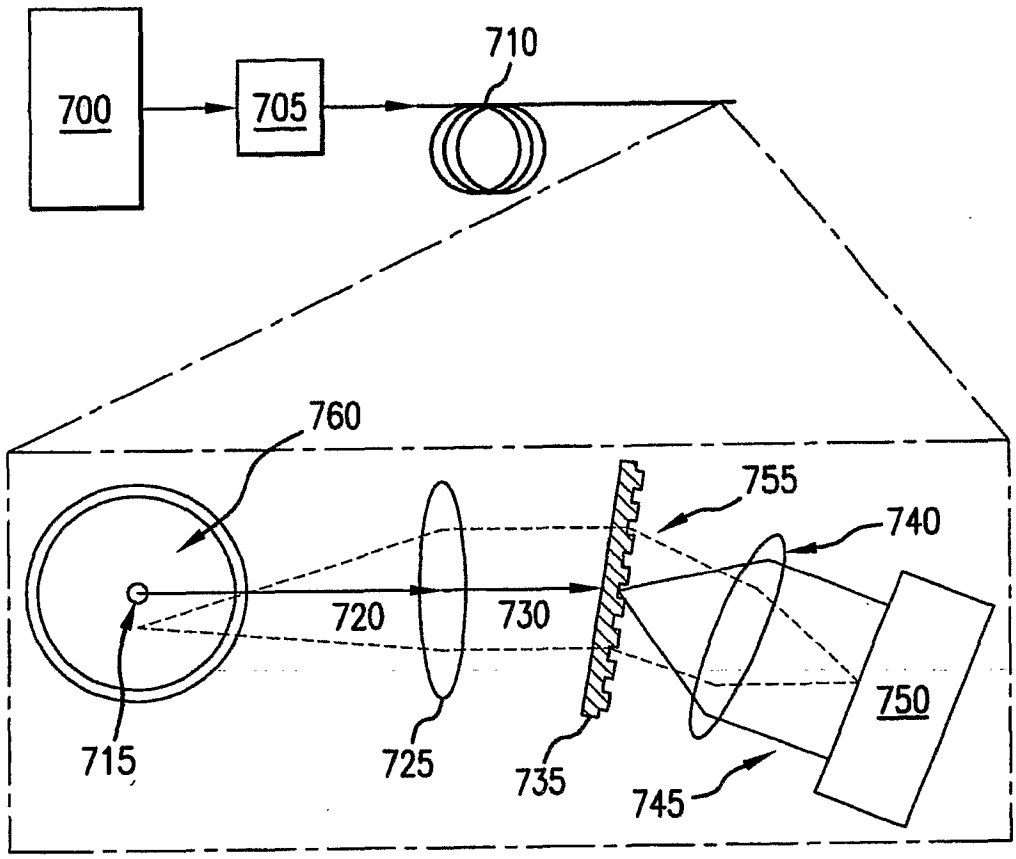

FIG.7

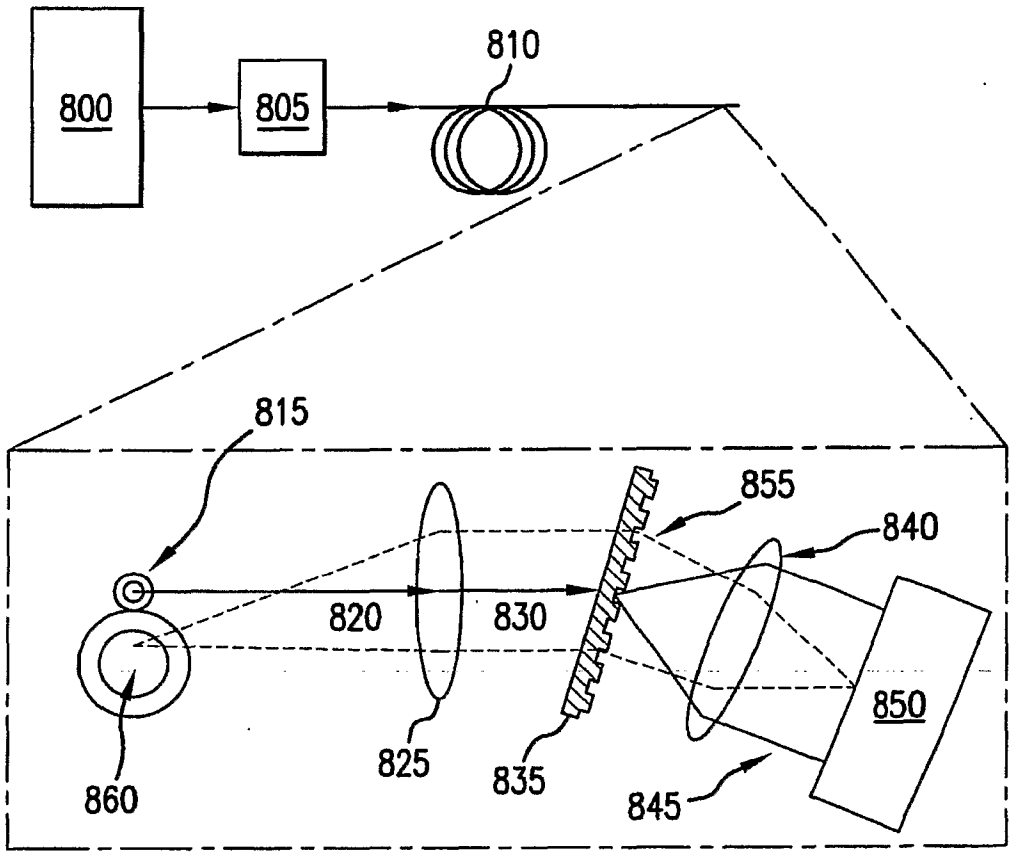

FIG.8

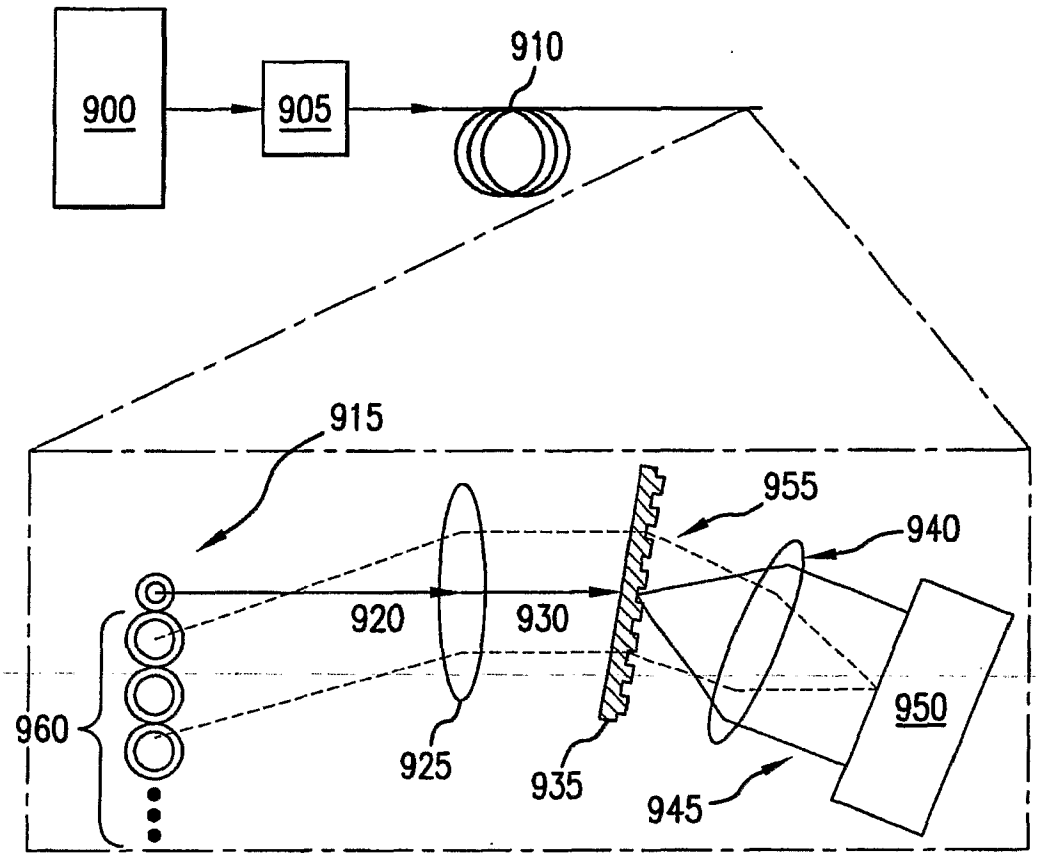

FIG.9

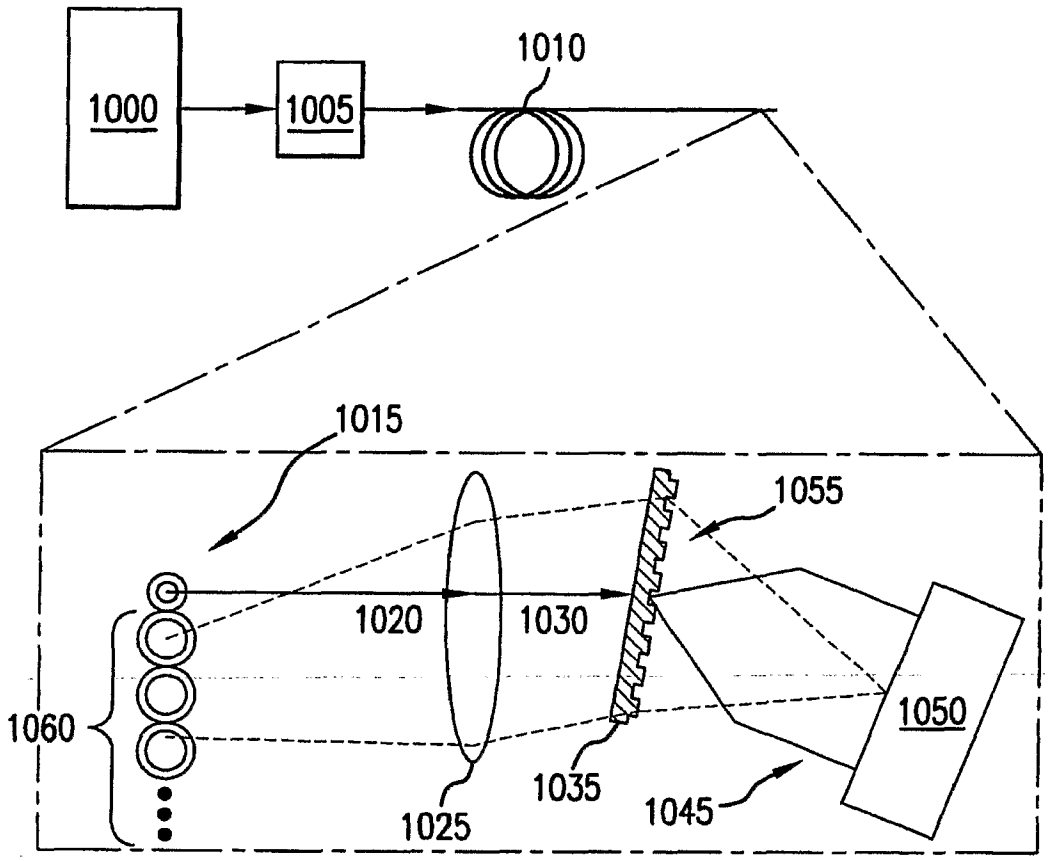

FIG. 10

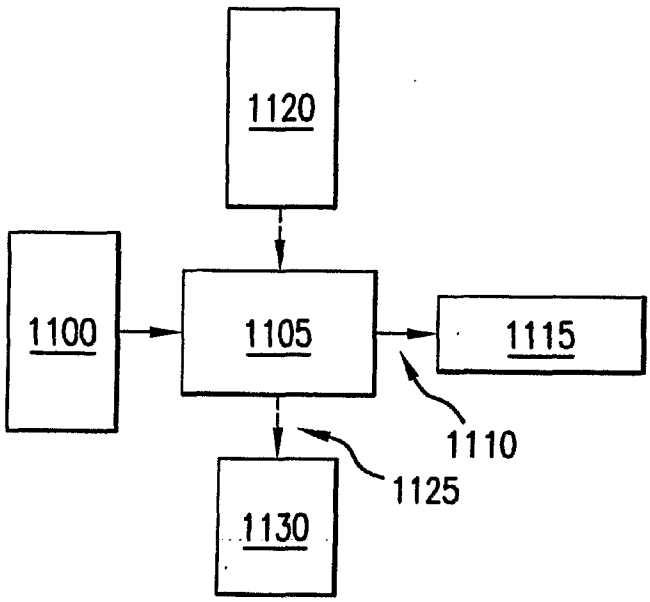

FIG.11

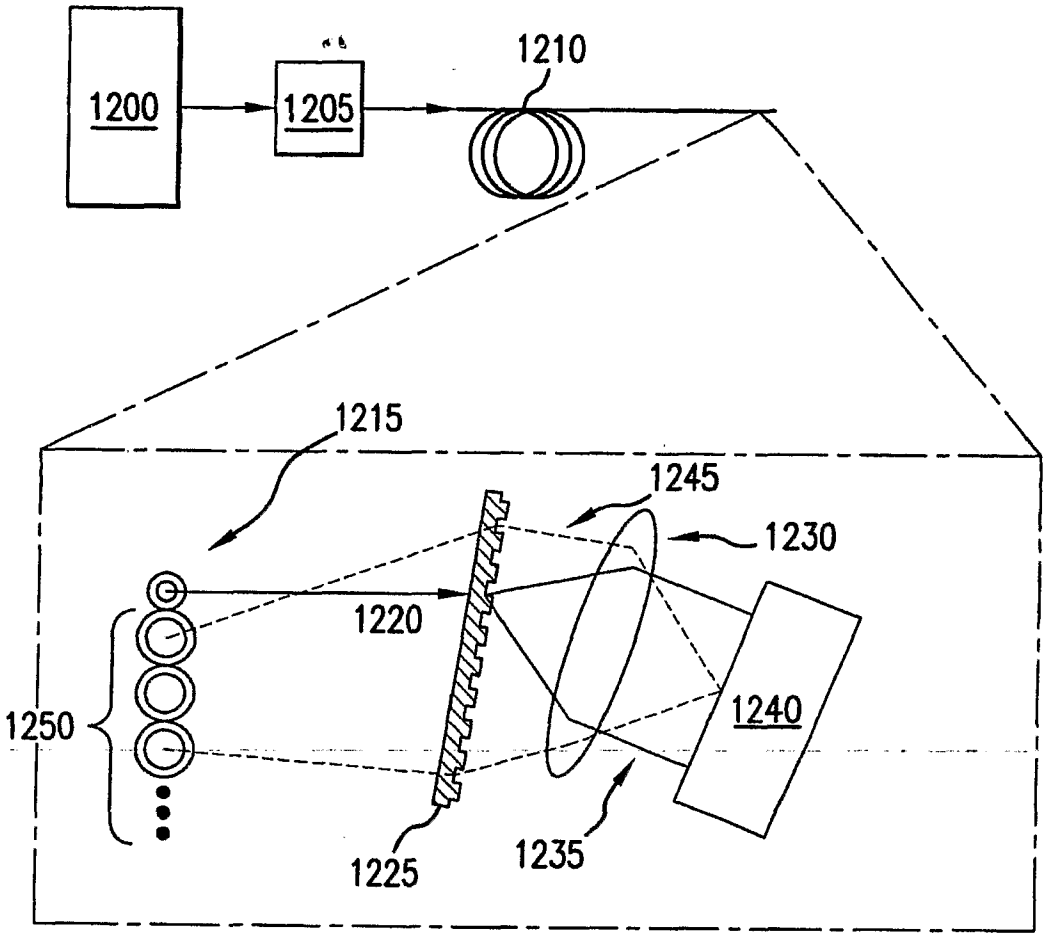

FIG.12

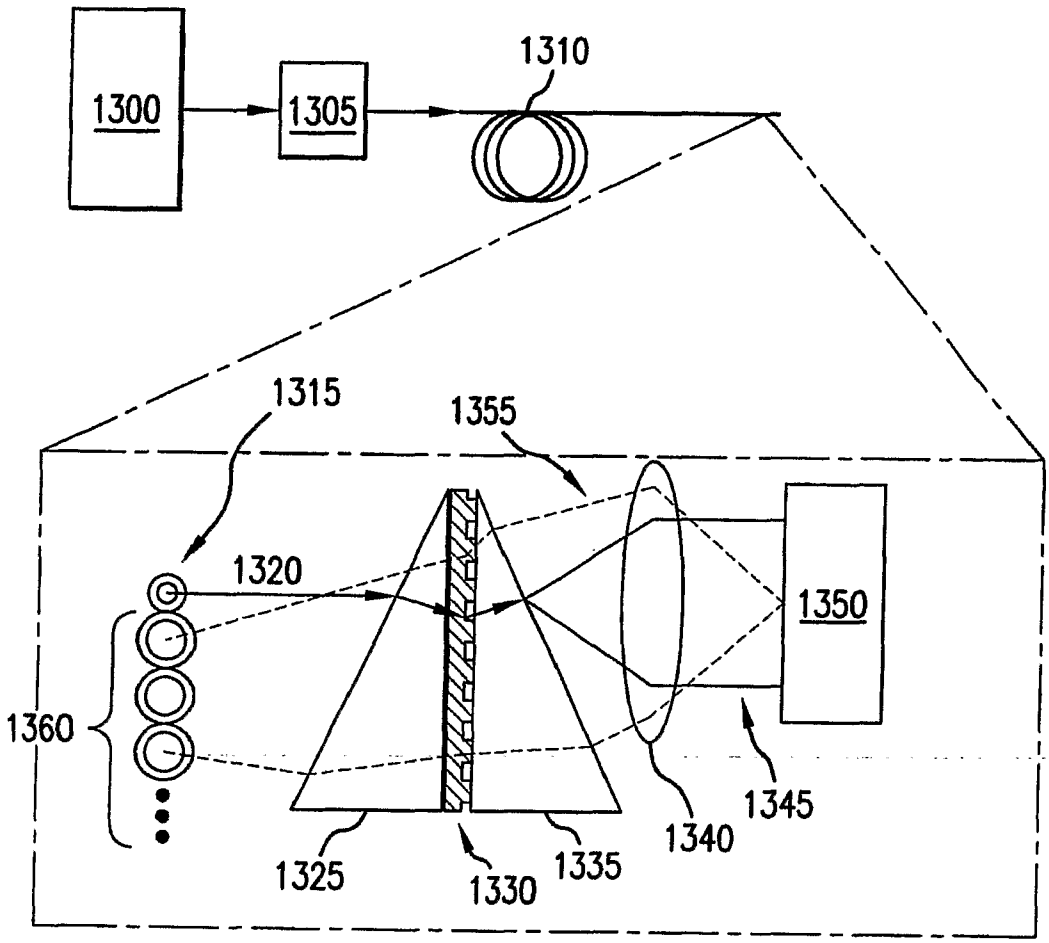
1300
1305
1310
1315
1355
1320
1350
1360
1325
1330
1335
1340
1345

FIG.13

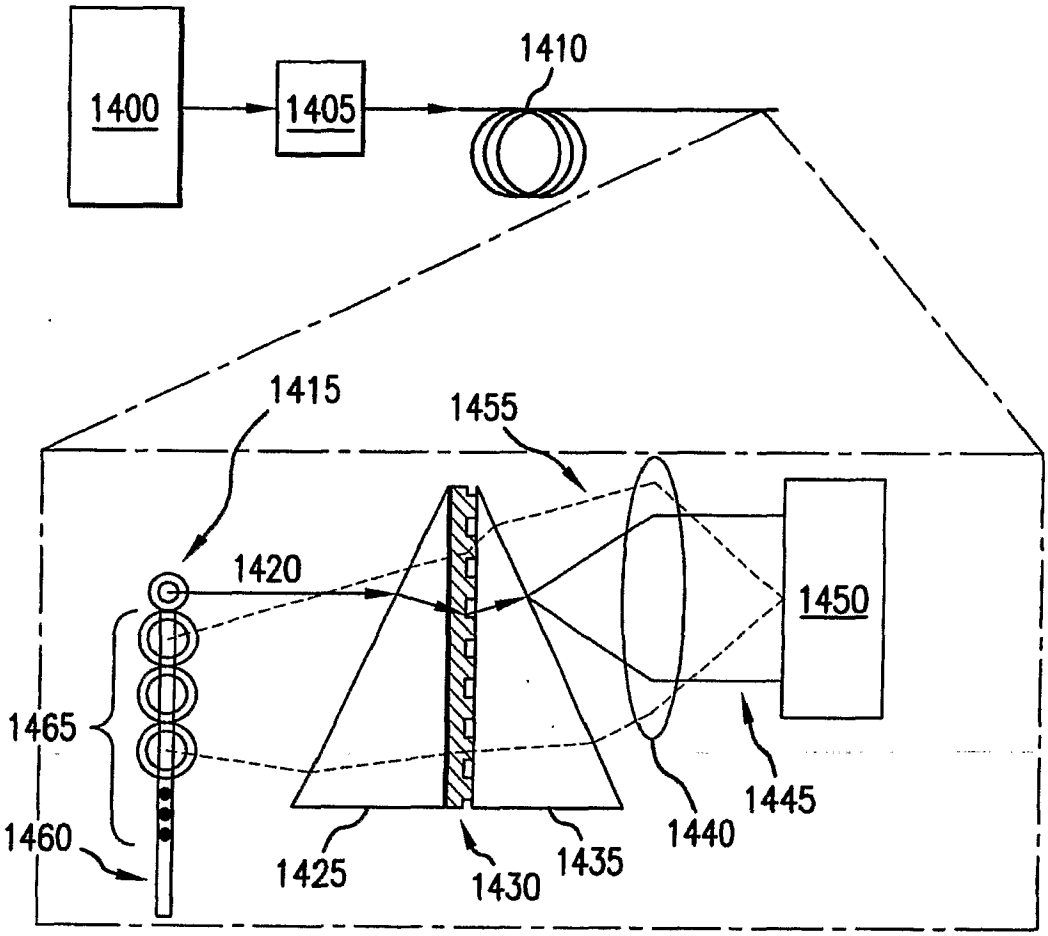
1400
1405
1410
1415
1455
1420
1450
1465
1460
1425
1430
1435
1440
1445

FIG.14

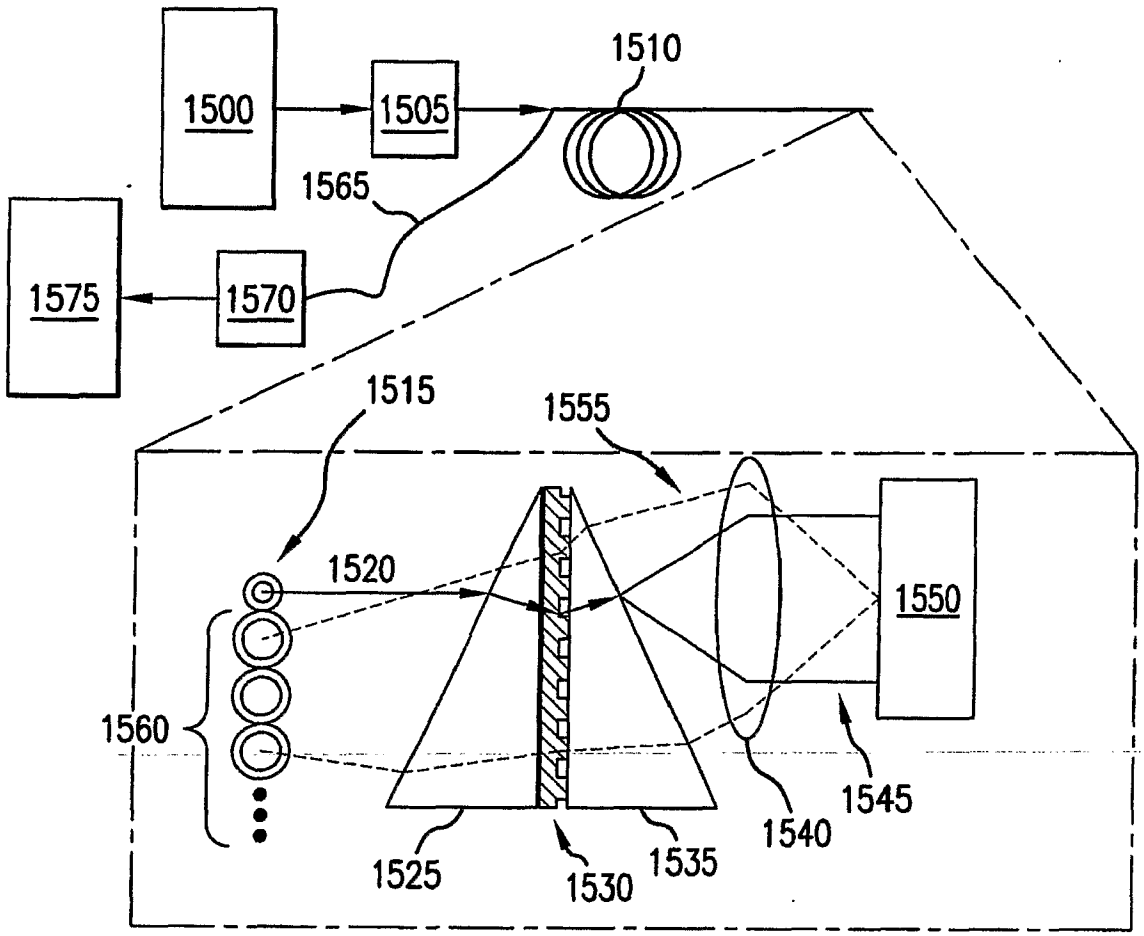
1500
1505
1510
1565
1575
1570
1515
1555
1520
1550
1560
1545
1540
1525
1530
1535

FIG. 15

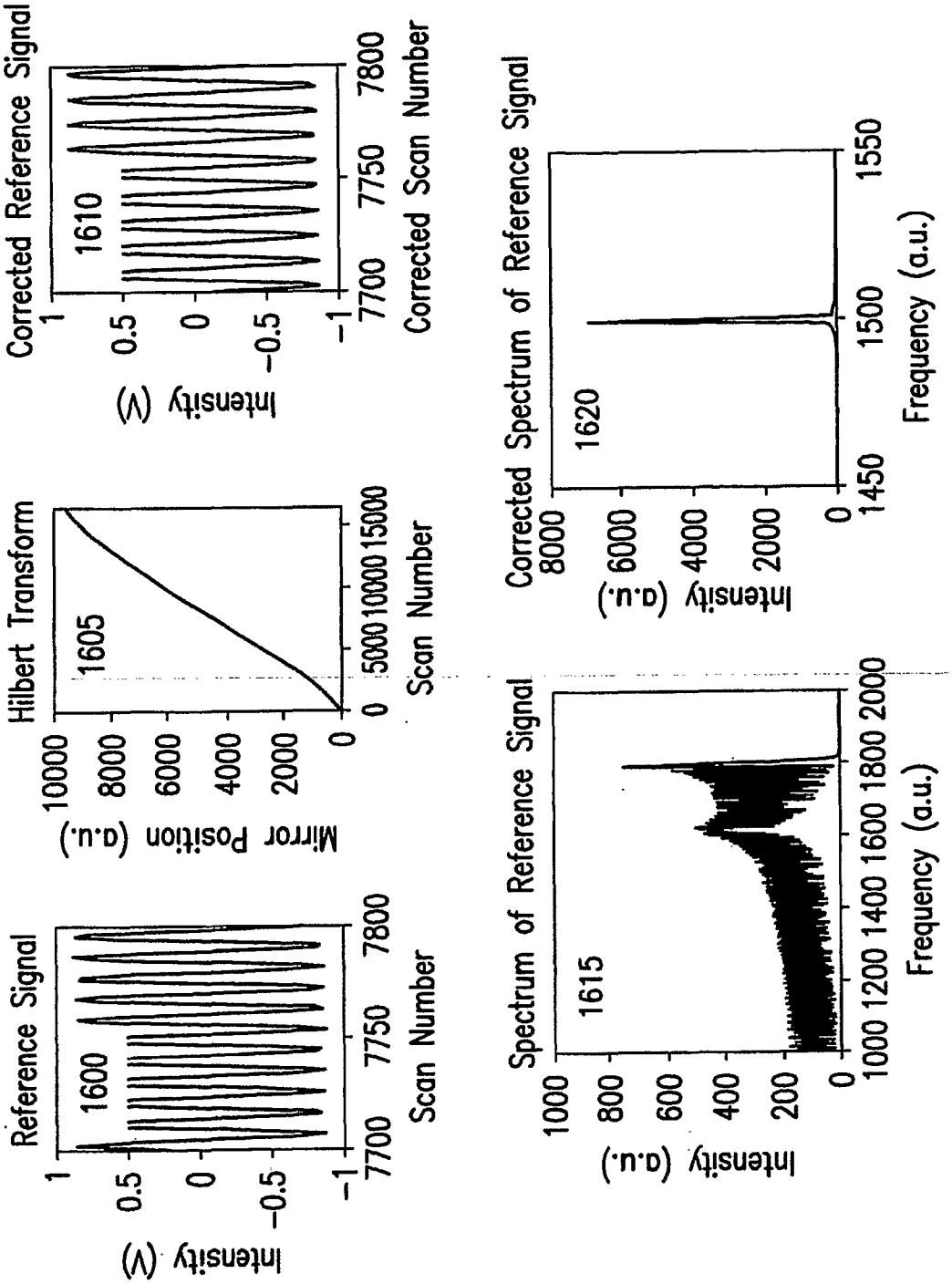
Reference Signal
Intensity (V)
1600
Scan Number
Hilbert Transform
Mirror Position (a.u.)
1605
Scan Number
Corrected Reference Signal
Intensity (V)
1610
Corrected Scan Number
Spectrum of Reference Signal
Intensity (a.u.)
1615
Frequency (a.u.)
Corrected Spectrum of Reference Signal
Intensity (a.u.)
1620
Frequency (a.u.)

FIG. 16

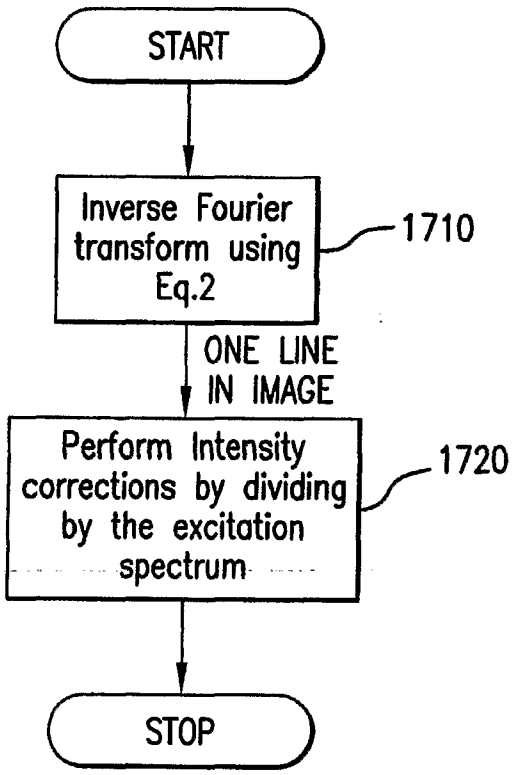
START
Inverse Fourier transform using Eq.2
1710
ONE LINE IN IMAGE
Perform Intensity corrections by dividing by the excitation spectrum
1720
STOP

FIG.17

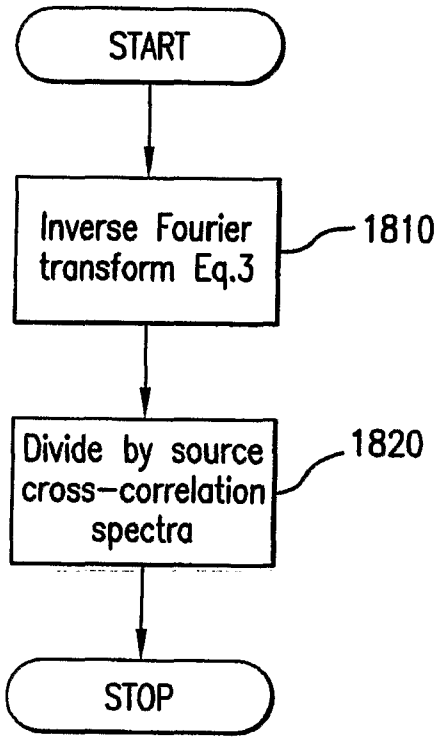
START
Inverse Fourier transform Eq.3
1810
Divide by source cross-correlation spectra
1820
STOP

FIG.18

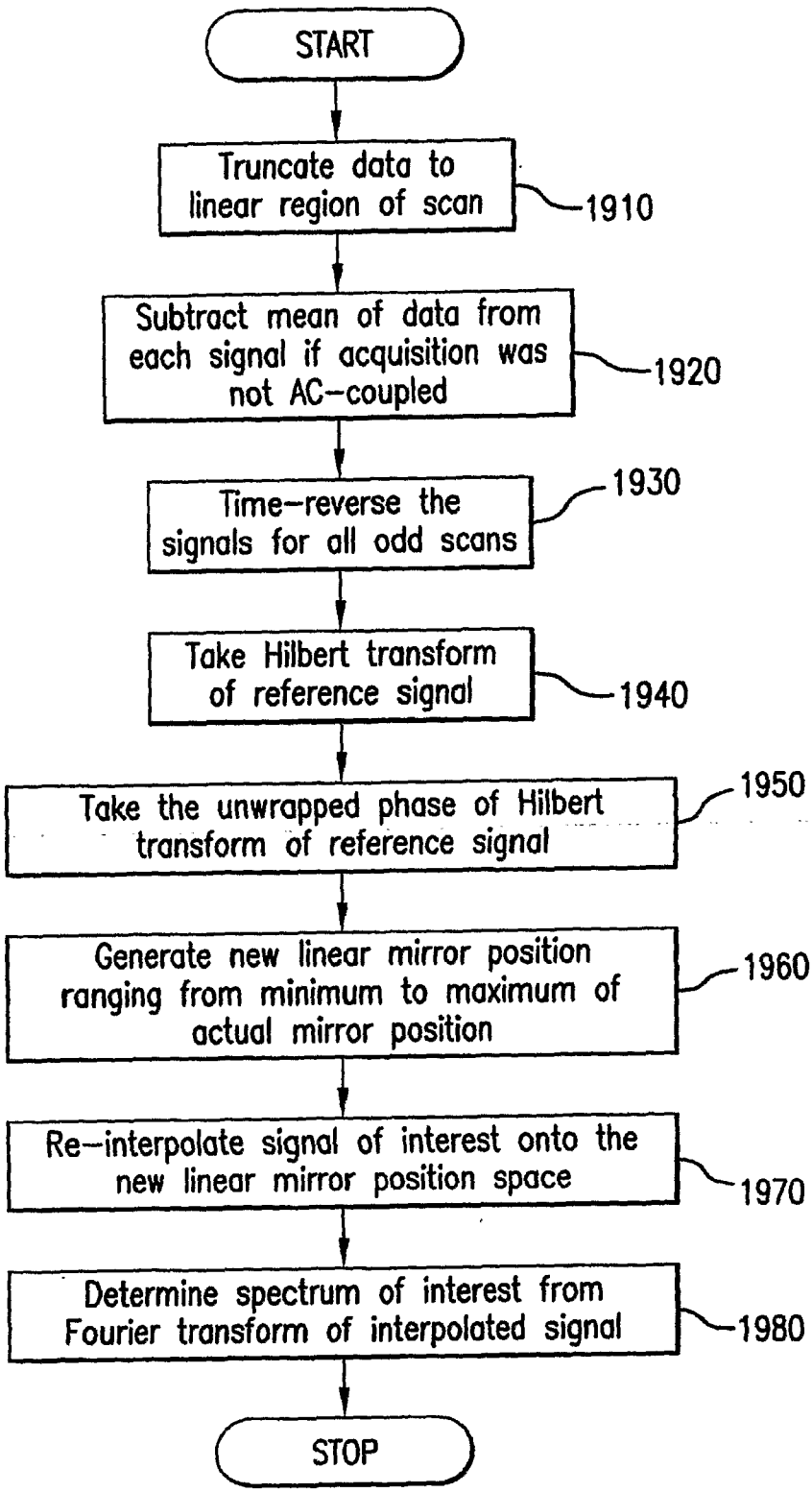
START
Truncate data to linear region of scan
1910
Subtract mean of data from each signal if acquisition was not AC-coupled
1920
Time-reverse the signals for all odd scans
1930
Take Hilbert transform of reference signal
1940
Take the unwrapped phase of Hilbert transform of reference signal
1950
Generate new linear mirror position ranging from minimum to maximum of actual mirror position
1960
Re-interpolate signal of interest onto the new linear mirror position space
1970
Determine spectrum of interest from Fourier transform of interpolated signal
1980
STOP

FIG.19

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/040584

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61B5/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61B

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, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/38040 A2 (GEN HOSPITAL CORP [US]; TEARNEY GUILLERMO J [US]; BOUMA BRETT EUGENE [) 16 May 2002 (2002-05-16)	1-13, 16-22
Y	page 7, line 5 - page 10, line 10 page 13, line 5 - page 16, line 2 figures 1-6	14,15
Y	US 2003/030816 A1 (EOM TAE BONG [KR] ET AL) 13 February 2003 (2003-02-13) paragraphs [0035] - [0040]	14
Y	US 2002/057431 A1 (FATELEY WILLIAM G [US] ET AL) 16 May 2002 (2002-05-16) paragraphs [0009], [0011] paragraphs [0057], [0058]	15

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

[X] See patent family annex.

* Special categories of cited documents :

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" 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 February 2007	05/03/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Völlinger, Martin

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/040584

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MOTZ J T ET AL: "SPECTRAL- AND FREQUENCY-ENCODED FLUORESCENCE IMAGING" OPTICS LETTERS, OSA, OPTICAL SOCIETY OF AMERICA, WASHINGTON, DC, US, vol. 30, no. 20, 15 October 2005 (2005-10-15), pages 2760-2762, XP001235409 ISSN: 0146-9592 page 2760, left-hand column, last paragraph - page 2761, right-hand column, paragraph 2 page 2762, right-hand column, paragraph 2	1-8, 10-14, 16-22

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2006/040584

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 1343411 A2	17-09-2003
US 2003030816 A1	13-02-2003	NONE	
US 2002057431 A1	16-05-2002	NONE	