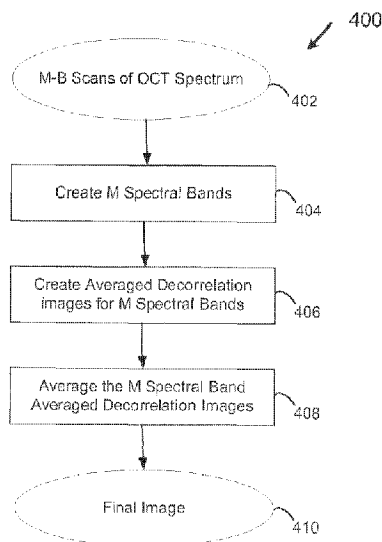




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(57) **Abrégé/Abstract:**

Amplitude decorrelation measurement is sensitive to transverse flow and immune to phase noise in comparison to Doppler and other phase-based approaches. However, the high axial resolution of OCT makes it very sensitive to the pulsatile bulk motion noise in the axial direction, resulting in unacceptable signal to noise ratio (SNR). To overcome this limitation, a novel OCT angiography technique based on the decorrelation of OCT signal amplitude due to flow was created. The full OCT spectrum can be split into several narrower spectral bands, resulting in the OCT resolution cell in each band being isotropic and less susceptible to axial motion noise. Inter-B-scan decorrelation can be determined using the narrower spectral bands separately and then averaged. Recombining the decorrelation images from the spectral bands yields angiograms that use the full information in the entire OCT spectral range. Such images showed significant improvement of SNR for both flow detection and connectivity of microvascular networks when compared to other amplitude-decorrelation techniques. Further, creation of isotropic resolution cells can be useful for quantifying flow having equal sensitivity to axial and transverse flow. Such improved non-invasive imagery can be useful in the diagnosis and management of a variety of diseases.

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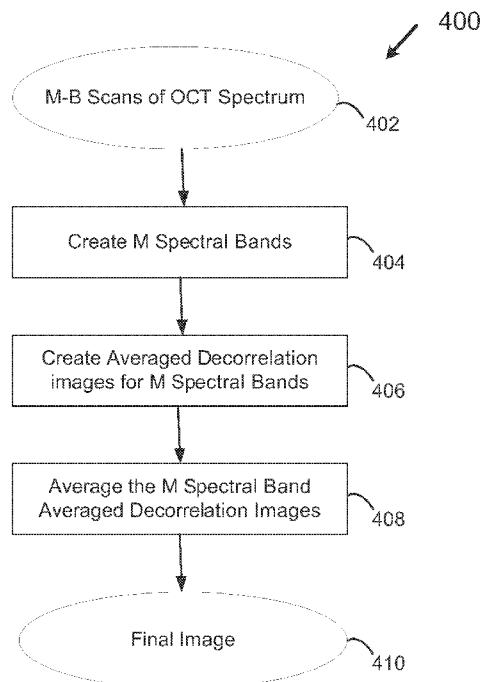
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IN VIVO OPTICAL FLOW IMAGING

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FIELD

This disclosure relates generally to the field of biomedical imaging, and more specifically to methods, apparatuses, and systems associated with optical coherence tomography and angiography.

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BACKGROUND

15

In vivo three-dimensional mapping of biologic tissue and vasculature is a challenging proposition due to the highly-scattering and absorptive nature of biologic tissue. Some current methods have slow scanning speeds making in vivo three-dimensional imaging difficult. Some other techniques having faster scanning speeds are still lacking due to their inability to scan deeply into biologic tissue without producing overlapped images, requiring the use of invasive procedures to scan the tissue of interest. Many techniques aimed at deeper imaging generally cannot provide deep imaging of tissue having moving material (e.g., blood flow). Therefore, methods to effectively image structure and/or tissue movement, such as blood flow, are of substantial clinical importance.

Optical coherence tomography (OCT) is an imaging modality for high-resolution, depth-resolved cross-sectional, and 3-dimensional (3D) imaging of biological tissue. Among its many applications, ocular imaging in particular has found widespread clinical use. In the last decade, due to the development of

5 light source and detection techniques, Fourier-domain OCT, including spectral (spectrometer-based) OCT and swept-source OCT, have demonstrated superior performance in terms of sensitivity and imaging speed over those of time-domain OCT systems. The high-speed of Fourier-domain OCT has made it easier to image not only structure, but also blood flow. This functional extension

10 was first demonstrated by Doppler OCT which images blood flow by evaluating phase differences between adjacent A-line scans. Although Doppler OCT is able to image and measure blood flow in larger blood vessels, it has difficulty distinguishing the slow flow in small blood vessels from biological motion in extravascular tissue. In the imaging of retinal blood vessels, Doppler OCT faces

15 the additional constraint that most vessels are nearly perpendicular to the OCT beam, and therefore the detectability of the Doppler shift signal depends critically on the beam incident angle. Thus, other techniques that do not depend on beam incidence angle are particularly attractive for retinal and choroidal angiography.

20 Several OCT-based techniques have been successfully developed to image microvascular networks in human eyes *in vivo*. One example is optical microangiography (OMAG), which can resolve the fine vasculature in both retinal and choroid layers. OMAG works by using a modified Hilbert transform to separate the scattering signals from static and moving scatters. By applying the

25 OMAG algorithm along the slow scanning axis, high sensitivity imaging of capillary flow can be achieved. However, the high-sensitivity of OMAG requires precise removal of bulk-motion by resolving the Doppler phase shift. Thus, it is susceptible to artifacts from system or biological phase instability. Other related methods such as phase variance and Doppler variance have been developed to

30 detect small phase variations from microvascular flow. These methods do not require non-perpendicular beam incidence and can detect both transverse and axial flow. They have also been successful in visualizing retinal and choroidal

microvascular networks. However, these phase-based methods also require very precise removal of background Doppler phase shifts due to the axial movement of bulk tissue. Artifacts can also be introduced by phase noise in the OCT system and transverse tissue motion, and these also need to be removed.

5 To date, most of the aforementioned approaches have been based on spectral OCT, which provides high phase stability to evaluate phase shifts or differentiates the phase contrast resulting from blood flow. Compared with spectral OCT, swept-source OCT introduces another source of phase variation from the cycle-to-cycle tuning and timing variabilities. This makes phase-based
10 angiography noisier. To use phase-based angiography methods on swept-source OCT, more complex approaches to reduce system phase noise are required. On the other hand, swept-source OCT offers several advantages over spectral OCT, such as longer imaging range, less depth-dependent signal roll-off, and less motion-induced signal loss due to fringe washout. Thus an
15 angiography method that does not depend on phase stability may be the best choice to fully exploit the advantages of swept-source OCT. In this context, amplitude-based OCT signal analysis may be advantageous for ophthalmic microvascular imaging.

One difficulty associated with OCT's application in microvascular imaging
20 comes from the prevalent existence of speckle in OCT images obtained from *in vivo* or *in situ* biological samples. Speckle is the result of the coherent summation of light waves with random path lengths and it is often considered as a noise source which degrades the quality of OCT images. Various methods have been developed to reduce speckle in spatial domain, such as angle
25 compounding, spectral compounding, and strain compounding. Speckle adds to "salt-and-pepper-like" noise to OCT images and induces random modulation to interferometric spectra which can significantly reduce contrast.

In spite of being a noise source, speckle also carries information. Speckle pattern forms due to the coherent superposition of random phasors. As a result
30 of speckle, the OCT signal becomes random in an area that is macroscopically uniform. If a sample under imaging is static, the speckle pattern is temporally stationary. However, when photons are backscattered by moving particles,

such as red blood cells in flowing blood, the formed speckle pattern will change rapidly over time. Speckle decorrelation has long been used in ultrasound imaging and in laser speckle technique to detect optical scattering from moving particles such as red blood cells. This phenomenon is also clearly exhibited by
5 real-time OCT reflectance images. The scattering pattern of blood flow varies rapidly over time. This is caused by the fact that the flow stream drives randomly distributed blood cells through the imaging volume (voxel), resulting in decorrelation of the received backscattered signals that are a function of scatterer displacement over time. The contrast between the decorrelation of
10 blood flow and static tissue may be used to extract flow signals for angiography.

The speckle phenomenon has been used in speckle variance OCT for the visualization of microvasculature. Speckle patterns at areas with flowing blood have a large temporal variation, which can be quantified by inter-frame speckle variance. This technique termed "speckle variance" has been used with
15 swept-source OCT demonstrating a significant improvement in capillary detection in tumors by calculation of the variance of the OCT signal intensity. A key advantage of the speckle variance method is that it does not suffer from phase noise artifacts and does not require complex phase correction methods. Correlation mapping is another amplitude-based method that has also recently
20 demonstrated swept-source OCT mapping of animal cerebral and human cutaneous microcirculation *in vivo*. These amplitude-based angiography methods are well suited to swept-source OCT and offer valuable alternatives to the phase-based methods. However, such methods still suffer from bulk-motion noise in the axial dimension where OCT resolution is very high. Therefore, an
25 amplitude-based swept-source angiography method that is able to reduce bulk-motion noise without significant sacrifice in the flow signal would be optimal. For example, imaging of retinal and choroidal flow could be particularly improved with such noise reduction, as in the ocular fundus the flow signal is predominantly in the transverse rather than axial dimension.

30

SUMMARY

Disclosed herein are methods, apparatuses, and systems for amplitude-based OCT angiography that utilize the splitting of the OCT spectrum to reduce the predominant bulk-motion noise in the axial dimension where OCT resolution is very high. For example, such methods, apparatuses and systems can be called "split-spectrum amplitude-decorrelation angiography" (SSADA).

A novel OCT angiography technique based on the decorrelation of OCT signal amplitude due to flow is described herein. By splitting the full OCT spectral interferograms into several wavenumber bands, the OCT resolution cell in each band is made isotropic and less susceptible to axial motion noise. Recombining the decorrelation images from the wavenumber bands yields angiograms that use the full information in the entire OCT spectral range. The isotropic resolution cell resulting from of the SSADA can be used to quantify flow with equal sensitivity to axial and transverse flow. SSADA can improve signal to noise ratio (SNR) of flow detection and vascular connectivity compared to existing amplitude-based swept-source angiography methods. Utilizing SSADA for non-invasive angiography of the ocular circulatory beds (e.g., peri- and parafoveal retinal microcirculatory networks) can be useful in the diagnosis and management of important blinding diseases such as glaucoma, diabetic retinopathy and age-related macular degeneration. SSADA can also be useful outside the eye, for example in the investigation of cerebral circulation and tumor angiogenesis.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present invention will be readily understood by the following detailed description in conjunction with the accompanying drawings. Embodiments of the invention are illustrated by way of example and not by way of limitation in the figures of the accompanying drawings.

Fig. 1 is a chart comparing prior art techniques and the present invention with regard to vascular connectivity and decorrelation signal/noise (DSNR).

Fig 2 schematically illustrates modification of an OCT imaging resolution cell to create an isotropic resolution cell utilizing a band-pass filter and the present invention.

Fig. 3 schematically illustrates M-B-scan mode for acquiring OCT spectrum.

Fig. 4 is a flowchart showing an exemplary method for creating a decorrelation (flow) image that uses split-spectrum techniques and the full
5 information in the entire OCT spectral range.

Fig. 5 is a flowchart showing additional exemplary methods of the exemplary method of Fig. 4.

Fig. 6 schematically illustrates a 2D spectral interferogram split into different frequency bands as described in the present invention.

10 Fig. 7 schematically illustrates the methods of Fig. 4 and Fig. 5 for creating a decorrelation (flow) image that uses split-spectrum techniques and the full information in the entire OCT spectral range.

Fig. 8 is a flowchart showing an exemplary method for eliminating decorrelation images with excessive motion noise.

15 Fig. 9 schematically illustrates an *in vivo* imaging system for collecting image information.

Fig. 10 illustrates an embodiment of an *in vivo* imaging system in accordance with various embodiments of the present invention.

20 Fig. 11 illustrates an embodiment of an article of manufacture for *in vivo* imaging in accordance with various embodiments of the present invention.

Fig. 12 illustrates *in vivo* 3-D volumetric structure images of the optic nerve head using imaging methods in accordance with various embodiments of the present invention.

25 Fig. 13 illustrates *in vivo* 3-D volumetric structure images of the macula using methods in accordance with various embodiments of the present invention.

Fig. 14 illustrates *in vivo* images of macular retinal circulation using methods in accordance with prior art methods and in accordance with various embodiments of the present invention.

30 Fig. 15 illustrates *in vivo* images depicting vascular connectivity and signal to noise ration (SNR) using methods in accordance with prior art methods and in accordance with various embodiment of the present invention.

DETAILED DESCRIPTION

In the following detailed description, reference is made to the accompanying drawings which form a part hereof and in which is shown by way of illustration embodiments in which the invention may be practiced. It is to be understood that other embodiments may be utilized and structural or logical changes may be made without departing from the scope of the present invention. Therefore, the following detailed description is not to be taken in a limiting sense, and the scope of embodiments in accordance with the present invention is defined by the appended claims and their equivalents.

Various operations may be described as multiple discrete operations in turn, in a manner that may be helpful in understanding embodiments of the present invention; however, the order of description should not be construed to imply that these operations are order dependent.

The description may use perspective-based descriptions such as up/down, back/front, and top/bottom. Such descriptions are merely used to facilitate the discussion and are not intended to restrict the application of embodiments of the present invention.

The description may use the phrases "in an embodiment," or "in embodiments," which may each refer to one or more of the same or different embodiments. Furthermore, the terms "comprising," "including," "having," and the like, as used with respect to embodiments of the present invention, are synonymous.

A phrase in the form of "A/B" means "A or B." A phrase in the form "A and/or B" means "(A), (B), or (A and B)." A phrase in the form "at least one of A, B and C" means "(A), (B), (C), (A and B), (A and C), (B and C) or (A, B and C)." A phrase in the form "(A) B" means "(B) or (A B)," that is, A is optional.

In various embodiments of the present invention, methods, apparatuses, and systems for biomedical imaging are provided. In exemplary embodiments of the present invention, a computing system may be endowed with one or more components of the disclosed articles of manufacture and/or systems and may be employed to perform one or more methods as disclosed herein.

5 In various embodiments, structure and/or flow information of a sample may be obtained using optical coherence tomography (OCT) (structure) and OCT angiography (structure and flow) imaging based on the detection of spectral interference. Such imaging may be two-dimensional (2-D) or three-dimensional (3-D), depending on the application. Structural imaging may be of an extended depth range relative to prior art methods, and flow imaging may be performed in real time. One or both of structural imaging and flow imaging as disclosed herein may be enlisted for producing 2-D or 3-D images.

10 Unless otherwise noted or explained, all technical and scientific terms used herein are used according to conventional usage and have the same meaning as commonly understood by one of ordinary skill in the art which the disclosure belongs. Although methods, systems, and apparatuses/materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods, systems, and apparatuses/materials are described below.

15 In case of conflict, the present specification, including explanation of terms, will control. In addition, the methods, systems, apparatuses, materials, and examples are illustrative only and not intended to be limiting.

20 In order to facilitate review of the various embodiments of the disclosure, the following explanation of specific terms is provided:

A-scan: A reflectivity profile that contains information about spatial dimensions and location of structures with an item of interest (e.g., an axial depth scan).

25 **Autocorrelation:** A cross-correlation of a signal with itself; the similarity between observations as a function of the time separation between them. For example, autocorrelation can be used to find repeating patterns, such as the presence of a periodic signal which has been buried under noise, or used to identify the missing fundamental frequency in a signal implied by its harmonic frequencies.

B-scan: A cross-sectional tomograph that may be achieved by laterally combining a series of axial depth scans (e.g., A-scans).

Cross-correlation: A measure of similarity of two waveforms as a function of a time-lag applied to one of the waveforms.

5 **Decorrelation:** A process that is used to reduce autocorrelation within a signal, or cross-correlation within a set of signals, while preserving other aspects of the signal. For example, decorrelation can be used to enhance differences found in each pixel of an image. A measure of a lack of correlation or similarity between corresponding pixels in two images can also describe
10 decorrelation. The end result of a decorrelation process is that faint information within a signal may be enhanced to bring out (e.g., present) subtle differences that may be meaningful. For example, one can calculate decorrelation to find a difference between images.

Illustrated in Fig. 1 is a comparison chart 100 of prior art amplitude-based
15 OCT signal analysis methods and the present invention based on vascular connectivity and decorrelation signal/noise (DSNR). Full-spectrum decorrelation method 100, for example, can be utilized as the baseline value for comparison purposes, however, as described previously, it is sensitive to axial bulk motion causing significant noise in the resulting images produced. In pixel averaging
20 method 112 the signal in several adjacent pixels is combined resulting in an improvement of decorrelation signal-to-noise ratio (DSNR). The improved DSNR of pixel averaging method 112 in turn leads to higher quality images of microcirculation (compared to full-spectrum decorrelation method 100), which can be assessed by measuring the vascular of the microvascular network
25 revealed in the OCT angiograms. As described herein, the present invention of split-spectrum decorrelation 122 further improves DSNR (compared to the improvement offered by pixel averaging method 112) by reducing the noise due to axial bulk motion. This can be accomplished by the methods described herein below (e.g., reducing the axial dimension of the effective resolution cell).
30 The improved DSNR of split-spectrum decorrelation method 122 in turn leads to even higher quality images of microcirculation (compared to full-spectrum decorrelation method 100 and pixel averaging method 112), which can be

assessed by measuring the vascular of the microvascular network revealed in the OCT angiograms. Such an improvement, can allow for images and information useful in for diagnostic and management of diseases in the eye, as well as investigations and analysis of circulation, angiogenesis and the other blood flow imaging analysis. Additionally, the split-spectrum decorrelation could be used to obtain angiography images that could be used to replace fluorescein and indocyanine green angiographies, with the additional advantage of being intrinsically 3-dimensional rather than 2-dimensional. Additional uses can include, but not be limited to, imaging of blood flow in other biological tissue and the imaging of flow in any system, living or nonliving.

In more detail, prior art full-spectrum decorrelation 102 achieves decorrelation purely through process the amplitude signal and does not require phase information. To evaluate the flow signals coming from the scattering tissue, an average decorrelation image $\overline{D}(x,z)$ at each position is obtained by averaging N-1 decorrelation image frames computed from N reflectance amplitude images frames from M-B mode scanning. Each decorrelation frame is computed from 2 adjacent amplitude frames: $A_n(x,z)$ and $A_{n+1}(x,z)$. Using the full spectrum decorrelation method 102, the decorrelation image it is given by the following equation

(1)

$$\overline{D}(x,z) = 1 - \frac{1}{N-1} \sum_{n=1}^{N-1} \frac{A_n(x,z)A_{n+1}(x,z)}{\left[\frac{1}{2}A_n(x,z)^2 + \frac{1}{2}A_{n+1}(x,z)^2\right]} \quad (N=0)$$

where x and z are lateral and depth indices of the B-scan images and n denotes the B-scan slice index. In this full spectrum equation, the decorrelation signal-to-noise ratio acquired from full spectrum can only be increased by increasing the number N of B-scans taken at the same position. However, more scans require more imaging time which may not be practical.

In more detail, prior art pixel averaging method 112 can produce decorrelation images given by the following equation

(2)

$$\overline{D}(x,z) = 1 - \frac{1}{N-1} \frac{1}{PQ} \sum_{p=1}^{P-1} \sum_{q=1}^{Q-1} \sum_{n=1}^{N-1} \frac{A_n(x+p,z+q)A_{n+1}(x+p,z+q)}{[\frac{1}{2}A_n(x+p,z+q)^2 + \frac{1}{2}A_{n+1}(x+p,z+q)^2]}$$

(P=1, Q=4, N=8)

where P and Q are the averaging window widths in the X and Z directions, as described in .J. Enfield, E. Jonathan, and M. Leahy, "In vivo
5 imaging of the microcirculation of the volar forearm using correlation mapping optical coherence tomography (cmoct)," Biomed. Opt. Express 2(5), 1184-1193 (2011). To suppress the spurious noise and discontinuities in the vasculature, P by Q window moving average can be implemented over the X - Z 2D map. To fairly compare the prior art pixel averaging method 112 with the split-spectrum
10 decorrelation 122 described herein, a 1 by 4 window can be created, which means pixel-averaging is only applied along the Z direction, the same direction used for splitting the spectrum in split-spectrum decorrelation 122.

In more detail, split-spectrum decorrelation 122 can produce decorrelation images given by the following equation,

15 (3)

$$\overline{D}(x,z) = 1 - \frac{1}{N-1} \frac{1}{M} \sum_{n=1}^{M-1} \sum_{m=1}^M \frac{A_n(x,z)A_{n+1}(x,z)}{[\frac{1}{2}A_n(x,z)^2 + \frac{1}{2}A_{n+1}(x,z)^2]}$$

(M = 4, N = 8)

After splitting the spectrum by applying M (for example, M can = 4 as
20 described in an exemplary example below) equally spaced bandpass filters, M individual decorrelation images can be obtained between each pair of B-scans, which can then be averaged along both the lateral (X) and axial (Z) directions to increase DSNR. In split-spectrum decorrelation 122, the average decorrelation image $\overline{D}(x,z)$ can be described as the average of decorrelation images from M
25 spectral bands. By increasing the number M (up to a point), the decorrelation signal-to-noise ratio can be improved without increasing the scan acquisition time.

Whichever decorrelation method is used (full-spectrum 102, pixel-averaging 112, and split-spectrum 122) the resulting average decorrelation image frame $\overline{D}(x, z)$ should be a value between zero and one, indicating weak and strong decorrelation, respectively. By describing the decorrelation methods in such detail above, it is possible to compare the methods to one another based on the resulting decorrelation images obtained as illustrated in chart 100 of Fig. 1. The split-spectrum method 122 suppresses noise from axial bulk motion and, in addition, makes use of information in the full k spectrum resulting in superior decorrelation signal-to-noise ratio for flow detection. Utilizing the split-spectrum method 122, axial bulk motion can be suppressed by the use of spectral (k) bandpass filters that increase the axial dimension of the resolution cell so that it can be equal (or substantially equal) to the transverse dimension of the resolution cell.

Illustrated in Fig. 2 is diagram 200 visually depicting modification of an OCT imaging resolution cell 202 via two distinct and separate techniques (band-pass filtering 204 and split-spectrum 206) to create an isotropic resolution cell 208. Each pixel in a B-scan OCT image is formed from backscattered signals of a 3D volume in space, referred to as a resolution cell (e.g., imaging resolution cell 202 in Fig. 2). The statistical changes in the envelope intensity are related to the motion of scatterers through the OCT resolution cell. For a typical swept-source OCT setup, the axial (Z direction) resolution, determined by the source central wavelength and its spectral bandwidth, is much higher than the lateral resolution determined by the laser beam profile in both X and Y directions. For example, in common swept source OCT systems, using the full-width-half-maximum (FWHM) amplitude profile definition, the axial resolution ($\sim 5 \mu\text{m}$) is four times higher than the lateral resolution ($\sim 18 \mu\text{m}$) if both are defined as full-width-half-maximum amplitude profiles (e.g., imaging resolution cell 202 depicts $x=y=4z$). This anisotropic resolution cell, with higher axial than transverse resolution, will result in higher decorrelation sensitivity for axial motion. In the fundus, ocular pulsation related to heart beat, driven by the retrobulbar orbital tissue, mainly occurs along the axial direction. The anisotropic resolution cell of retinal OCT imaging is very sensitive to this axial motion noise. On the other

hand, retinal and choroidal blood flow vectors are primarily transverse to the OCT beam, along the wider (less sensitive) dimensions of the OCT resolution cell. Therefore, to improve the signal-to-noise ratio (SNR) of flow detection, it is desirable to lower the axial resolution and dampen the axial decorrelation sensitivity. This reduces the axial motion noise without sacrificing the transverse flow signal.

One straightforward way to achieve this resolution modification is band-pass filtering of the spectral interferogram (e.g., band-pass filtering 204). Unfortunately, this also sacrifices most of the speckle information in the spectral interferogram and decreases the flow signal. Thus, this is not an effective way to increase the SNR of flow (decorrelation) detection. A better way to decrease axial resolution without losing any speckle information is to split the spectrum into different frequency bands (e.g., split-spectrum 206) and calculate decorrelation in each band separately. The decorrelation (flow) images from the multiple spectral bands can then be averaged together to make full use of the speckle information in the entire OCT spectrum. The details of the split-spectrum procedure are explained herein and below (e.g., split-spectrum decorrelation 122 of Fig. 1 can be utilized).

Illustrated in Fig. 3 is a visual 300 of one 3D volumetric data 302 comprising data obtained via an exemplary embodiment M-B-scan mode from an OCT system. N consecutive B-scans at a single Y position comprise M-B-scan 306 (e.g., in some exemplary embodiments described herein, N= eight (8), but is not limited to any specific N). Therefore, for each 3D volumetric data 302, in the fast scan (x) axis, a single B-scan comprises a plurality of A-scans 304, and in the slow scan (y) axis, there are a number of M-B-scans 306 comprising N consecutive B-scans.

Fig. 4 shows an exemplary method 400 for creating a decorrelation (flow) image that uses split-spectrum techniques and the full information in the entire OCT spectral range. The method 400 can be performed, for example, by *in vivo* imaging systems described herein below. Portions of method 400 and any of the other methods (or portion of methods) described herein can be performed

by computer-executable instructions stored on computer-readable media and articles of manufacture for *in vivo* imaging.

At 402, M-B scans of OCT spectrum are received. For example, M-B scans as depicted in visual 300 of Fig. 3 can be received from an OCT *in vivo* imaging system.

At 404, M spectral bands can be created from the M-B scans of OCT spectrum 402. For example, split spectrum 206 of Fig. 2 can be utilized to create the M spectral bands.

At 406, averaged decorrelation images for each spectral band of the M spectral bands can be created. For example, split spectrum decorrelation 122 described in Fig. 1 can be utilized to create decorrelation images for the M spectral bands and then for each spectral band those decorrelation images can be averaged.

At 408, the averaged decorrelation images for each spectral band created at 406 can be averaged to create a single final image (e.g., final decorrelation image) 410.

Fig. 5 shows additional exemplary methods 500, including reference to similar methods within method 400 of Fig. 4, for creating a decorrelation (flow) image that uses split-spectrum techniques and the full information in the entire OCT spectral range. The method 500 can be performed, for example, by *in vivo* imaging systems described herein below. Portions of method 500 and any of the other methods (or portion of methods) described herein can be performed by computer-executable instructions stored on computer-readable media and articles of manufacture for *in vivo* imaging.

Fig. 6 schematically illustrates via visual 600 a 2D spectral interferogram split into different frequency bands as described in methods 400 of Fig. 4 and 500 of Fig. 5.

Fig. 7 schematically illustrates via visual 700 the methods 400 of Fig. 4 and 500 of Fig. 5 for creating a decorrelatin (flow) image that uses split-spectrum techniques and the full information in the entire OCT spectral range.

Fig. 8 is a flowchart 800 showing an exemplary method for eliminating decorrelation images with excessive motion noise (e.g., as described in method 500 of Fig. 5).

Continuing with the method 500 of Fig. 5, at 502, M-B scans of OCT spectrum are received. For example, M-B scans of OCT spectrum 402 can be received from an OCT *in vivo* imaging system, as depicted in Fig. 7. In more detail, for example, spectral interference signal recorded by a high speed digitizer in swept-source OCT, after subtracting background and autocorrelation terms, can be received and simply given by the following equation

(4)

$$I(x, k) = \int_{-\infty}^{\infty} R(k) A(x, k, z) \cos(2kz) dz$$

where x is the transverse position of focus beam spot on the sample along the fast scan axis, k is the wavenumber, $I(x, k)$ is the light intensity, $R(k)$ is the amplitude of light reflected from the reference arm, $A(x, k, z)$ is the amplitude of the light backscattered from the sample, and z is the optical delay mismatch between the sample reflections and the reference reflection in the free space equivalent.

At 504, overlapping filters (M) covering the entire spectrum can be created. Additionally, at 506, band pass filtering along k can be conducted. Collectively, creating overlapping filters 504 and band pass filtering 506 can result in creating M spectral bands 507 as depicted in Fig. 7 (e.g., as described in creating M spectral bands 404 in method 400 of Fig. 4). Following along with the example provided above of the spectral interference signal represented by equation (4), the Gaussian shape above the 2D interferogram $I(x, k)$ (e.g., 2 D interferogram 605 of Fig. 6) can be used to express the received interferometric fringe at one position. The bandwidth of this full-spectrum fringe can first be

defined, and then a filter bank created to divide this full-spectrum fringe into different bands (e.g., creating overlapping filters (M) 504 of method 500). The specifications of this filter bank can depend on several factors, including, but not limited to, 1) filter type, 2) bandwidth of each filter, 3) overlap between different

5 bands, and 4) number of bands. In one exemplary embodiment, a Gaussian filter can be introduced whose function was defined by the following equation

(5)

$$G(n) = \exp\left[-\frac{(k-m)^2}{2\sigma^2}\right]$$

10 where n is the spectral element number that varies from 1 to 1400 and is linearly mapped to wavenumber k . The range of sampled k can be 10000 to 9091 cm^{-1} , corresponding to a wavelength range of 1000 to 1100 nm. The bandwidth, referred to as “BW,” (e.g., as depicted in 604 of Fig. 600) of the full spectrum can be 69 nm, which can provide a FWHM axial spatial resolution of 5.3 μm . m

15 is the position of the spectral peak. In an exemplary embodiment, the peaks of the spectral Gaussian filters can be placed at 9784, 9625, 9466, and 9307 cm^{-1} . σ^2 is the variance of the Gaussian filter in terms of the number of spectral elements. In an exemplary embodiment, the FWHM amplitude bandwidth, referred to as “bw,” of the bandpass filters can equal to $2\sqrt{2\ln 2}\sigma$, covering 378

20 spectral elements, corresponding to a wavelength range of 27nm or a wavenumber range of 245 cm^{-1} . The four (4) bandpass filters (e.g., as depicted in 608, 610, 612, and 614 of Fig. 6), described in such an exemplary embodiment, can overlap so that none of the frequency components of the original signal are lost in the processing. Fig. 6 visually displays a 2D spectral

25 interferogram 605 split at 606 (e.g., via 404 of method 400 of Fig. 4) into four new spectra with smaller k bandwidth, with “BW” 604 indicating the bandwidth of a full-spectrum filter and multiple “bw”s 608, 610, 612, and 614 being the bandwidth of multiple Gaussian filters, respectively, and regions of non-zero values in the data block are indicated by the dark shaded patterns 616, 618,

30 620, and 622 (similarly visually depicted, for example in Fig. 7).

At 508, the M spectral bands 507 from each individual frequency band can be passed into conventional Fourier-domain OCT algorithms to Fourier transform along k. Additionally, phase information can be dropped to result in amplitude information for each spectral band 509 (e.g., as depicted in Fig. 7).

- 5 For example, the OCT signals therefore can be directly calculated from the decomposed interferograms $I(x, k)$ by applying Fourier transform upon wavenumber k . The computed OCT signal can be a complex function, $I(x, z)$, which can be written as the following equation

(6)

10
$$I(x, z) = FFT(I(x, k)) = A(x, z) \exp [i\varphi(x, z)]$$

where $\varphi(x, z)$ is the phase of the analytic signal $I(x, z)$. The amplitudes of the OCT signals, $A(x, z)$, can be used while the phase information can be selectively disregarded.

- At 510, a fixed value can be set for removal of high decorrelation generated by background noise. Decorrelation of OCT signal amplitude between B-scans taken at the same nominal position can be caused by several sources: (1) flow, (2) bulk tissue motion or scanner position error, and (3) background noise. To help accentuate true flow measurement in the images created and improve the signal-to-noise ratio for flow detection, removal of high decorrelation generated by background noise is desirable. Background noise is random and therefore has high decorrelation between B-scan frames. Noise predominates in pixels with low OCT signal amplitude and therefore flow cannot be assessed in these pixels with any accuracy. A fixed decorrelation value of zero (0) can be assigned to these pixels with low OCT signal amplitude. For example, this can be achieved by setting the low signal pixels a constant amplitude value. The threshold value, for example, can then be chosen to be two standard deviations above the mean background value measured when the sample beam was blocked.
- 15
20
25

- At 512, decorrelation calculation can be obtained between adjacent amplitude frames. For example, split-spectrum decorrelation 122 as described
- 30

in Fig. 1 can be utilized to produce decorrelation images for each spectral band 513 (e.g., as depicted in Fig. 7 visually).

At 514, decorrelation images for each spectral band 513 having excessive motion noise can be eliminated. To help accentuate true flow measurement in the images created and improve the signal-to-noise ratio for flow detection, removal of decorrelation generated by bulk tissue motion or scanner position is desirable. Saccadic and micro-saccadic eye movements are rapid and cause a high degree of decorrelation between B-scans, as depicted, for example, in flowchart 800 of Fig. 8. Such movements can be seen in visual 802 which displays three frames of a set of seven (7) decorrelation images 804 (D_n) of the region around the optic nerve head (ONH), computed from eight (8) OCT B-scans at the same Y location. Each decorrelation image frame depicted can be calculated from a pair of adjacent B-scan amplitude frames, for example as described using the methods described above. In six (6) of the seven (7) decorrelation frames, flow pixels could be distinguished from non-flow pixels by their higher decorrelation values. However, in frame D4 806, both flow (vessel) and non-flow (bulk tissue) pixels had high decorrelation values possibly due to rapid eye movement (e.g., saccadic). To detect bulk motion, the median decorrelation value in the highly reflective portion of the imaged tissue structures (between the region noted as 808) can be determined. High bulk motion in frame D4 806 can be detected by high median decorrelation value in pixel histogram analysis 810. Histogram analysis can be performed within a high reflectivity band starting at the retinal inner limiting membrane and spanning 30 pixels below (within region 808 of 802. By comparing the median decorrelation value 814 to a preset threshold 812 (e.g., in one exemplary embodiment the threshold was set at 0.15, two standard deviations above the mean median decorrelation value), it can be determined that a frame (e.g. frame D4) is a statistical outlier and should be eliminated. Visual 816 depicts the result after the removal of the outlier frame D4.

At 516, the decorrelation images at each spectral band that remain after images with excessive motion noise have been removed can be averaged to create an average decorrelation image for each spectral band, therefore

resulting in multiple averaged decorrelation images (i.e., one average decorrelation for each spectral band as visualized in Fig. 7).

At 518, the averaged decorrelation images from M spectral bands are averaged to create one final decorrelation image 410 (e.g., as visualized in Fig. 7 and also described in method 400, step 408 of Fig. 4).

Returning back to flowchart 800 of Fig. 8, after removing frame D4 806 as an outlier, the remaining six (6) decorrelation images can be averaged to produce a cleaned decorrelation image 818 which displays high contrast between flow pixels (e.g., bright area in retinal vessels and choroid) and non-flow dark regions. An uncleaned decorrelation image 820 depicts a final decorrelation image had outlier frame D4 806 remained showing less contrast between flow (vessels) and non-flow (static tissue) pixels compared to the cleaned decorrelation image 818, as evident by the lack of completely dark space between retinal vessels in the peripapillary areas circled at 822 and 824.

Utilizing method 500, a 3D dataset comprising a stack of two hundred (200) corrected average decorrelation cross-sectional images, along with the associated average reflectance images, that together spans 3 mm in the slow transverse scan (Y) direction can be obtained. In some embodiments it may be desirable to separate the 3D data into retinal and choroidal regions with the dividing boundary set at the retina pigment epithelium (RPE). The depth (Z position) of the highly reflective RPE can be identified through the analysis of the reflectance and reflectance gradient profiles in depth. The region above the RPE is the retinal layer and the region below is the choroidal layer. The *en face* X-Y projection angiograms can then be produced by selecting the maximum decorrelation value along the axial (Z) direction in each layer. In ONH scans, the RPE depth just outside the disc boundary can be used to set an interpolated RPE plane inside the disc.

Fig. 9 schematically illustrates an *in vivo* imaging system 900 for collecting OCT image information. For example, a high-speed swept-source OCT system 900 (e.g., as described in B. Potsaid, B. Baumann, D. Huang, S. Barry, A. E. Cable, J. S. Schuman, J. S. Duker, and J. G. Fujimoto, "Ultrahigh speed 1050nm swept source / fourier domain oct retinal and anterior segment

imaging at 100,000 to 400,000 axial scans per second," Opt. Express 18(19), 20029-20048 (2010)) can used to demonstrate the methods described above for imaging of microcirculation in the human ocular fundus. High speed swept-source OCT system 900 comprises a tunable laser 901. For example, tunable
5 laser 901 (e.g., a tunable laser from Axsun Technologies, Inc, Billerica, MA, USA) may have a wavelength of 1050 nm with 100 nm tuning range, a tuning cycle with a repetition rate of 100 kHz and a duty cycle of 50%. Such OCT system 900 can produce a measured axial resolution of 5.3 μm (full-width-half-maximum amplitude profile) and an imaging range of 2.9 mm in tissue. Light
10 from swept source 901 can be coupled into a two by two fiber coupler 902 through single mode optical fiber. One portion of the light (e.g., 70%) can proceed to the sample arm (i.e., the patient interface), and the other portion of the light (e.g., 30%) can proceed to the reference arm.

In the sample arm, a sample arm polarization control unit 903 can be
15 used to adjust light polarization state. The exit light from the fiber coupler 902 can then be couple with a retinal scanner whereby the light is collimated by sample arm collimating lens 904 and reflected by mirror 905 and two dimensional galvo scanner 909 (e.g., an XY galvonanometer scanner). Two lenses, first lense 906 (e.g., an objective lense) and second lense 907 (e.g., an
20 ocular lense) can relay probe beam reflected by galvo scanner 909 into a human eye 908. For example, a focused spot diameter of 18 μm (full-width-half-maximum amplitude profile) can be calculated on the retinal plane based on an eye model. The average light power (i.e., output power of the laser) onto human eye can be 1.9 mW, which is consistent with safe ocular exposure limit set by
25 the American National Standard Institute (ANSI).

The reference arm can comprise a first reference arm collimating lens 913, a water cell 912, a retro-reflector 911, a glass plate 914 and a second reference arm collimating lens 915. Glass plate 914 can be used to balance the dispersion between the OCT sample arm and reference arm. Water cell 912 can
30 be used to compensate the influence of dispersion in the human eye 908. Retro-reflector 911 can be mounted on a translation stage 910 which can be moved to adjust the path length in the reference arm.

Light from sample and reference arm can interfere at beam splitter 917. A reference arm polarization control unit 916 can be used to adjust the beam polarization state in the reference arm to maximum interference signal. The optical interference signal from beam splitter 917 (e.g., a 50/50 coupler) can be
5 detected by a balanced detector 918 (e.g., a balanced receiver manufactured by Thorlabs, Inc, Newton, NJ, USA), sampled by an analog digital conversion unit 919 (e.g., a high speed digitizer manufactured by Innovative Integration, Inc.) and transferred into computer 920 for processing. For example, computer 920 can be used for storing instruction for and implementing the methods described
10 herein. Interference fringes, for example, can be recorded by analog digital conversion unit 919 at 400 MHz with 14-bit resolution, with the acquisition driven by the optical clock output of tunable laser 901. In such an exemplary setup, imaging system 900, sensitivity can be measured with a mirror and neutral density filter at 95 dB, with a sensitivity roll-off of 4.2 dB/mm.

15 While a swept-source OCT system has been described above, the technology disclosed herein can be applied to any Fourier-domain OCT system. In Fourier-domain OCT systems the reference mirror is kept stationary and the interference between the sample and reference reflections are captured as spectral interferograms, which are processed by Fourier-transform to obtain
20 cross-sectional images. In the spectral OCT implementation of Fourier-domain OCT, a broad band light source is used and the spectral interferogram is captured by a grating or prism-based spectrometer. The spectrometer uses a line camera to detect the spectral interferogram in a simultaneous manner. In the swept-source OCT implementation of Fourier-domain OCT, the light source
25 is a laser that is very rapidly and repetitively tuned across a wide spectrum and the spectral interferogram is captured sequentially. Swept-source OCT can achieve higher speed and the beam can be scanned transversely more rapidly (with less spot overlap between axial scans) without suffering as much signal loss due to fringe washout compared to other Fourier-domain OCT systems.
30 However, a very high speed spectral OCT system could also be utilized with the technology disclosed herein.

Any one or more of various embodiments as previously discussed may be incorporated, in part or in whole, into a system. Fig. 10 illustrates an exemplary embodiment of an *in vivo* imaging system (e.g. an OCT system) 1000 in accordance with various embodiments of the present invention. In the
5 embodiments, OCT system 1000 may comprise an OCT apparatus 1002 and one or more processors 1012 coupled thereto. One or more of the processors 1012 may be adapted to perform methods in accordance with various methods as disclosed herein. In various embodiments, OCT system 1000 may comprise a computing apparatus including, for example, a personal computer in any form,
10 and in various ones of these embodiments, one or more of the processors may be disposed in the computing apparatus. OCT systems in accordance with various embodiments may be adapted to store various information. For instance, an OCT system may be adapted to store parameters and/or instructions for performing one or more methods as disclosed herein.

15 In various embodiments, an OCT system may be adapted to allow an operator to perform various tasks. For example, an OCT system may be adapted to allow an operator to configure and/or launch various ones of the above-described methods. In some embodiments, an OCT system may be adapted to generate, or cause to be generated, reports of various information
20 including, for example, reports of the results of scans run on a sample.

 In embodiments of OCT systems comprising a display device, data and/or other information may be displayed for an operator. In embodiments, a display device may be adapted to receive an input (e.g., by a touch screen, actuation of an icon, manipulation of an input device such as a joystick or knob,
25 etc.) and the input may, in some cases, be communicated (actively and/or passively) to one or more processors. In various embodiments, data and/or information may be displayed, and an operator may input information in response thereto.

 Any one or more of various embodiments as previously discussed may
30 be incorporated, in part or in whole, into an article of manufacture. In various embodiments and as shown in Fig. 11, an article of manufacture 1100 in accordance with various embodiments of the present invention may comprise a

storage medium 1112 and a plurality of programming instructions 1102 stored in storage medium 1112. In various ones of these embodiments, programming instructions 1102 may be adapted to program an apparatus to enable the apparatus to perform one or more of the previously-discussed methods.

5 In various embodiments, an OCT image may provide data from which a diagnosis and/or evaluation may be made. In embodiments, such determinations may relate to biologic tissue structure, vasculature, and/or microcirculation. For example, in some embodiments, 3-D *in vivo* imaging of a biologic tissue and quantifying flow of blood through individual vessels therein
10 may be useful in understanding mechanisms behind a number of disease developments and treatments including, for example, ischemia, degeneration, trauma, seizures, and various other neurological diseases. In still other embodiments, an OCT image and techniques herein disclosed may be used to identify cancer, tumors, dementia, and ophthalmologic diseases/conditions
15 (including, e.g., glaucoma, diabetic retinopathy, age-related macular degeneration). Still further, in various embodiments, OCT techniques as herein disclosed may be used for endoscopic imaging or other internal medicine applications. The foregoing illustrative embodiments of diagnosis and/or evaluation are exemplary and thus embodiments of the present invention are
20 not limited to the embodiments discussed.

 Although certain embodiments have been illustrated and described herein for purposes of description of the preferred embodiment, it will be appreciated by those of ordinary skill in the art that a wide variety of alternate and/or equivalent embodiments or implementations calculated to achieve the
25 same purposes may be substituted for the embodiments shown and described without departing from the scope of the present invention. Those with skill in the art will readily appreciate that embodiments in accordance with the present invention may be implemented in a very wide variety of ways. This application is intended to cover any adaptations or variations of the embodiments discussed
30 herein. Therefore, it is manifestly intended that embodiments in accordance with the present invention be limited only by the claims and the equivalents thereof.

EXEMPLARY EMBODIMENTS

Macular and ONH imaging were performed on three normal volunteers using a swept-source OCT system 900 described herein, as approved by an Institutional Review Board (IRB). For each imaging, the subject's head was stabilized by chin and forehead rests. A flashing internal fixation target was projected by an attenuated pico projector using digital light processing (DLP) technology (Texas Instruments, Dallas, TX, USA). The imaging area on the fundus was visualized by the operator using real-time *en face* view of a 3 mm×3 mm OCT preview scan

The swept-source OCT system was operated at 100-kHz axial scan repetition rate. In the fast transverse scan (X) direction, the B-scan consisted of 200 A-scans over 3 mm. In the slow transverse scan (Y) direction, there were 200 discrete sampling planes over 3 mm. Eight consecutive B-scans were acquired at each Y position. This is referred to as the "M-B-scan mode" (e.g., as illustrated in Fig. 3) because it enables detection of motion between consecutive B-scans at the same position. Thus, it took 3.2 sec to obtain a 3D volumetric data cube comprised of 1600 B-scans and 32,000 A-scans. Under this scanning protocol, methods described herein were applied to the repeated frame sequences at each step. Finally, the 200 calculated B-scan frames were combined to form 3D blood perfusion images of posterior part of the human eye.

Fig. 12 illustrates *in vivo* 3-D volumetric structure images (3.0 (x) X 3.0 (y) X 2.9 (z) mm) of the optic nerve head (ONH) in the right eye of a myopic individual using imaging methods in accordance with various embodiments of the present invention. From one 3D volumetric dataset, both reflectance intensity images and decorrelation (angiography) images were obtained. For the optical nerve head (ONH) scan, the *en face* maximum projection of reflectance intensity 1202 showed the major retinal blood vessels and the second order branches 1204, but finer branches and the microcirculation of the retina, choroid, and optic disc were not visible. In the vertical cross-sectional intensity image 1208 taken from plane 1206 of projection 1202, the connective tissue struts (bright) and pores (dark) of the lamina cribosa could be visualized deep within the optic disc. Around the disc, the retina, choroid, and sclera can be

delineated. The ONH angiogram obtained by the methods described herein showed both many orders of vascular branching as well as the microcirculatory network. The *en face* maximum decorrelation projection angiogram 1210 showed many orders of branching from the central retinal artery and vein, a
5 dense capillary network in the disc, a cilioretinal artery (reference by an arrow in angiogram 1210 at the nasal disc margin), and a near continuous sheet of choroidal vessels around the disc, much of which could not be visualized well on the *en face* intensity image 1202. The vertical SSADA cross-section decorrelation image 1212 (in the same plane 1206 as 1208 displayed) created
10 showed blood flow in blood vessels in the disc (represented by arrows), retinal vessels, and choroid that form columns from the surface to a depth of ~1.0 mm. It may be unclear if this represents deep penetrating vessels or if this is represents decorrelation projection artifact. Projection artifact refers to the fact that light reflected from deeper static structures may show decorrelation due to
15 passing through a more superficial blood vessel. This type of artifact is evident where the peripapillary retinal vessels seem thicker than they should be, for example in fly-through movie still frame image 1216 and in decorrelation image 1212. Due to this artifact, these vessels extended down the full depth of the nerve fiber layer (NFL), and the decorrelation signal appeared in the subjacent
20 pigment epithelium (RPE), which should be avascular.

To separately view the retinal vessels and superficial disc vessels, pixels were removed below the level of the peripapillary RPE to remove the choroid. The resulting *en face* angiogram 1214 showed that the superficial vascular network nourishes the disc ends at the disc boundary. By comparison, the
25 choroidal circulation formed an almost continuous sheet of blood flow under the retina as shown in 1210. The *en face* images 1202, 1210, and 1214 show RPE atrophy in a temporal crescent just outside the disc margin. Inside the crescent there was also a small region of choriocapillaris atrophy (see the arrow region within 1210). Overlaying the cross-sectional gray scale reflectance intensity
30 image with the color scale flow (decorrelation) image showed that the major retinal branches vessels were at the level of the peripapillary NFL, as shown in fly-through movie still frame image 1216 (i.e., how the disc, retina, and choroid

are perfused in a 3D volumetric fashion). It also showed the blood flow within the full thickness of the choroid. The combined image 1216 also showed that the deeper disc circulation resides primarily in the pores of the lamina cribosa and not in the connective tissue struts. This may be the first time that the disc
5 microcirculation has been visualized noninvasively in such a comprehensive manner. The horizontal line across the image was a result of a fixed pattern artifact that originated from the swept laser source.

Another exemplary example utilizing the invention disclosed herein was demonstrated in macular angiography. The macular region of the fundus is responsible for central vision. Capillary dropout in the macular region due to
10 diabetic retinopathy is a major cause of vision loss. Focal loss of the choriocapillaris is a possible causative factor in the pathogenesis of both dry and wet age-related macular degeneration, the leading cause of blindness in industrialized nations. Thus macular angiography is important. The technology
15 described herein was used to demonstrate macular angiography of both the retinal and choroidal circulations in a normal eye as shown in the *in vivo* 3-D volumetric structure images (3.0 (x) X 3.0 (y) X 2.9 (z) mm) of the macula in Fig. 13.

The vascular pattern and capillary networks visualized using the
20 technology disclosed herein were similar to those previously reported using phase-based OCT angiography techniques. The flow pixels formed a continuous microcirculatory network in the retina. There was an absence of vascular network in the foveal avascular zone (as shown in *en face* maximum decorrelation projection angiogram 1302) of approximately 600 μ m diameter, in
25 agreement with known anatomy. There were some disconnected apparent flow pixels within the foveal avascular zone due to noise. Horizontal OCT cross section through the foveal center (upper dashed line in 1302) with merged flow information (decorrelation represented in bright/color scale) and structure information (reflectance intensity represented in gray/darker scale) is
30 represented with foveal center image 1304. Inspection of foveal center image 1304 shows these false flow pixels to be decorrelation noise in the high reflectance layers of the RPE and photoreceptors. The choriocapillaris layer

forms a confluent overlapping plexus, so it is to be expected that the projection image of the choroid circulation (see *en face* maximum decorrelation projection angiogram of the choroidal circulation 1306) shows confluent flow. Similar to 1304, a merged horizontal OCT cross section of the inferior macula (lower dashed line in 1302) is represented with inferior macula image 1308. The cross section images 1304 and 1308 showed retinal vessels from the NFL to the outer plexiform layer, in agreement with known anatomy. The flow in the inner choroid had higher velocity as based on decorrelation seen in the bright/color scale. The volume was also greater than the retinal circulation (as shown in the cross section images 1304 and 1308), again consistent with known physiology that the choroidal circulation has much higher flow than the retinal circulation. There were signal voids in the outer choroid which may be due to fringe washout from high flow velocity and the shadowing effect of overlying tissue. The cross section images 1304 and 1308 also showed a few spots of decorrelation in the RPE layer. These are likely artifacts because the RPE is known to be avascular. As mentioned previously, this is likely due to the projection of decorrelation of flow in a proximal layer (i.e., inner retinal layers) onto distal layers with a strong reflected signal (i.e., RPE). There was also a tendency for vessels to form vertical arrays in the inner retina, which may in some instances be due to the projection artifact as well.

Another exemplary example utilizing the invention disclosed herein was demonstrated to appreciate the differences between full-spectrum, pixel-averaging, and split-spectrum techniques (as described in Fig. 1) for decorrelation-based angiography. To obtain angiograms, the methods described above, in particular with description to Fig. 1 and as described by equations (1)-(3), respectively. For fair comparison, identical motion error reduction, noise threshold, and *en face* projection methods were used.

Fig. 14 illustrates *en face* angiograms of macular retinal circulation using methods in accordance with prior art methods full-spectrum (1402) and pixel-averaging (1404) and in accordance with various embodiments of the present invention (1408). While the prior art methods and present invention provided good visualization of major macular vessels, the capillary network looked the

cleanest and most continuous in split-spectrum angiogram 108 generated with the split-spectrum present invention. The pixel-averaging method producing pixel-averaging angiogram 1404 displays the second cleanest and continuous capillary network. The full-spectrum method producing full-spectrum angiogram 1402 showed significantly more disconnected flow pixels that were likely to be noise. The noise can be most easily appreciated in the foveal avascular zone (inside the yellow circles of 1402A, 1402B, and 1408C images of 600-um diameter), which should not have any retinal vessels, including capillaries. In the split-spectrum angiogram 1408, there was a near continuous visualization of the capillary network just outside the avascular zone, while this loop appeared broken up using the other two prior art techniques. The cross-sectional angiograms for each method as displayed in 1402D, 1404E, and 1408F (all scanned across a horizontal dashed line as shown in 1402A) showed that the split-spectrum method provided the cleanest contrast between distinct retinal vessels and dark background. Again, the pixel-averaging method was second best, and the full-spectrum method showed visible snow-like background noise.

To obtain quantitative figures of merit to compare the three decorrelation-based angiography techniques, we made use of two pieces of anatomic knowledge. One is that the retinal vessels form a continuous network, and the other is that there are no retinal vessels within the foveal avascular zone. Fig. 15 illustrates *in vivo* images depicting vascular connectivity and signal to noise ratio (SNR) using methods in accordance with prior art methods and in accordance with various embodiment of the present invention. In Fig. 15, images 1502A1- 1502A4 were obtained using the full-spectrum method (all in row 1502), images 1504B1-1504B4 were obtained using the pixel-averaging method (all in row 1504), and images 1506C1-C4 were obtained using the split-spectrum technology described herein. To assess vessel connectivity, the projection images (1402A, 1404B, and 1408C of Fig. 14) obtained by the three different methods were converted to binary images (e.g., binerized) (as shown in the images in first column 1508 of Fig. 15, images 1502A1, 1504B1, and 1506C1) based on a fixed threshold. Then a skeletonizing morphological operation (e.g., skeletonized) was applied to obtain a vascular network made of

1-pixel wide lines and dots (as shown in the images in second column 1510 of Fig. 15, images 1502A2, 1504B2, and 1506C2). Next the unconnected flow pixels were separated from the connected flow skeleton (e.g., filtered to remove unconnected flow pixels) (as shown in the images in third column 1512 of Fig. 15, images 1502A3, 1504B3, and 1506C3). The vascular connectivity was defined as the ratio of the number of connected flow pixels to the total number of flow pixels on the skeleton map. Connectivity was analyzed on the OCT macular angiograms of six eyes of the three participants (see Table 1 below). A comparison of the three techniques based on paired t -tests showed that the split-spectrum technology had significantly better connectivity relative to the pixel-averaging ($p = 0.037$) and full-spectrum ($p = 0.014$) techniques. The split-spectrum technology disclosed herein reduced the number of unconnected flow pixels (18%) by more than a factor of 2 when compared with the full-spectrum prior art technique (39%).

To compute a signal to noise (SNR) for the decorrelation signal, it was necessary to define relevant signal and noise regions. For the macula, fortuitously, the central foveal avascular zone (FAZ) is devoid of blood vessels, including capillaries. The parafoveal capillary network nourishes the fovea and the loss of these capillaries in diabetic retinopathy is an important mechanism in the loss of vision. Thus the ratio of decorrelation value in the parafoveal region relative to the FAZ can be a clean and clinically relevant way to compute SNR. In the fourth column 1512 of Fig. 15, images 1502A4, 1504B4, and 1506C4, show decorrelation SNR, where the noise region was inside the foveal avascular zone (displayed as inner dotted circles with radius R_1) and the signal region was the parafoveal annulus (as displayed the grayed region between radius R_2 and radius R_3). The radius of the FAZ (R_1) is approximately 0.3 mm. Therefore, it was chosen that the central FAZ with a radius of 0.3 mm was the noise region and the annular parafoveal region between 0.65 (R_2) and 1.00 mm (R_3) radii was the signal region. Therefore, the decorrelation signal-to-noise ratio $DSNR$ can be represent using the following formula,

(7)

$$DSNR = \frac{\bar{I}_{\text{parafovea}} - \bar{I}_{\text{FAZ}}}{\sqrt{\sigma_{\text{FAZ}}^2}}$$

where $\bar{I}_{\text{parafovea}}$ and $\bar{I}_{\text{FAZ}}$ are the average decorrelation values within the parafoveal annulus and FAZ, respectively; and σ_{FAZ}^2 is the variance of decorrelation values within the FAZ. These computations were performed over the *en face* maximum projection images.

The *DSNR* was analyzed on the OCT macular angiograms performed on six eyes of the three participants (see Table 1 below). The paired *t*-test showed that the *DSNR* of the split-spectrum technology was significantly higher than the pixel-averaging technique ($p = 0.034$) and the full-spectrum technique ($p = 0.012$). The split-spectrum technology improved the *DSNR* by more than a factor of 2 compared to the full-spectrum technique.

Table 1. Vascular Connectivity and Signal-to-Noise Ratio of Three Angiography Algorithms

Amplitude decorrelation	Connectivity (mean±sd)	Improvement of connectivity	<i>DSNR</i> (mean±sd)	Improvement of <i>DSNR</i>
full-spectrum	0.61±0.08	N/A	3.30±0.81	N/A
pixel-averaging	0.70±0.06	14.8%	4.57±1.08	38.5%
split-spectrum	0.82±0.07	34.4%	6.78±0.82	105%

DSNR = decorrelation signal-to-noise ratio. Statistical analysis is based on 6 eyes of 3 normal human subjects.

Utilizing the technology disclosed, visualization of both larger blood vessels and the capillary network in the retinal and choroidal circulations has been demonstrated. This visualization can also be achieved using Doppler and other phase-based flow detection techniques, however the SSADA (i.e., the split-spectrum) techniques disclosed have several potential advantages over

phase-based techniques. Insensitivity to phase noise is one advantage. Another advantage includes the ability to quantify microvascular flow. Because the effective resolution cell is made isotropic (having the same size in X, Y, and Z dimensions, as described in Fig. 2), it is equally sensitive to transverse (X, Y) and axial (Z) flow. This contrasts with all phase-based techniques, which are intrinsically more sensitive to flow in the axial direction over which Doppler shift occurs. Thus utilizing the technology disclosed results in the decorrelation value as a function of the flow velocity regardless of direction. The faster blood particles move across the laser beam, the higher the decorrelation index of the received signals within a velocity range set by the scan parameters. In theory the saturation velocity should be approximately the size of the resolution cell (0.018 mm) divided by the interframe time delay (0.002 sec), or 9 mm/sec. The minimum detectable flow velocity can be determined by the decorrelation noise floor, which can be based on the decorrelation distribution statistics of the non-flow tissue voxels. In this example, the projection view of split-spectrum technology showed the vascular pattern within the macular capillary zone (parafoveal region). This describes that the split-spectrum technology disclosed is able to detect retinal capillary flow, which is within the range of 0.5-2 mm/sec. Calibration of velocity to decorrelation values using *in vitro* flow phantom experiments can be done to further determine the minimum detectable flow velocity.

The projection of flow from proximal (shallower) layers to distal (deeper) layers can be challenging. Flow in the major peripapillary retinal arteries and veins (as shown in Fig.12) and larger macular vessels in the inner retina (as shown in Fig.13) projects onto the highly reflective RPE, which should not contain any blood vessels. There were also probable projection of flow from the more superficial inner retinal layers (i.e. nerve fiber layer and ganglion cell layer) to the deeper inner retinal layers (i.e. inner and outer plexiform layers). This does not affect the accuracy of *en face* projection of the retinal circulation, but it could affect the accuracy of cross-sectional angiograms and *en face* projection of the choroidal circulation. One can raise the threshold decorrelation value for flow identification in deeper voxels if a more superficial voxel has a

suprathreshold decorrelation value; however, this can inevitably introduce a potential shadow artifact in place of a flow projection artifact. Thus, images of deeper vessels can be interpreted with this artifact in mind.

Noise from bulk tissue motion, while dramatically reduced using the
5 technology disclosed herein, may not be entirely eliminated. As described in the
examples disclosed, no attempt was made to compensate for X-Z motion
between consecutive B-scan frames by the use of frame-shift registration. This
registration can likely reduce the effect of bulk motion in the X-Z dimensions
(though not in the Y direction) and improve the accuracy of flow detection
10 further. It is also apparent from the *en face* angiograms that there are saccadic
motion artifacts in the 3D dataset. This can likely be reduced by the use of 3D
registration algorithms. .

The disclosure set forth above encompasses multiple distinct
15 embodiments. While each of these embodiments have been disclosed in its
preferred form, the specific embodiments as disclosed and illustrated herein are
not to be considered in a limiting sense as numerous variations are possible.
The subject matter of the present disclosure includes all novel and non-obvious
combinations and subcombinations of the various elements, features, functions
20 and/or properties disclosed herein. Similarly, where any claim recites “a” or “a
first” element or the equivalent thereof, such claim should be understood to
include incorporation of one or more such elements, neither requiring nor
excluding two or more such elements.

What is claimed is:

1. A method of imaging, comprising:
 - scanning, by an optical coherence tomography (OCT) system, a flowing
 - 5 sample to obtain a spectral interference signal;
 - detecting the spectral interference signal to obtain M-B scans of OCT spectrum;
 - splitting, after the detecting, the M-B scans of OCT spectrum into M spectral bands;
 - 10 creating decorrelation images for the M spectral bands; and
 - combining the decorrelation images for the M spectral bands to create a flow image.
2. The method of claim 1, wherein splitting the M-B scans of OCT spectrum
- 15 into M spectral bands comprises:
 - creating overlapping filters covering the OCT spectrum; and
 - filtering the OCT spectrum with the overlapping filters.
3. The method of claim 1, wherein creating decorrelation images for M
- 20 spectral bands comprises:
 - determining amplitude information for each spectral band; and
 - calculating decorrelation between adjacent amplitude frames for each spectral band.
- 25 4. The method of claim 3, further comprising removing background noise.
5. The method of claim 1, wherein combining the decorrelation images for the M spectral bands to create a flow image comprises:
 - averaging the decorrelation images for each spectral band to create an
 - 30 average decorrelation image for each spectral band; and
 - averaging the averaged decorrelation images from the M spectral bands.
6. The method of claim 5, further comprising eliminating decorrelation images for each spectral band having motion noise above a threshold.

7. A system for in vivo imaging, comprising:
an optical coherence tomography (OCT) apparatus; and
one or more processors coupled to the OCT apparatus and adapted to
- 5 cause the apparatus to:
detect a spectral interference signal associated with a flowing sample to
obtain M-B scans of OCT spectrum from the flowing sample;
split the M-B scans of OCT spectrum into M spectral bands;
create decorrelation images for the M spectral bands; and
- 10 combine the decorrelation images for the M spectral bands to create a flow
image.
8. The system of claim 7, wherein the one or more processors adapted to
cause the OCT apparatus to split the M-B scans of OCT spectrum into M spectral
- 15 bands further comprises being adapted to cause the OCT apparatus to:
create overlapping filters covering the OCT spectrum; and
filter the OCT spectrum with the overlapping filters.
9. The system of claim 7, wherein the one or more processors adapted to
- 20 cause the apparatus to create decorrelation images for M spectral bands further
comprises being adapted to cause the apparatus to:
determine amplitude information for each spectral band; and
calculate decorrelation between adjacent amplitude frames for each
spectral band.
- 25
10. The system of claim 7, wherein the one or more processors adapted to
cause the apparatus to combine the decorrelation images for the M spectral
bands to create a flow image further comprises being adapted to cause the OCT
apparatus to:
- 30 average the decorrelation images for each spectral band to create an
average decorrelation image for each spectral band; and
average the averaged decorrelation images from the M spectral bands.

FIG. 1

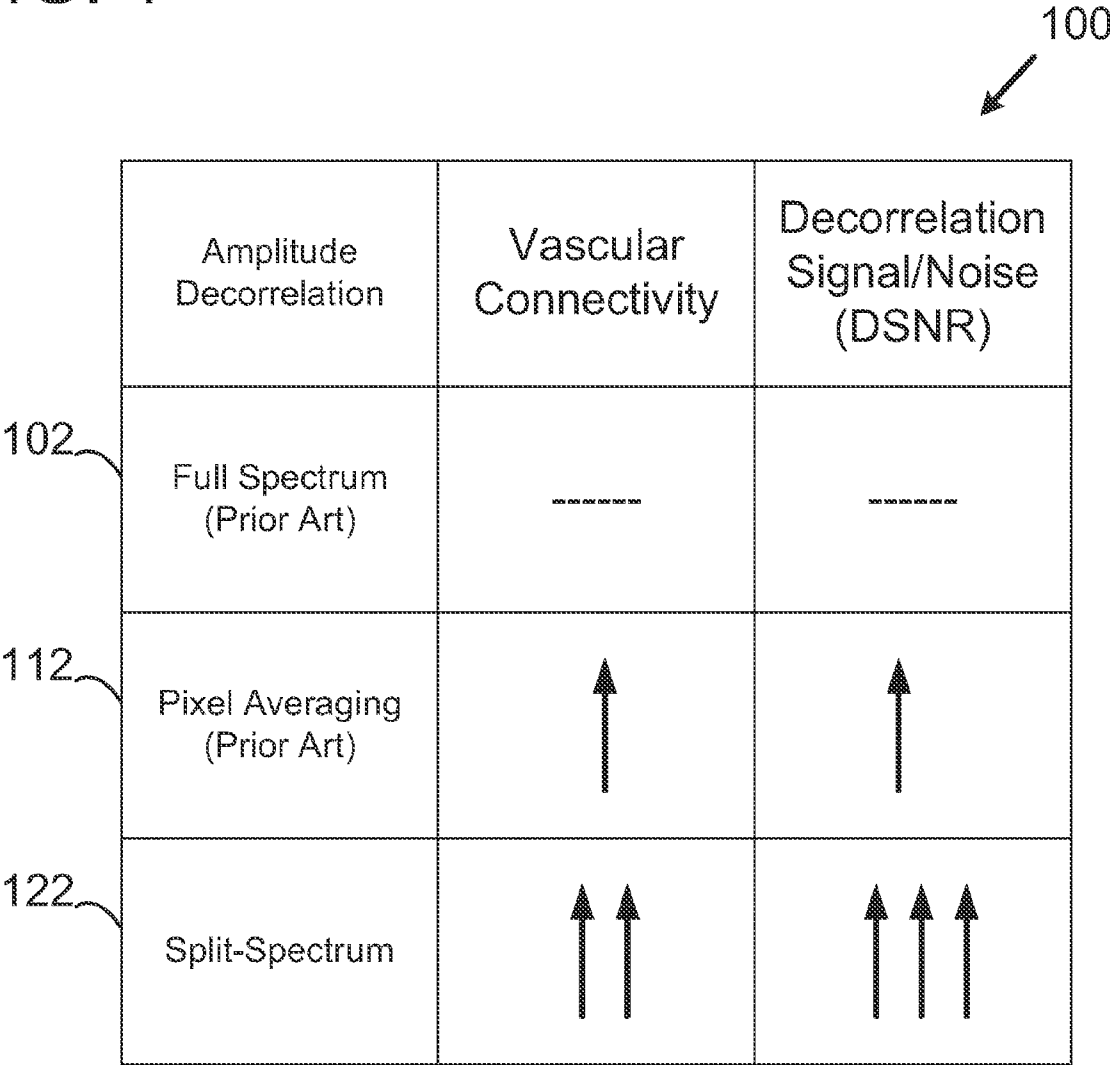


FIG. 2

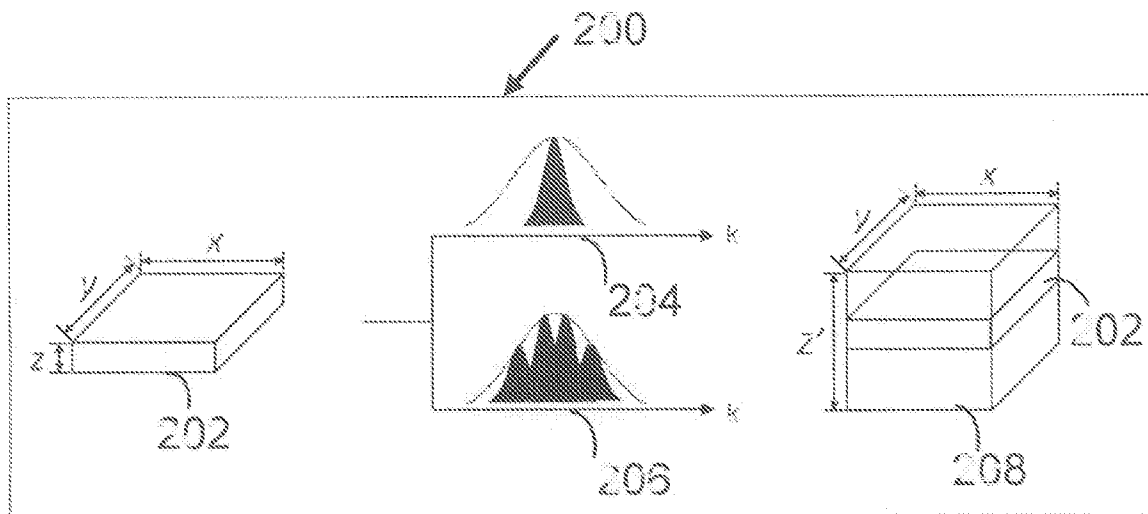


FIG. 3

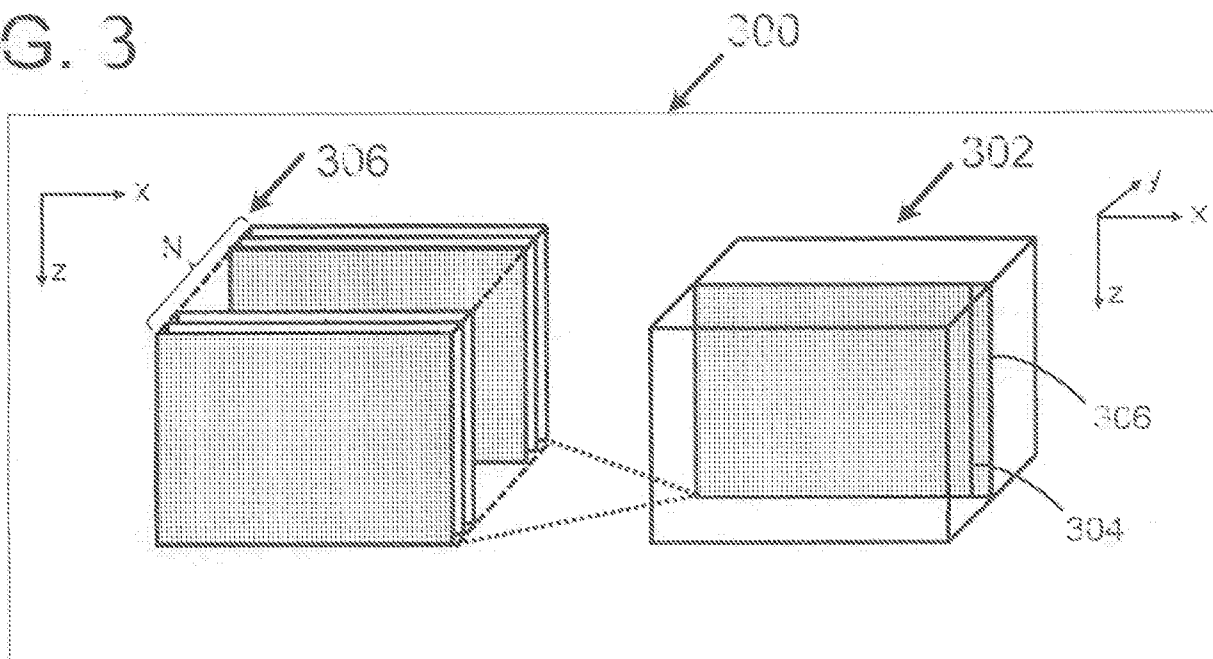


FIG. 4

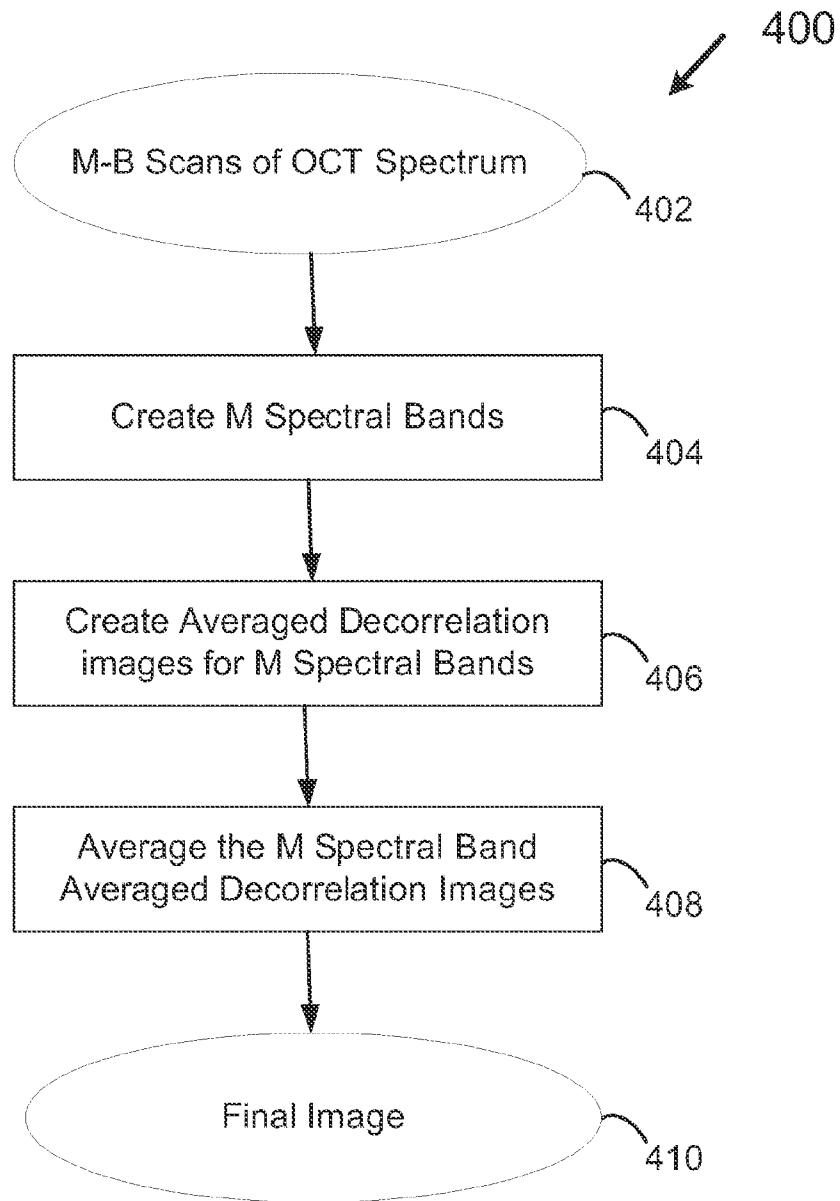


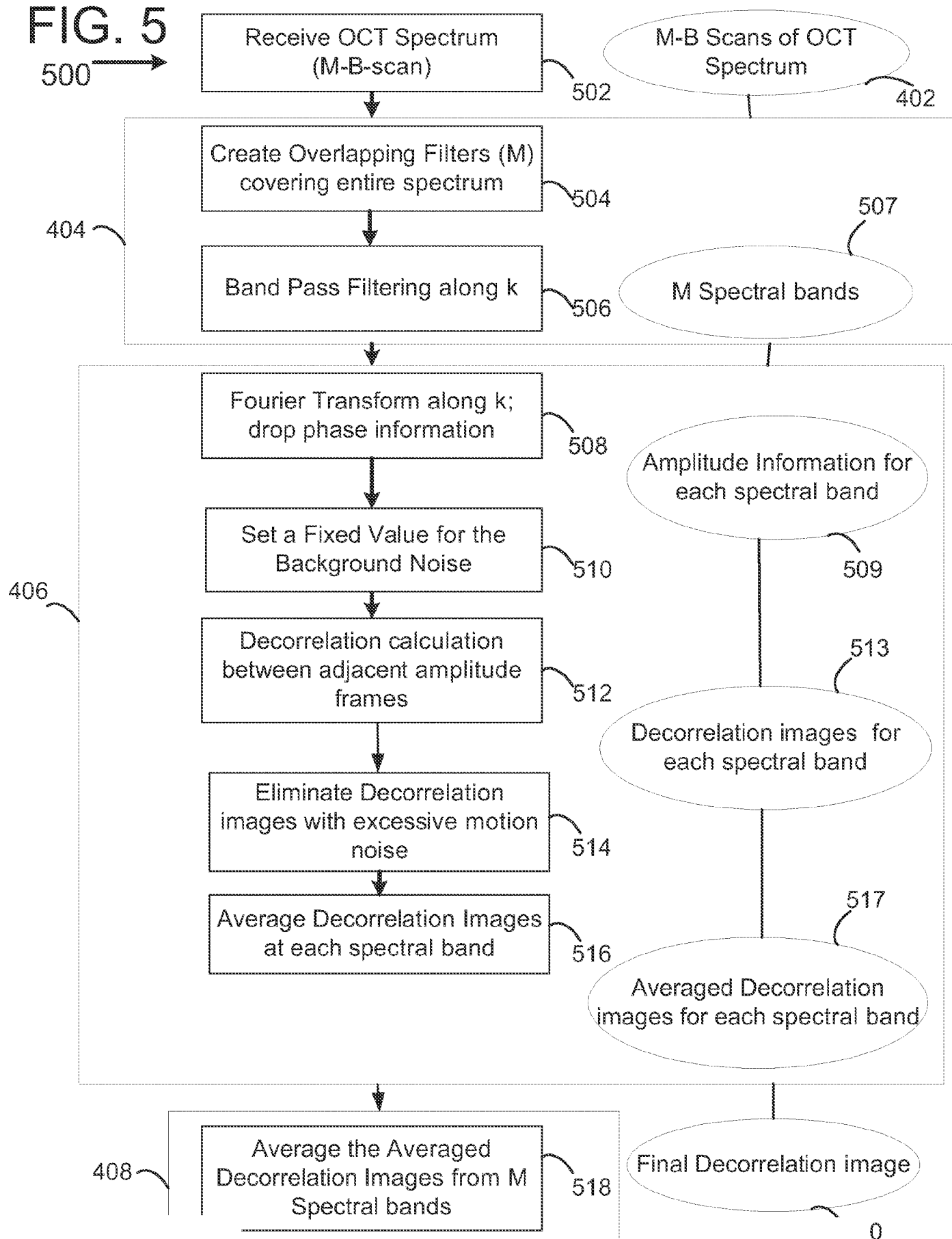
FIG. 5

FIG. 6

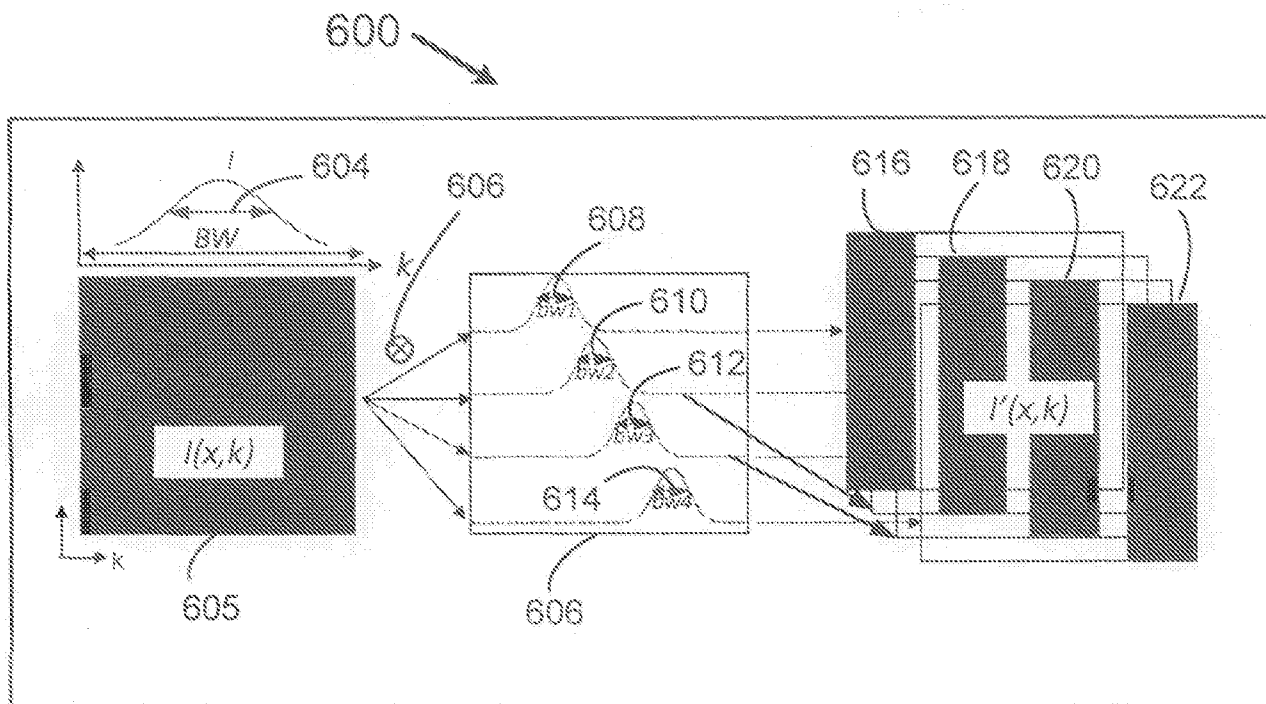


FIG. 7

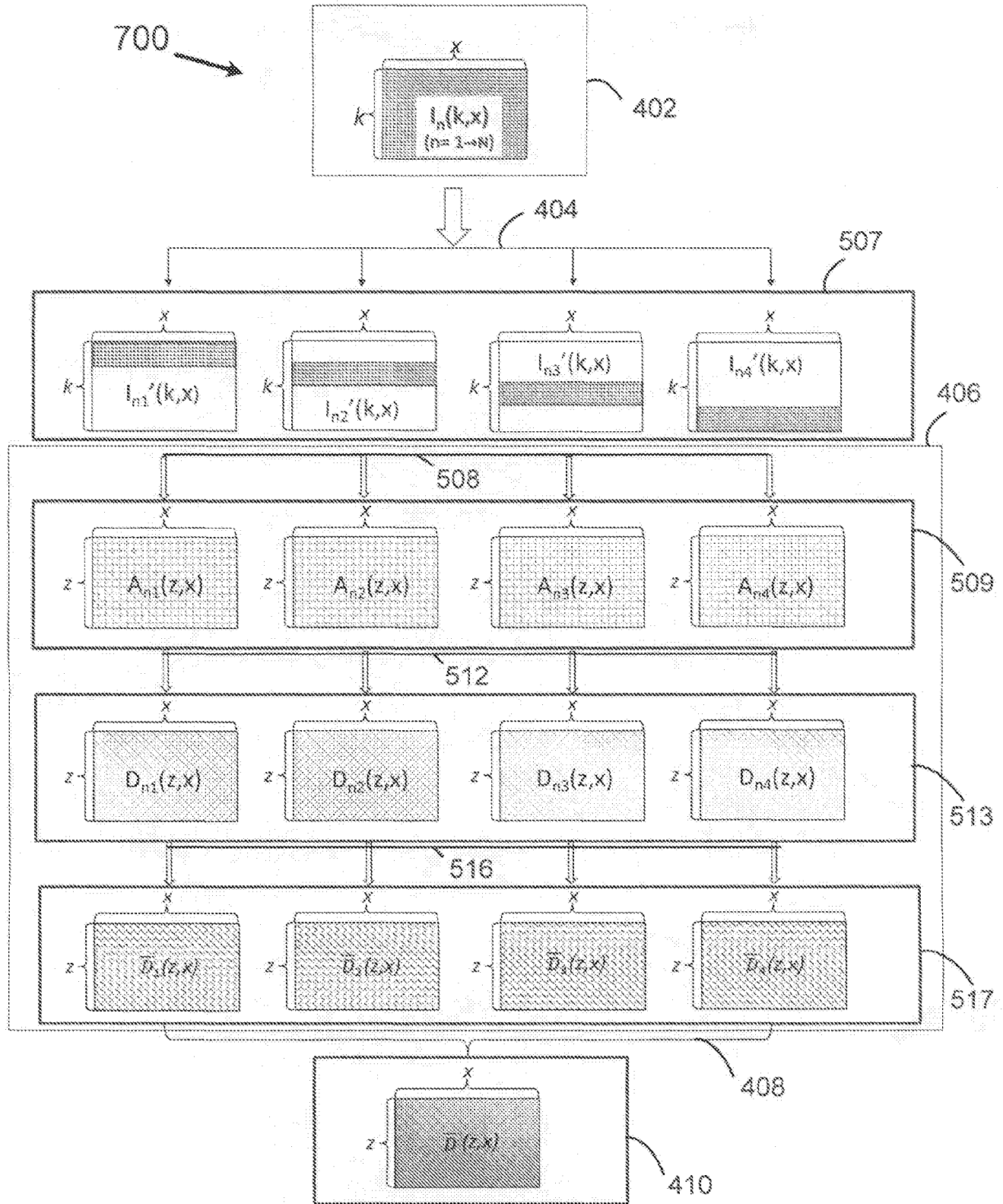


FIG. 8

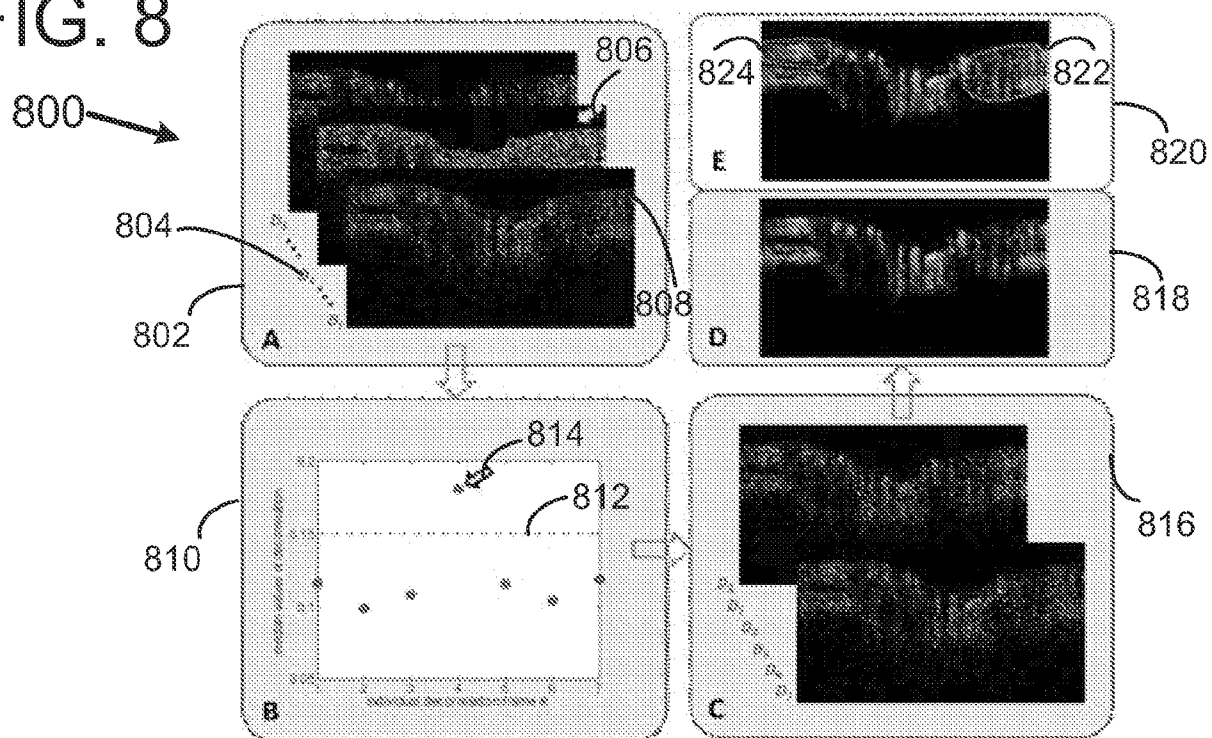


FIG. 9

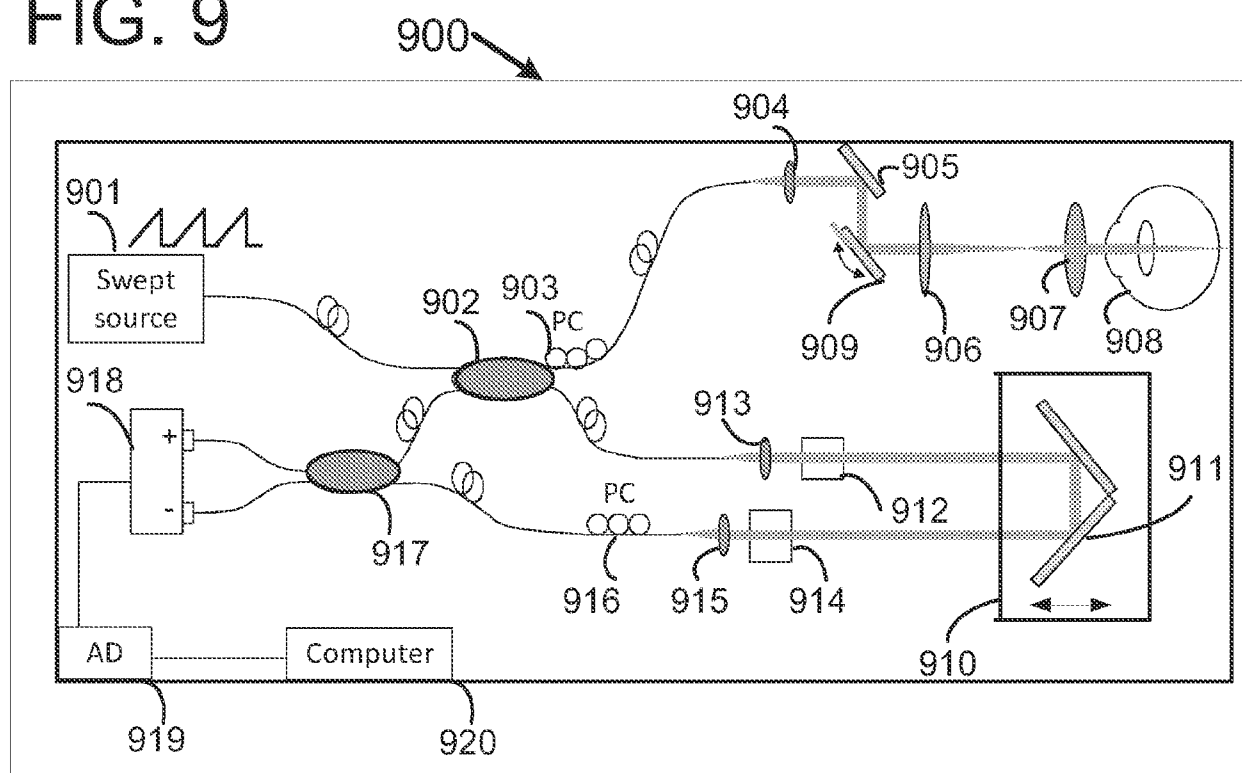


FIG.
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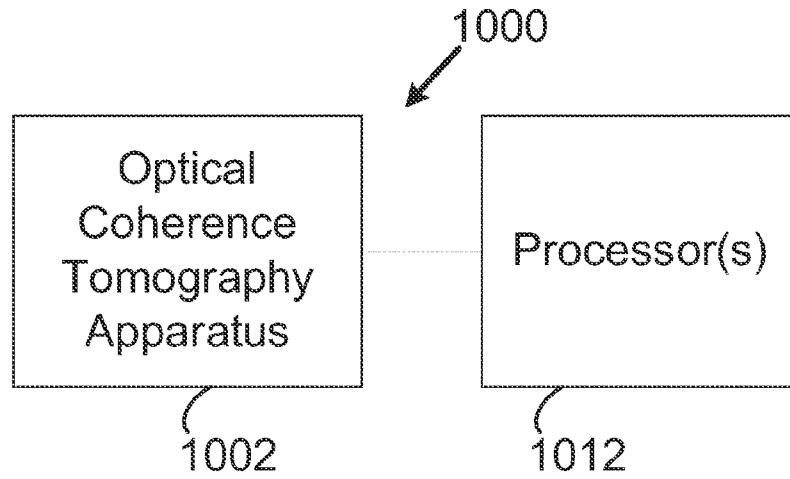


FIG.
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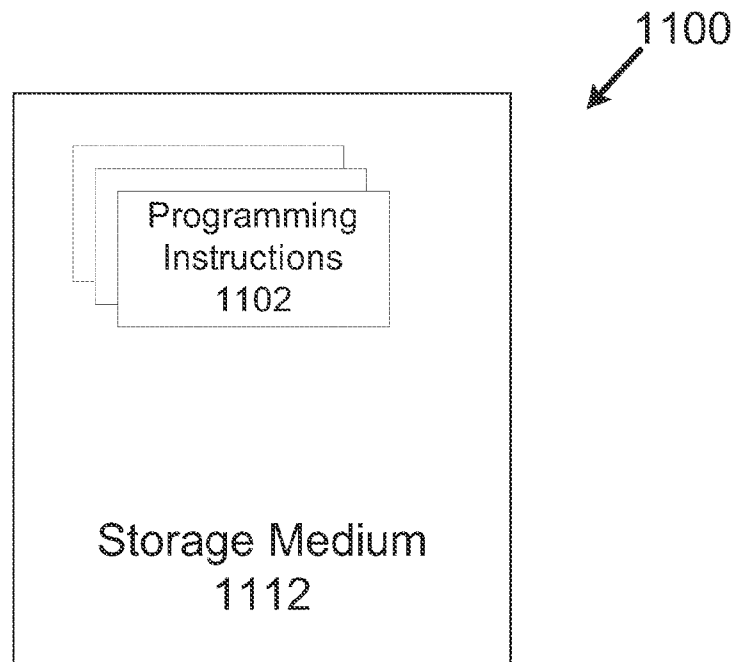


FIG. 12

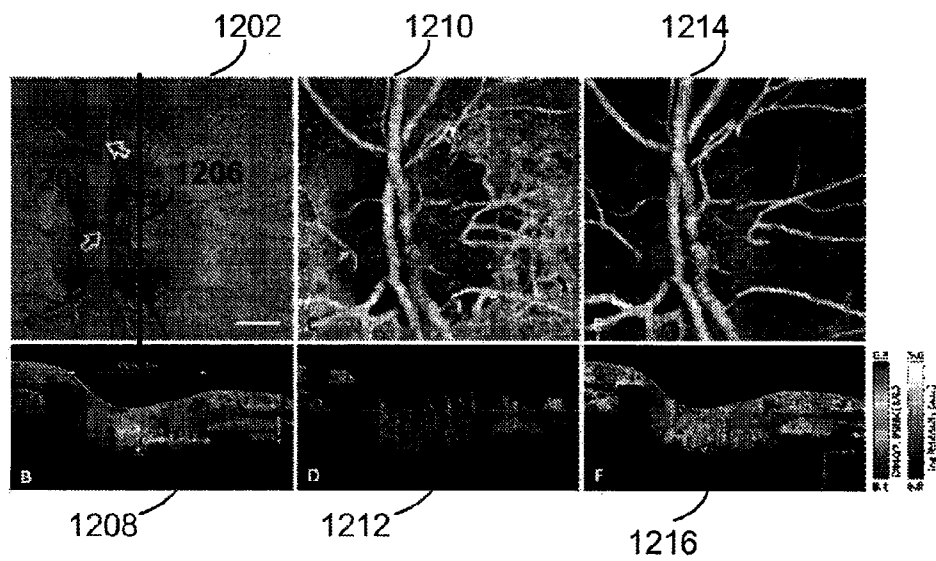


FIG. 13

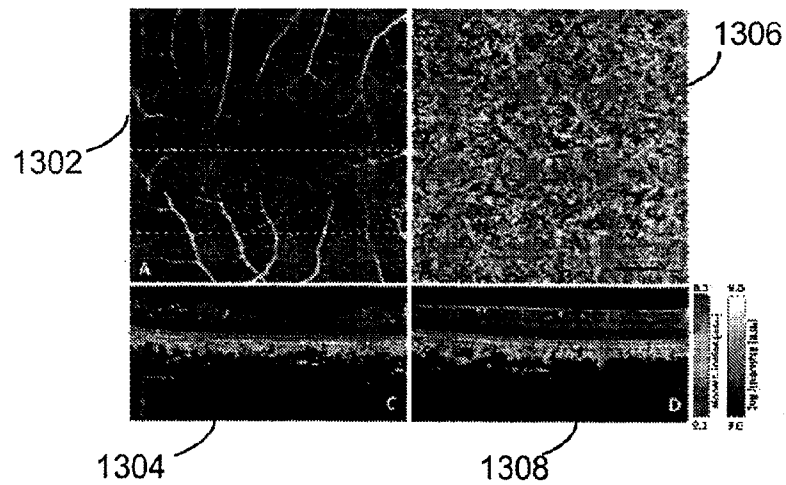


FIG. 14

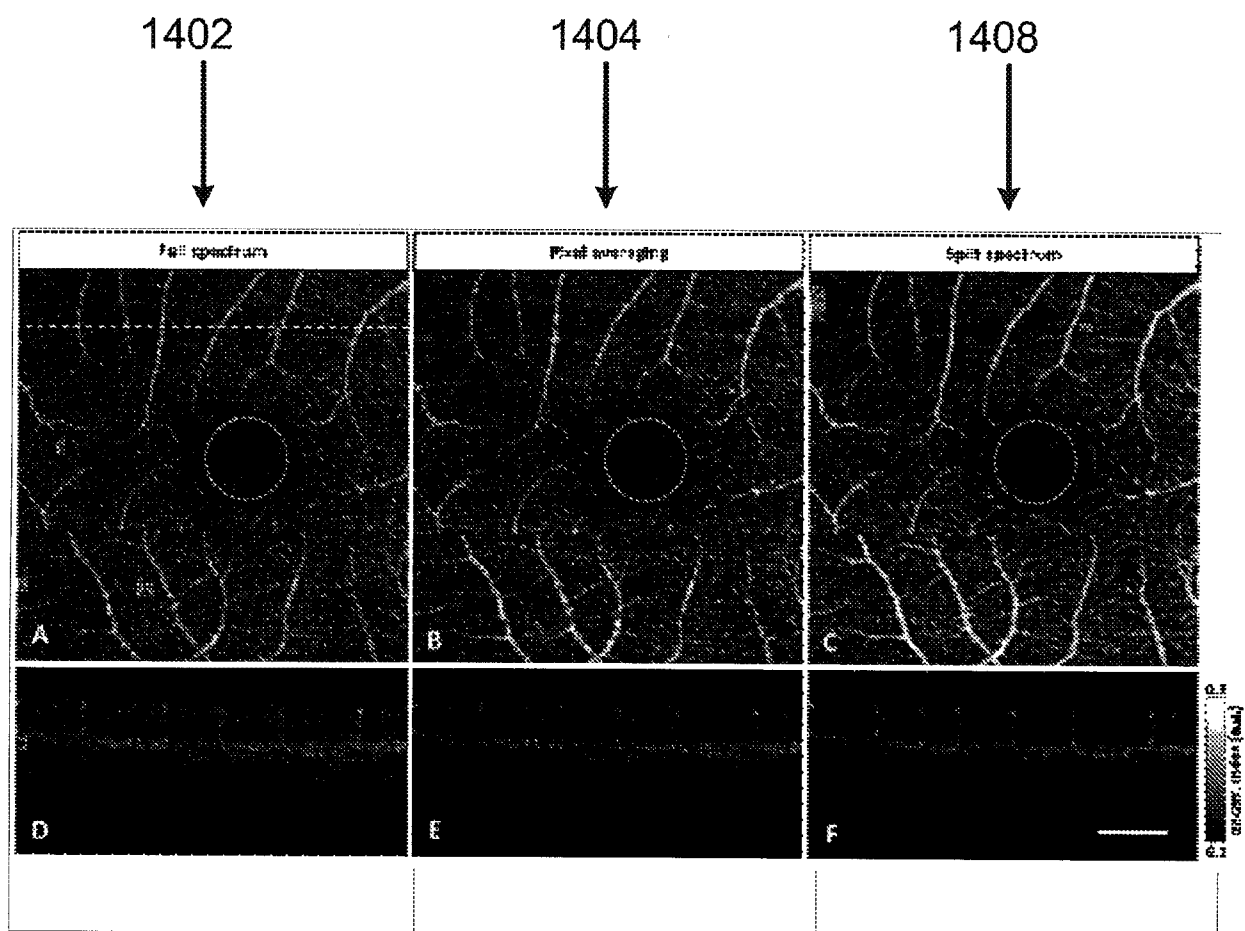
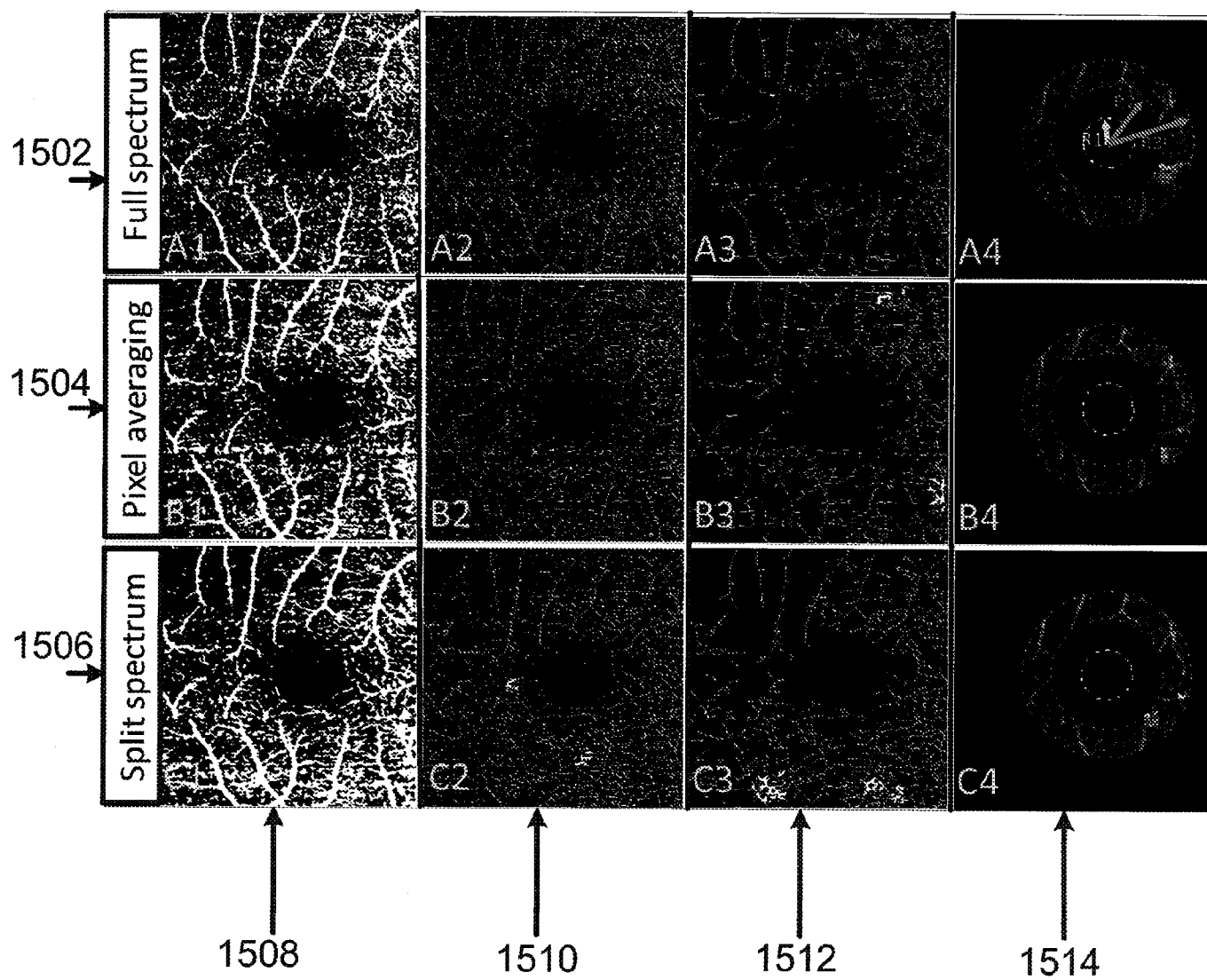


FIG. 15



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