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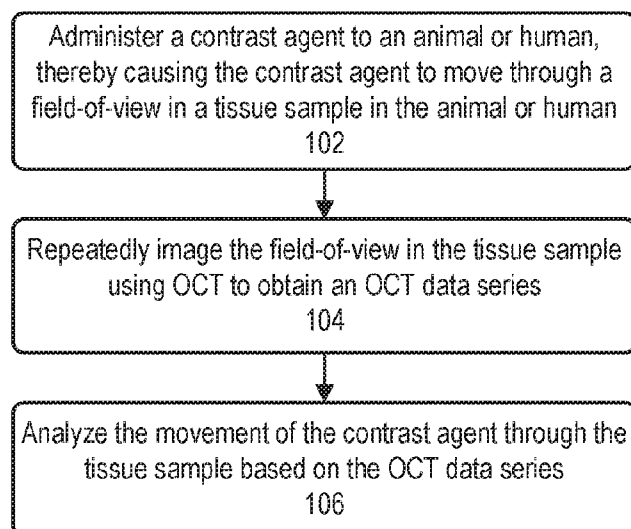
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**FIG. 1A**

(57) **Abstract:** The disclosed embodiments relate to analyzing tissue samples. A contrast agent can be administered to an animal or human, thereby causing the contrast agent to move through a field-of-view in a tissue sample in the animal or human. Next, the field-of-view can be repeatedly imaged in the tissue sample using, e.g., optical coherence tomography (OCT). The one or more images can then be used to analyze the movement of the contrast agent through the tissue sample. In some embodiments, the contrast agent is endogenously derived from the animal or human to which or whom it is administered, thereby ensuring that the contrast agent is fully biocompatible and non-immunogenic with respect to the animal or human.

DYNAMIC CONTRAST OPTICAL COHERENCE TOMOGRAPHY AND ENDOGENOUSLY-DERIVED CONTRAST AGENTS

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BACKGROUND

Field

10 [001] The disclosed embodiments generally relate to analyzing a tissue sample in an animal or human. More specifically, the disclosed embodiments relate to using dynamic contrast optical coherence tomography (OCT) for analyzing a tissue sample in an animal or human, and using endogenously-derived contrast agents during imaging.

15 Related Art

 [002] OCT is an established medical imaging technique that uses light to capture micrometer-resolution, three-dimensional images from within optical scattering media (e.g., biological tissue). Optical coherence tomography is based on low-coherence interferometry, typically employing near-infrared light. The use of relatively long wavelength light allows it to
20 penetrate into the scattering medium. Commercially available optical coherence tomography systems are employed in diverse applications, including diagnostic medicine, notably in ophthalmology and optometry where it can be used to obtain detailed images from within the retina. Doppler OCT, decorrelation-based OCT, and Count-based OCT have all been developed for assessing blood flow, but all these methods require calibration of the focal spot, which may
25 be difficult to perform accurately. There is also a loss of spatial resolution over depth, so it is challenging to quantify blood passage in deep capillaries.

 [003] Due to the large regulatory hurdles, there are relatively few FDA-approved exogenous optical imaging contrast agents for humans. Even these contrast agents, which are in routine clinical use, can sometimes induce adverse reactions.

30 [004] Therefore, what are needed are imaging techniques and contrast agents without the above-mentioned drawbacks.

SUMMARY

[005] In some embodiments, a contrast agent can be administered to an animal or human, thereby causing the contrast agent to move through a field-of-view in a tissue sample in the animal or human. Next, the field-of-view in the tissue sample can be repeatedly imaged using OCT to obtain an OCT data series. The OCT data series can then be used to analyze the movement of the contrast agent through the tissue sample, e.g., by computing a parameter associated with the movement of the contrast agent through the tissue sample.

[006] In some embodiments, the field-of-view in the tissue sample can be repeatedly imaged as the contrast agent moves through the field-of-view in the tissue sample.

[007] In some embodiments, the parameter is one of: (1) a transit time distribution associated with the movement of the contrast agent through the tissue sample, (2) a flow rate associated with the movement of the contrast agent through the tissue sample, or (3) a volume associated with the contrast agent that moves through the tissue sample.

[008] In some embodiments, the contrast agent can be introduced into the bloodstream.

[009] In some embodiments, the contrast agent can be endogenously derived from the animal or human.

[0010] In some embodiments, repeatedly imaging the field-of-view in the tissue sample can commence either prior to or immediately following the administration of the contrast agent to the animal or human.

[0011] In some embodiments, said field-of-view is repeatedly imaged at a single point, line, 2-D area, or 3-D volume.

[0012] In some embodiments, imaging is sufficiently fast to acquire at least one complete time point in the data series during the first passage of the contrast agent through the field of view, but before recirculation.

[0013] In some embodiments, angiography techniques based on OCT amplitude/intensity and/or phase are applied to the data obtained during the first passage of the contrast agent.

[0014] In some embodiments, angiography techniques based on OCT amplitude/intensity and/or phase are applied to the data obtained after the first passage of the contrast agent.

[0015] In some embodiments, said analyzing can include using a tracer kinetics analysis technique to characterize the movement of the contrast agent through the tissue sample.

[0016] In some embodiments, said analyzing can include computing a baseline measurement value before the contrast agent arrives at the field-of-view.

[0017] In some embodiments, the contrast agent is one of: (1) Intralipid with a 10%-30% concentration, (2) a substance that increases or decreases scattering within the sample, (3) a substance that is polarization sensitive, (4) a substance that causes the OCT signal to deviate

from baseline due to passage of the tracer, or (5) a mixture of contrast agents for OCT and one or more other imaging modalities for multi-modal imaging.

[0018] In some embodiments, the OCT technique involves using low-coherence optical interferometry that uses near infrared light to capture images with micrometer resolution from the tissue sample.

[0019] In some embodiments, a first tissue sample can be extracted from an animal or human. Next, the first tissue sample can be processed to obtain a processed tissue sample that is fully biocompatible and non-immunogenic with respect to the animal or human. The processed tissue sample can then be administered to the animal or human, thereby causing the processed tissue sample to move through a field-of-view in a second tissue sample in the animal or human. Next, one or more images can be obtained by imaging the field-of-view in the second tissue sample at different time instances. The one or more images can be obtained by using a suitable imaging technique. Examples of suitable imaging techniques include, but are not limited to, OCT, near-infrared spectroscopy (NIRS), and diffuse correlation spectroscopy (DCS). The embodiments can then analyze the movement of the processed tissue sample through the second tissue sample based on the one or more images (e.g., some embodiments can compute a parameter associated with the movement of the processed tissue sample through the second tissue sample). Specifically, in some embodiments, a contrast between multiple images can be analyzed, wherein the contrast between the multiple images is created by the movement of the processed tissue sample through the field-of-view in the second tissue sample.

BRIEF DESCRIPTION OF THE FIGURES

[0020] FIGs. 1A-1B illustrate a process for performing dynamic contrast OCT in accordance with some embodiments described here.

[0021] FIG. 2 illustrates an imaging mechanism for performing DyC-OCT in accordance with some embodiments described herein.

[0022] FIGs. 3A-3F illustrate an analysis of an OCT data series in accordance with some embodiments described herein.

[0023] FIGs. 4A-4B illustrate a process for using an endogenously-derived contrast agent during imaging in accordance with some embodiments described herein.

[0024] FIG. 5 illustrates blood cloudiness levels corresponding to different lipid concentration levels in accordance with some embodiments described herein.

[0025] FIG. 6 illustrates a computer system in accordance with some embodiments described herein.

DETAILED DESCRIPTION

[0026] The following description is presented to enable any person skilled in the art to make and use the present embodiments, and is provided in the context of a particular application and its requirements. Various modifications to the disclosed embodiments will be readily apparent to those skilled in the art, and the general principles defined herein may be applied to other embodiments and applications without departing from the spirit and scope of the present embodiments. Thus, the present embodiments are not limited to the embodiments shown, but are to be accorded the widest scope consistent with the principles and features disclosed herein.

[0027] The data structures and code described in this detailed description are typically stored on a computer-readable storage medium, which may be any device or medium that can store code and/or data for use by a computer system. The computer-readable storage medium includes, but is not limited to, volatile memory, non-volatile memory, magnetic and optical storage devices such as disk drives, magnetic tape, CDs (compact discs), DVDs (digital versatile discs or digital video discs), or other media capable of storing computer-readable media now known or later developed.

[0028] The methods and processes described in the detailed description section can be embodied as code and/or data, which can be stored in a computer-readable storage medium as described above. When a computer system reads and executes the code and/or data stored on the computer-readable storage medium, the computer system performs the methods and processes embodied as data structures and code and stored within the computer-readable storage medium. Furthermore, the methods and processes described below can be included in hardware modules. For example, the hardware modules can include, but are not limited to, application-specific integrated circuit (ASIC) chips, field-programmable gate arrays (FPGAs), and other programmable-logic devices now known or later developed. When the hardware modules are activated, the hardware modules perform the methods and processes included within the hardware modules.

Overview

[0029] Some embodiments disclosed herein feature dynamic contrast OCT (DyC-OCT), which is an improved method of measuring the capillary network especially for tissues, for example, deep in the brain and optic nerve heads. It involves injecting a chemically inert “contrast agent” into a tissue sample and closely monitoring for the passage of the contrast agent across a field of view. Dynamics of the contrast agent movement are collected by repeated imaging of the field of view. The collected OCT data series (or time series of OCT data) can be analyzed to determine blood flow dynamics including, but not limited to, transit time distribution,

presence at an extravascular location (for rate of vascular leakage), diffusion rates, volume or flow rate associated with the movement of the contrast agent through the field of view, and permeability of the vasculature.

[0030] An important aspect of DyC-OCT is the concept of real time imaging of the contrast agent movement: that is, injecting the bolus/contrast agent and precisely monitoring its presence at a specific site by repeated imaging by OCT. Imaging is started either prior to bolus injection or just after bolus injection. Typically data acquisition continues until recirculation. Different data processing techniques may be used to visualize and analyze the images.

[0031] While the technique is amenable to multiple contrast agents and data analysis, some embodiments disclosed herein use a tracer kinetics method to measure the dynamics of the intralipid contrast agent movement.

[0032] The embodiments disclosed herein have a number of advantages when compared to other existing approaches, including the following: (1) no calibration of the focal spot is required, (2) the embodiments facilitate higher spatial resolution of deep tissue like inner retina, choroid, choriocapillaris, and (3) the embodiments provide the ability to quantify microvascular dynamics.

[0033] Optical Coherence Tomography is the optical analog of ultrasound, and generates depth-resolved images of backscattering or backreflection from biological tissue. For example, see Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, et al. "Optical Coherence Tomography." Science. 1991;254(5035):1178-81. PubMed PMID: ISI:A1991GQ83400038. In the brain, imaging depths of up to and exceeding 1 mm are possible with OCT. A number of OCT methods have since been developed for the visualization of blood vessels within scattering tissue. For example, see (1) Fingler J, Schwartz D, Yang C, Fraser SE. Mobility and transverse flow visualization using phase variance contrast with spectral domain optical coherence tomography. Opt Exp. 2007;15:12636-53, (2) Wang RK, Jacques SL, Ma Z, Hurst S, Hanson SR, Gruber A. Three dimensional optical angiography. Optics express. 2007;15(7):4083-97. PubMed PMID: ISI:000245406400046, (3) Mariampillai A, Standish BA, Moriyama EH, Khurana M, Munce NR, Leung MKK, et al. Speckle variance detection of microvasculature using swept-source optical coherence tomography. Opt Lett. 2008;33:1530-2, (4) Tao YK, Davis AM, Izatt JA. Single-pass volumetric bidirectional blood flow imaging spectral domain optical coherence tomography using a modified Hilbert transform. Optics express. 2008;16(16):12350-61. Epub 2008/08/06. doi: 170291 [pii]. PubMed PMID: 18679512, and (5) Vakoc BJ, Lanning RM, Tyrrell JA, Padera TP, Bartlett LA, Stylianopoulos T, et al. Three-dimensional microscopy of the tumor microenvironment in vivo using optical frequency

domain imaging. Nat Med. 2009;15(10):1219-23. Epub 2009/09/15. doi: nm.1971 [pii]
10.1038/nm.1971. PubMed PMID: 19749772; PubMed Central PMCID: PMCPMC2759417.

[0034] Conventional techniques all share the feature that motion is used as a contrast mechanism. Recently, a highly sensitive method was proposed to separate both dynamic and static signals in cortical tissue (see Radhakrishnan H, Srinivasan VJ. Compartment-resolved imaging of cortical functional hyperemia with OCT angiography. Biomedical optics express. 2013;4(8):1255-68. doi: 10.1364/BOE.4.001255. PubMed PMID: 24009990; PubMed Central PMCID: PMC3756578).

[0035] Maps of dynamic scattering, or “angiograms,” can be considered as perfusion maps, where the source of contrast arises from a combination of motion and scattering. While OCT has traditionally used endogenous contrast, an FDA-approved intravenous nutritional supplement called Intralipid was recently shown to enhance contrast in Doppler OCT images following injection in steady state (see e.g., Pan Y, You J, Volkow ND, Park K, Du C. Ultrasensitive detection of 3D cerebral microvascular network dynamics in vivo. NeuroImage. 2014;103:492-501. doi: 10.1016/j.neuroimage.2014.08.051. PubMed PMID: 25192654; PubMed Central PMCID: PMC4252474; see also, PCT Publication No. WO 2014/012042, entitled “Intralipid as a contrast agent to enhance subsurface blood flow imaging,” by inventors Yingtian Pan, Congwu Du, Hugang Ren, and Nora Volkow).

[0036] Some embodiments described herein use the OCT angiography technique to observe the time-resolved changes in the dynamic signal during the first pass of an Intralipid tracer. Indicator dilution theory can then be applied to this dynamic contrast signal to extract additional information (e.g., mouse cortical hemodynamics) about the movement of the contrast agent through the tissue sample.

Dynamic Contrast OCT

[0037] FIGs. 1A-1B illustrate a process for performing dynamic contrast OCT in accordance with some embodiments described here. As shown in FIG. 1A, the process can begin by administering a contrast agent to an animal or human, thereby causing the contrast agent to move through a field-of-view in a tissue sample in the animal or human (operation 102). Next, the field-of-view in the tissue sample can be repeatedly imaged using optical coherence tomography (OCT) to obtain an OCT data series (operation 104). The process can then analyze the movement of the contrast agent through the tissue sample based on the OCT data series (operation 106), e.g., by computing a parameter associated with the movement of the contrast agent through the tissue sample.

[0038] For example, as shown in FIG. 1B, a contrast agent can be administered into a tissue sample of an animal or human 156 using administering mechanism 152. The contrast agent can then move through field-of-view 154 in the tissue sample. Imaging apparatus 158 (e.g., OCT) can then be used to repeatedly image field-of-view 154 in the tissue sample, thereby generating data series 160. Data series 160 can then be provided to computing mechanism 162 which can compute a parameter associated with the movement of the contrast agent through the tissue sample based on data series 160.

An Example of an Embodiment

[0039] The following description provides details of one embodiment that was used to image a mouse cortex using DyC-OCT. FIG. 2 illustrates an imaging mechanism for performing DyC-OCT in accordance with some embodiments described herein. In FIG. 2, “SLED1” and “SLED2” are superluminescent diodes, “LSC” is a Line Scan Camera, “DG” is a Diffraction Grating, “DM” is a Dichroic Mirror, “GM” is a Galvanometer Mirror, “DGM” is a Dichroic Galvanometer Mirror, “LPF” is a Long-Pass Filter, “OBJ” is an Objective Lens, “VIS” refers to Visible, “BS” is a Beam Splitter, and “CCD” is a Charge-Coupled Device Camera. Components “470nm LED,” “DM,” “Beam Dump,” and “LPF” were added to enable fluorescence imaging. Due to the spatial constraints imposed by the commercial probe, the long-pass filter, used to reject stray excitation light, was placed in the OCT beam path. This caused an estimated 15% excess loss in OCT signal intensity. Components for fluorescence imaging were removed when the fluorescence channel was not used to avoid these losses. The lines in FIG. 2 denote light propagation. Specifically, excitation light is denoted by (1) the lines between “470nm LED” and “DM,” and (2) the lines between “DM” and “Sample” represent. Light with a wavelength in the emission spectrum is denoted by (1) the lines between “CCD” and “VIS OBJ,” (2) the lines between “VIS OBJ” and “DGM,” and (3) the parallel lines between “OBJ” and “Sample.” All other lines denote the OCT beam with a center wavelength of 1325 nm.

[0040] Specifically, as shown in FIG. 2, a 1325 nm spectral/Fourier domain OCT microscope was used to image the mouse cortex. The light source consisted of two superluminescent diodes (SLED1 and SLED2 shown in FIG. 2) combined using a 50/50 fiber coupler to yield a bandwidth of over 150 nm. The axial (depth) resolution, after computational spectral shaping of the raw spectrum, was 7 μm in air (5.3 μm in tissue). A spectrometer with a 1024 pixel InGaAs line scan camera operated at 91 kHz. Imaging was performed with a 5x objective, yielding a transverse resolution of 15 microns, and a 10x objective, yielding a transverse resolution of 7.5 microns. The system sensitivity was measured to be approximately 91 dB.

Modified Setup for Simultaneous OCT and Fluorescence Imaging

[0041] In order to acquire spatially and temporally coregistered fluorescence and OCT data, some modifications had to be made to the imaging setup. The OCT system already has a wide-field camera aligned and coregistered with the OCT beam. A narrow-bandwidth 470-nm LED light source was used for excitation and was additionally filtered with a reflective 490-nm short-pass filter. To reject excitation light from the camera, an absorptive 515-nm long-pass filter was placed in front of the camera. Due to the spatial restrictions of the enclosed commercial OCT system, this filter had to be placed in the path of the OCT beam which is believed to cause ~8% round-trip drop in OCT signal.

Animal Preparation

[0042] Male C57BL Mice (n = 10; 20-35 grams) were used. The mice were initially anesthetized with 1.5% v/v isoflurane with a gas mixture of 80% air and 20% oxygen. After successful induction of anesthesia, the mice were mounted on a stereotactic frame with continued delivery of 1-1.5% v/v isoflurane, modulated as necessary to maintain healthy and steady breathing throughout the surgical and imaging procedures. Once in the frame, the scalp was retracted, the fascia was removed, and the skull was cleaned and dried using a gauze pad. Using a progressively finer series of dental burrs, a $\sim 3 \times 3 \text{ mm}^2$ region of the skull lateral to the midpoint between lambda and bregma was thinned to within $\sim 30 \text{ }\mu\text{m}$ in thickness. This made the skull more transparent, and enabled imaging of the cortex through the thinned region without requiring an invasive craniotomy. A 5 mm diameter coverglass was glued to the thinned region to enhance light transmission to the cortex. A large ball joint attached to the stereotactic frame is used to position the mouse such that the coverglass is perpendicular to the beam path. Throughout the surgical and imaging procedures, the animal's core temperature was maintained at 37 degrees Celsius using a heating blanket (Harvard Apparatus USA).

Bolus Injection Protocol

[0043] A tracer bolus was injected into the tail vein using either a 27 or 31 gauge syringe. The bolus was restricted to 100 μL ($\sim 3 \text{ mL/kg}$) to minimize changes in cortical hemodynamics due to the bolus itself. Furthermore, the bolus was injected quickly ($\sim 0.5 \text{ sec}$) to best approximate a delta input function and maximize the DyC-OCT signal. For the DyC-OCT measurements, Intralipid 20% was used as the contrast agent. For the simultaneous DyC-OCT and fluorescence measurements, a 4:1 mixture of Intralipid 20% and saline-dissolved fluorescein

isothiocyanate–dextran (FITC-dextran) was used. The final FITC-dextran concentration was 5% weight by volume.

DyC-OCT Imaging Protocol and Data Processing

5 **[0044]** To capture the change in scattering signal as the bolus passes through the vasculature, repeated B-scans of the same area were acquired to generate a 2-D time-resolved cross-section of the cortex. 1000 B-scans were acquired every 13 ms along a 1.2-2 mm region containing both arteries and veins to observe the dynamic change in signal due to Intralipid contrast across a time window of 13 seconds starting from the time of tracer injection. In some
10 embodiments, to validate the DyC-OCT process, spatially and temporally coregistered DyC-OCT and time-resolved fluorescence measurements can be acquired. A custom LabView program can be used to simultaneously acquire wide-field fluorescence images and B-scans over the same region-of-interest at a rate of 30 Hz.

15 **[0045]** The resulting OCT data was processed both parametrically and non-parametrically on a pixel by pixel basis. A number of data processing techniques were applied to the data. Some of these data processing techniques are now described.

OCT Angiography

20 **[0046]** OCT angiograms can be produced by removing the static scattering component of the OCT signal. This can be accomplished by performing a complex subtraction of repeated B-scans. This subtraction removes the static scattering components which do not change over time while highlighting the areas where the scattering signal does change, as is seen in the vasculature due to blood flow. This angiogram signal can be further improved through averaging and by applying a phase correction before the complex subtraction to account for sub-pixel movements.

DyC-OCT Signal

25 **[0047]** FIGs. 3A-3F illustrate an analysis of an OCT data series in accordance with some embodiments described herein. As shown in FIG. 3A, the DyC-OCT data is acquired as a 3-D stack of repeated B-scans 302. In the figure, $A(x, z, t)$ represents a pixel at location (x, z) at time
30 t . First the static-scattering components of the signal can be removed using angiography methods to improve detection of the tracer signal. As shown in FIG. 3B, the values 304 of a given pixel, e.g., pixel (x, z) , in each angiogram can be extracted, and these values can be used to obtain the time-resolved signal at the given pixel. The passage of the tracer at a given pixel causes a dynamic change in signal, which is shown in FIG. 3C that has several distinct features. There is
35 a baseline value attributed to the red blood cell (RBC) signal before the tracer arrives (shown as

“Baseline” in FIG. 3C), there is a sharp increase in signal as the tracer passes through for the first time followed by a slower decrease (shown as “First Passage” in FIG. 3C), and there is settling of the signal at a level higher than baseline (shown as “Recirculation” in FIG. 3C), which is due to recirculation of the tracer. This occurs when the injected tracer passes through the body, returns to the heart, and is pumped back through the ROI. This typically happens before complete decay of the tracer signal when the tracer is injected via the tail vein. For all methods, the data can be averaged 3x laterally 3x axially and 3x temporally to reduce noise.

Signal Increase Mask

[0048] The first step in visualizing this data is to look at the increase in the angiogram signal caused by the Intralipid bolus. A baseline signal is determined by averaging the first 1 to 2 seconds of data before the bolus arrives. Then the global maximum of the signal during the measured time course is determined after additional temporal averaging to reduce noise. The maximum signal divided by the baseline yields the relative increase in signal. The next step is to threshold out the background noise to create a mask of the vasculature where the tracer has traveled. To do this, the mean and standard deviation of the noise in the signal increase map is measured by using a region of air above the skull. The threshold for the mask was set at 4 standard deviations above this noise, ~35% increase in signal, and this mask is used for all of the parametrically determined data in order to reduce processing time. The temporal increase in scattering or the metrics derived from this change may be displayed in color encoded maps.

Parametric Model

[0049] To quantify parameters such as bolus duration and arrival time, a second-order plus dead time (SOPDT) model can be used after subtracting off the baseline signal. This phenomenological model shown in FIG. 3D uses arrival time (θ), signal frequency (ω), amplitude (A), and dampening (ξ) variables to generate a curve similar to the angiogram signal over time for each point:

$$g(t) = Au(t - \theta) \left[\frac{e^{\omega(t-\theta)(\sqrt{\xi^2-1}-\xi)} - e^{\omega(\theta-t)(\sqrt{\xi^2-1}+\xi)}}{2\omega\sqrt{\xi^2-1}} \right]$$

[0050] In the above equation, u is the Heaviside (unit step) function. Indicator-dilution models have been compared, and it has been shown that the SOPDT model fits indicator-dilution curves better than the traditional gamma-variate model (see Chinta LV, Lindvere L, Stefanovic

B., "Robust quantification of microvascular transit times via linear dynamical systems using two-photon fluorescence microscopy data. Journal of cerebral blood flow and metabolism," Official Journal of the International Society of Cerebral Blood Flow and Metabolism. 2012;32(9):1718-24. doi: 10.1038/jcbfm.2012.86. PubMed PMID: 22714047; PubMed Central PMCID:

- 5 PMC3434637). The variables in the SOPDT model can be optimized using a non-linear least-squares method. For example, FIG. 3D illustrates SOPDT model fitting and extraction of features such as arrival time, FWHM, and peak time.

Adaptive Data Selection

- 10 [0051] Because recirculation of the Intralipid particles confounds the fit of the model after the recirculation time, only the portion of the data before recirculation occurs is used. For example, curve 322 in FIG. 3E uses all of the data and results in a poor fit. On the other hand, curve 324 uses only the first 5 seconds of data and provides a better fit. If a single recirculation cutoff time is used across the entire data set, the fit will be poor in some of the vessels. For
- 15 example, when the first 5 seconds of data are used for fitting the data for a vein, the resulting curve 332 in FIG. 3F will provide incorrect or misleading results. Therefore, to get the best fit, the model is applied twice under different conditions. First, a general recirculation-time cutoff is used for every point in the map. For some points, this recirculation time will be too early and for some it will be too late, so the fit of the model will not be ideal, but a relatively accurate arrival
- 20 time can be extracted. Next the model is fit using an adaptive recirculation time determined by adding a constant to the arrival time determined by the first fit. This second fit (shown as adaptive fit 334 in FIG. 3F) is more accurate across the wide range of arrival times. For example, a 5 second cut-off time (shown as cutoff time 326) is used for the artery data shown in FIG. 3E, but a longer cutoff time (shown as cutoff time 336) is used for the vein data shown in
- 25 FIG. 3F.

Depth-Dependent Analysis

- [0052] Features of the DyC-OCT data can be analyzed as a function of depth in the cortex. Here, the larger vessels can be filtered out and the DyC-OCT signal can be examined in
- 30 the microvasculature to study the hemodynamic trends associated with different layers of the cortex.

Transit Time Distribution Quantification

- [0053] Indicator-dilution theory shows that if a tracer is injected into a vascular network,
- 35 and the tracer concentration is measured at an upstream location and a downstream location, then

the transit time distribution (TTD), or transport function, of the network connecting the two points can be calculated. The venous signal (proportional to the venous concentration) is equal to the TTD convolved with the arterial signal (proportional to the arterial concentration) as shown here where c_v and c_a are the venous and arterial concentrations respectively, “*” denotes a

5 convolution, and $h(t)$ is the TTD: $c_v(t) = (c_a * h)(t)$.

[0054] Furthermore, the central volume theorem allows us to calculate the CBF with knowledge of the mean transit time (MTT) and cerebral blood volume (CBV), or the CBV given the CBF and MTT: $CBV = CBF \times MTT$.

[0055] The relationship between the DyC-OCT signal in venous vasculature related to the
10 signal in connected arterial vasculature is given by a convolution with the TTD described in the above equation for $c_v(t)$. To extract a quantitative measure of the TTD from two measured signals, a discrete deconvolution can be performed either on the raw data or on a fit to the data. The noise and recirculation effects in the raw signal may cause poor deconvolution and extraction of the TTD, so the modeled signals may also be used. Because a convolution in the
15 time domain is the same as a multiplication in the transform domain, the deconvolution can also be directly calculated from the SOPDT model as a division of the venous signal by the arterial signal in the frequency domain followed by an inverse Laplace transform as follows:

$$h(t) \approx \mathcal{L}^{-1} \left\{ \frac{\omega_v^2}{\omega_a^2} e^{-(\theta_v - \theta_a)s} \frac{s^2 + 2\xi_a \omega_a s + \omega_a^2}{s^2 + 2\xi_v \omega_v s + \omega_v^2} \right\}.$$

20 [0056] In the above equation, $h(t)$ is the TTD, $\mathcal{L}^{-1}$ denotes the inverse Laplace transform, and “a” and “v” subscripts denote arterial and venous/capillary fitted parameters respectively. As $h(t)$ is typically taken to be a probability density function, it has been normalized to ensure an area of one. The MTT can then be determined as the centroid of the TTD.

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Quantifying Plasma Volume from the Steady State Signal

[0057] The baseline backscattering DyC-OCT signal is assumed to arise from endogenous blood cells (predominantly RBCs) and tracer, with backscattering coefficients of $\mu_{b,RBC}(z, x)$ and $\mu_{b,i}(z, x, t)$, respectively. Assuming that backscattering coefficients add, the
30 OCT angiogram signal is described as the product of a position-dependent sensitivity factor $h(z, x)$ and the total backscattering coefficient:

$$I_{OCTA}(z, x, t) = h(z, x) [\mu_{b,RBC}(z, x) + \mu_{b,i}(z, x, t)].$$

35 [0058] The factor $h(z, x)$ in the above equation defines the space-variant signal change

measured by the OCT instrument in response to a local increase in backscattering. It can account for a variety of effects, including spectrometer roll-off, focusing, vignetting, and attenuation due to scattering and/or absorption, all of which are spatially-dependent and subject-dependent.

Specifically,

$$h(z, x) = h_{\text{spec}}(z)h_{\text{focus}}(z)h_{\text{vign}}(x)h_{\text{atten}}(z, x).$$

[0059] The backscattering coefficient of the tracer is assumed to equal the time-dependent tracer concentration times the backscattering cross-section of the tracer:

$$\mu_{b,i}(z, x, t) = c_i(z, x, t)\sigma_{b,i}.$$

[0060] Similarly, the backscattering coefficient of endogenous blood is assumed to equal to the mean RBC concentration times the backscattering cross-section of RBCs:

$$\mu_{b,\text{RBC}}(z, x) = c_{\text{RBC}}(z, x)\sigma_{b,\text{RBC}}.$$

[0061] Note that the mean RBC concentration is equivalent to the local hematocrit in a voxel if the voxel samples only blood. If a voxel samples blood and tissue, the RBC concentration is lower than the local hematocrit. The mean RBC concentration, and therefore, backscattering, are assumed to be constant throughout the experiment, though stochastic fluctuations in instantaneous RBC concentration over time are a source of noise. Also, the change in RBC concentration caused by the volume of injected tracer is neglected. The proportionate scaling of RBC backscattering coefficient with concentration (see the equation shown above for $\mu_{b,\text{RBC}}(z, x)$) and the superposition of backscattering from RBCs and tracer (see the equation shown above for $I_{\text{OCTA}}(z, x, t)$) are both assumptions that may not fully hold in practice due to dependent scattering and shadowing effects in blood.

[0062] There is initially no tracer present in the field-of-view, so $c_i(z, x, 0) = 0$, and therefore, $\mu_{b,i}(z, x, 0) = 0$. The change in single-pass DyC-OCT signal over time can thus be written as:

$$\Delta I_{\text{OCTA}}(z, x, t) = I_{\text{OCTA}}(z, x, t) - I_{\text{OCTA}}(z, x, 0) = h(z, x)\mu_{b,i}(z, x, t) = K(z, x)c_i(z, x, t)$$

[0063] In the above expression, $K(z, x) = h(z, x)\sigma_{b,i}$ represents an unknown calibration factor relating the measured DyC-OCT signal to plasma tracer concentration, the quantity of interest.

[0064] Next, two methods are disclosed by which quantitative plasma volume can be extracted from the DyC-OCT signal either in individual vessels or across entire vascular

networks. In the first method, the steady state DyC-OCT signal, the signal following repeated recirculation of the tracer, is compared to baseline and calibrated to yield quantitative plasma volume:

$$\mu_{b,i,ss}(z, x) = c_{i,ss}(z, x)\sigma_{b,i}$$

- 5 **[0065]** Note that $\mu_{b,i}(z, x, t \rightarrow \infty) = 0 \neq \mu_{b,i,ss}(z, x)$ and $c_i(z, x, t \rightarrow \infty) = 0 \neq c_{i,ss}(z, x)$, since single-pass quantities decay to zero, while steady state quantities involve recirculation. The plasma tracer assumption can be expressed as $c_{i,ss}(z, x) = \alpha c_p(z, x)$, where $c_p(z, x)$ is the fractional plasma volume. Moreover, the coefficient, α , relating tracer concentration $c_{i,ss}$ to plasma concentration c_p depends on the total plasma volume (related to the total blood volume and hematocrit) of the subject and the amount of tracer injected. Thus,

$$\Delta I_{OCTA,ss}(z, x) = I_{OCTA,ss}(z, x) - I_{OCTA}(z, x, 0) = h(z, x)\mu_{b,i,ss}(z, x) = K_{ss}(z, x)c_p(z, x)$$

[0066] In the above expression, $K_{ss}(z, x) = h(z, x)\sigma_{b,i}\alpha$ represents an unknown constant relating the measured DyC-OCT signal to plasma volume, the quantity of interest.

- 15 **[0067]** To calibrate the DyC-OCT signal, we integrate the DyC-OCT signal over a vessel ROI with known hematocrit (being careful to avoid vessel edges with partial volume effects).

$$\Delta I_{OCT,ROI,ss}(z, x) = \frac{1}{A_{ROI}} \iint_{ROI} \Delta I_{OCT,ss}(z + \Delta z, x + \Delta x) d\Delta z d\Delta x$$

- [0068]** Based on this calibration and the known hematocrit of the vessel, it is possible to estimate the calibration factor $K(z, x)$. For instance, macro-vessels (vitreal arteries and veins) in the retina are known to have hematocrits close to the systemic hematocrit. Hence the fractional plasma may be estimated as $c_p = 1 - HcT$. For instance, if $HcT = 0.4$, then $c_{p,ROI} = 0.6$.

$$K_{ss}(z, x) \cong \frac{\Delta I_{OCT,ROI,ss}(z, x)}{c_{p,ROI}}$$

[0069] The plasma volume for individual vessels can then be expressed using the following integral where $PV_{V,ss}$ has units of plasma volume per unit vessel length:

25

$$PV_{V,ss} = \iint_{\text{vessel ROI}} \frac{\Delta I_{OCTA,ss}(z, x)}{K_{ss}(z, x)} dz dx$$

[0070] Similarly, plasma volume for different layers of the retinal vasculature can be expressed using the following integral where $PV_{L,ss}$ has units of plasma volume per unit *en face* area:

$$PV_{L,ss} = \frac{1}{X_{layer}} \iint_{layer\ ROI} \frac{\Delta I_{OCTA,ss}(z, x)}{K_{ss}(z, x)} dz dx$$

5

[0071] It should be emphasized that this calibration would ideally be performed at every voxel (z, x) in the image. However, since macro-vessels with known hematocrits are not uniformly available throughout the imaged field-of-view, this is not possible. In this work, we use a large vitreal artery or vein at the center of the field-of-view to perform the calibration.

10 Plasma volume measurements are expected to be most quantitatively accurate in the immediate vicinity of the vessel that was used for calibration. While plasma volume measurements can also be mapped on a voxel by voxel basis using this method, speckle noise is reduced when integrating the signal over a vessel or layer.

15 Layer-based flow and volume quantification

[0072] Here we assume that a vascular bed is characterized by a transport function $h_{av}(t)$, or distribution of arteriovenous transit times given an impulsive arterial input at $t = 0$. Thus, $h_{av}(t) = 0$, for $t < 0$, by causality. Since $h_{av}(t)$ is a distribution, $\int_{-\infty}^{\infty} h_{av}(t) dt = 1$. The transport function is characterized by a mean transit time (MTT), defined as $MTT =$

20 $\int_{-\infty}^{\infty} t h_{av}(t) dt$. To calculate layer-resolved flow and volume, we define a tissue concentration integrated over a layer, as shown below.

$$C_t(t) = \frac{1}{X_{layer}} \iint_{layer\ ROI} c_i(z, x, t) dz dx$$

[0073] $C_t(t)$ has units of indicator per unit *en face* tissue area. Likewise, an arterial input

25 is defined as:

$$c_a(t) = \frac{1}{A_{artery}} \iint_{arterial\ ROI} c_i(z, x, t) dz dx$$

[0074] If we are careful to avoid edges when selecting the arterial ROI, $c_a(t)$ has units of concentration (indicator per unit plasma volume). Based on indicator-dilution theory,

$$C_t(t) = c_a(t) * [PF \times R(t)]$$

5 [0075] The residue $R(t) = 1 - \int_{-\infty}^t h_{av}(\tau) d\tau$ is the fraction of tracer remaining in the vascular bed after an impulsive arterial input at $t = 0$. As such it is a unitless function that is 0 for $t < 0$ and decreases monotonically from 1 (at $t = 0$) to 0 (as $t \rightarrow \infty$). Given these units, and noting that convolution is with respect to time, plasma flow (PF) is obtained in units of plasma volume per unit *en face* tissue area per unit time. The solution of this equation for PF requires a
10 deconvolution if $R(t)$ is not known *a priori*. Deconvolution techniques can be divided into model-dependent and model-independent approaches. Finally, it is important to note that if $K(z, x)$ is similar for both the arterial ROI and layer ROI, $\Delta I_{OCT}(z, x, t)$ can replace $c_i(z, x, t)$ in the above integrals, and the unknown calibration factor cancels out in the deconvolution.

[0076] Moreover by making use of the fact that areas add under convolution and taking
15 into account the central volume principle, $PV = PF \times MTT$, with $MTT = \int_{-\infty}^{\infty} R(t) dt$, the following relationship is obtained:

$$PV = \frac{\int_{-\infty}^{\infty} C_t(t) dt}{\int_{-\infty}^{\infty} c_a(t) dt}$$

[0077] The units of PV are plasma volume per unit *en face* area. If $K(z, x)$ is similar for
20 both the arterial ROI and layer ROI, $\Delta I_{OCT}(z, x, t)$ can replace $c_i(z, x, t)$ in the above integrals, and the unknown calibration factor cancels out in the normalization. Thus, provided that appropriate arterial inputs can be defined, both plasma flow and plasma volume can be determined quantitatively in absolute units. Blood flow and blood volume may then be readily determined by assuming a hematocrit for the capillary bed.

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Endogenously-derived contrast agents for angiographic and dynamic-contrast imaging

[0078] Contrast agents are routinely used in preclinical research to enhance specificity or information content of *in vivo* images. However, due to the large regulatory hurdles, there are relatively few FDA-approved exogenous optical imaging contrast agents for humans. This short
30 list includes fluorescent molecules such as fluorescein and indocyanine green (ICG), both of which are routinely used as dynamic contrast agents to study perfusion. However, even these

contrast agents, which are in routine clinical use, can sometimes induce adverse reactions. This section describes a novel approach for deriving dynamic contrast agents endogenously. These contrast agents may have a significantly lower risk of adverse reaction.

[0079] As explained above, OCT performs cross-sectional imaging, three-dimensional imaging, or data acquisition in materials or biological tissue by measuring the magnitude and time delay of backscattered or backreflected light from inside the sample. OCT performs imaging or measurement by directing a light beam across a field-of-view on the sample, measuring the backscattered or backreflected signal from the sample as a function of the optical delay (known as an axial scan or A-scan), and scanning the OCT beam incident on the tissue or material to generate a two or three dimensional dataset which represents cross-sectional or volumetric information about the internal structure of the sample. In the case where the dataset includes a set of axial scans at sequential transverse positions, it is usually displayed as false color or gray scale images that represent cross-sections through the sample. Spectral / Fourier domain and swept source / Fourier domain detection have enabled dramatic improvements in imaging speed and sensitivity, and are now the most widespread OCT detection techniques.

[0080] Fluorescence angiography is routinely used qualitatively to assess arteriovenous transit times or vascular permeability in clinical examination. This method is based on assessing the transit of a fluorescent tracer (fluorescein or ICG) through the vasculature. However, because fluorescein angiography images are essentially photographs of the fundus, no depth resolution is achieved. Quantification of transit times at the capillary level, as well as the resolution of flow in small vessels in different layers of the eye (i.e. retina vs. choroid), remains challenging, even with indocyanine green. Moreover, these fluorescent contrast agents, while FDA-approved, can have adverse effects.

[0081] As explained above, while methods of assessing blood flow including Doppler, decorrelation, and RBC-passage based methods have been demonstrated using OCT, these remain limited in their capabilities. Thus, improved methods of assessing blood flow, particularly in capillary beds deep in scattering tissue (choroid and optic nerve head), are required. DyC-OCT can address these needs. In the embodiments described above, an exogenous contrast agent is introduced that enhances the OCT signal within the vasculature, but also can provide information on transport dynamics and blood flow. In essence, DyC-OCT is the OCT version of fluorescence angiography, but provides depth resolution. As explained above, one particular embodiment of DyC-OCT works by injecting a contrast agent into the vasculature upstream of the region of interest and repeatedly imaging the same region using OCT scans to track the change in signal as the tracer passes through the vasculature for the first time. The signal enhancement capability of exogenous contrast agents such as Intralipid, may be

particularly advantageous in that they facilitate higher spatial resolution imaging of deep tissue like inner retina, choroid, choriocapillaris. The shape of this signal can then be used to quantify the transit time distribution and other hemodynamic parameters with micron scale depth resolution.

5 **[0082]** In some embodiments described herein, an animal-derived or a human-derived endogenous tissue-sample can be extracted, and the extracted sample (either as such or after processing) can be administered to the animal or human to generate contrast for imaging, typically dynamic contrast imaging. The sample is typically purified prior to re-administration as a contrast agent. The sample may be reconstituted or otherwise prepared prior to re-
10 administration as a contrast agent for dynamic contrast imaging; however the source of contrast comes from the sample and not an outside source. The contrast agent should be fully biocompatible and non-immunogenic as it was derived from the original subject.

[0083] In one embodiment, blood is drawn from a subject, and purified or processed to isolate large lipid-based or lipoprotein particles (chylomicrons and/or VLDLs). Chylomicrons
15 have size and chemical composition that imbue them with optical scattering properties that are very similar to those of Intralipid (previously shown to be a viable contrast agent for DyC-OCT), which is an emulsion of lipid particles. Moreover, as these are endogenously derived in a subject-specific manner, they can be re-administered back into the original subject without significant risk of adverse reaction. In one embodiment, once purified, these chylomicrons
20 (and/or VLDLs) are subsequently resuspended and injected back into the body during dynamic contrast OCT imaging.

[0084] Methods of purification and processing may include, but are not limited to: a combination of centrifugation, freezing, filtering, chromatography, microfluidics, and optical or acoustic forces. One method involves centrifuging the blood to obtain an emulsion of plasma
25 and lipids which can then be used or further purified. Another method uses ultrasonic waves to separate lipids from blood in a microfluidic channel.

[0085] In another aspect, this method can extract components of blood that can then be used as contrast agents during imaging. The order of events is blood collection, component isolation or purification (if necessary), and finally, injection of said components back into the
30 subject as a contrast agent during dynamic contrast imaging. The contrast agent should be fully biocompatible and non-immunogenic as it was derived from the original subject.

[0086] FIGs. 4A-4B illustrate a process for using an endogenously-derived contrast agent during imaging in accordance with some embodiments described herein. As shown in FIG. 4A, the process can begin by extracting a first tissue sample from an animal or human (operation
35 402). Next, the extracted first tissue sample can be processed to obtain a processed tissue sample

that is fully biocompatible and non-immunogenic with respect to the animal or human (operation 404). The processed tissue sample can then be administered to the animal or human, thereby causing the processed tissue sample to move through a field-of-view in a second tissue sample in the animal or human (operation 406). Next, one or more of images can be obtained by imaging the field-of-view in the second tissue sample at one or more time instances (operation 408). The process can then analyze the movement of the processed tissue sample through the second tissue sample based on the one or more images (operation 410), e.g., the process can compute a parameter associated with the movement of the processed tissue sample through the second tissue sample based on the one or more images. Specifically, in some embodiments, a contrast between multiple images can be analyzed, wherein the contrast between the multiple images is created by the movement of the processed tissue sample through the field-of-view in the second tissue sample.

[0087] For example, as shown in FIG. 4B, blood extraction 452 can be performed on an animal or human by using an extracting mechanism, e.g., a syringe. Next, the extracted blood 454 can be processed. For example, a blood component isolation 456 process can be performed to produce an isolated blood component 458. Next, the isolated blood component can be administered 460 to the animal or human, and one or more images can be obtained 462 as the isolated blood component moves through a field-of-view in the animal or human.

[0088] In yet another embodiment, the subject is first asked to eat a high-fat meal and blood is drawn at a timepoint at which blood cloudiness peaks. In another embodiment, the dynamic contrast OCT imaging is performed at a timepoint at which blood cloudiness bottoms out. Such judicious selection of timepoints ensures maximal contrast-to-noise ratio. FIG. 5 illustrates blood cloudiness levels corresponding to different lipid concentration levels in accordance with some embodiments described herein. Note that the lipemic blood sample is cloudier than the non-lipemic blood sample.

[0089] If optical imaging such as dynamic contrast OCT is performed, the illumination and detection geometries of the optical system can be optimized to maximize signal detected from more isotropically scattering lipid particles, which are typically smaller than a wavelength, and minimize signal detected from anisotropic red blood cells, which are larger than a wavelength. Such an optimization may be performed based on the scattering phase functions of lipid particles versus red blood cells. Certain geometries, for instance, back-scattering or side-scattering, will increase the ratio of signal detected from lipid particles relative to that from red blood cells. Such geometries will maximize the signal-to-noise ratio of measurements based on small lipid particles in blood.

[0090] Another example is isolating human serum albumin which autofluoresces and

could potentially be used to perform time resolved fluorescence angiography. The purification step may include, but is not limited to, a combination of centrifugation, cold ethanol fractionation (also known as the Cohn method), freezing, filtration, and chromatography. Intravenous injection of purified albumin would be expected to increase the observed autofluorescence intensity and/or change the fluorescence lifetime. These changes would be observed using time resolved fluorescence angiography methods.

[0091] In yet another embodiment, a subject consumes a high fat meal. After processing of lipids through the small intestine, blood cloudiness begins to increase. The large lipid-based particles causing this cloudiness (chylomicrons, for instance) constitute the contrast agent.

Imaging of vasculature (with, for instance, OCT) can be performed at one or more time points over time to monitor the time course of blood scattering. In yet another embodiment, the optical scattering properties of blood in the post-prandial state, measured by OCT or OCT angiography or another imaging modality over time, can be used to assess lipoprotein processing and lipemia, and potentially, risk for subsequent cardiovascular disease. These measurements can be referenced to a baseline state to isolate the effect of the meal on blood scattering over time. Lymphatics, which are normally transparent, can also be imaged in a similar manner.

[0092] In one embodiment, a subject consumes a high fat meal. Imaging of vasculature (using, for instance, OCT angiography) is timed to coincide with maximal blood lipid and lipoprotein scattering (likely arising from large VLDL and chylomicrons) in the post-prandial state. Lymphatics (particularly those draining from the small intestine) can also be imaged in a similar manner, by timing the imaging to coincide with peak lymph cloudiness.

[0093] In another embodiment, the dynamic contrast agent can be used with near-infrared spectroscopy (NIRS) or diffuse correlation spectroscopy (DCS). Contrast agent injection would increase the scattering coefficient as well as probability of scattering from blood, both of which could be quantified over time to extract flow parameters using Beer-Lambert techniques with NIRS or DCS.

Computer System

[0094] FIG. 6 illustrates a computer system in accordance with some embodiments described herein. Computer system 602 can include processor 604, memory 606, and storage device 608. Specifically, memory locations in memory 606 can be addressable by processor 604, thereby enabling processor 604 to access (e.g., via load/store instructions) and manipulate (e.g., via logical/floating point/arithmetic instructions) the data stored in memory 606. Computer system 602 can be coupled to display device 614, keyboard 610, and pointing device 612. Storage device 608 can store operating system 616, analysis software tool 618, and data 620.

Data 620 can include input required by analysis software tool 618 and/or output generated by analysis software tool 618.

[0095] Computer system 602 may automatically (or with user help) perform one or more operations that are implicitly or explicitly described in this disclosure. For example, computer
5 system 602 can load analysis software tool 618 into memory 606, and analysis software tool 618 can then be used to analyze an OCT data series.

[0096] Various modifications to the disclosed embodiments will be readily apparent to those skilled in the art, and the general principles defined herein may be applied to other embodiments and applications without departing from the spirit and scope of the present
10 invention. Thus, the present invention is not limited to the embodiments shown, but is to be accorded the widest scope consistent with the principles and features disclosed herein.

[0097] The foregoing descriptions of embodiments have been presented for purposes of illustration and description only. They are not intended to be exhaustive or to limit the present description to the forms disclosed. Accordingly, many modifications and variations will be
15 apparent to practitioners skilled in the art. Additionally, the above disclosure is not intended to limit the present description. The scope of the present description is defined by the appended claims.

What Is Claimed Is:

1. A method, comprising:

administering a contrast agent to an animal or human, thereby causing the contrast agent
5 to move through a field-of-view in a tissue sample in the animal or human;
repeatedly imaging the field-of-view in the tissue sample using optical coherence
tomography (OCT) to obtain an OCT data series;
analyzing the movement of the contrast agent through the tissue sample based on the
OCT data series.

10 2. The method of claim 1, wherein the field-of-view in the tissue sample is
repeatedly imaged as the contrast agent moves through the field-of-view in the tissue sample.

3. The method of claim 1, wherein the parameter is one of: (1) a transit time
distribution associated with the movement of the contrast agent through the tissue sample, (2) a
flow rate associated with the movement of the contrast agent through the tissue sample, or (3) a
15 volume associated with the contrast agent that moves through the tissue sample.

4. The method of claim 1, wherein the contrast agent is introduced into the
bloodstream of the animal or human.

5. The method of claim 1, wherein the contrast agent is endogenously derived from
the animal or human.

20 6. The method of claim 1, wherein said repeatedly imaging the field-of-view in the
tissue sample commences either prior to or immediately following the administration of the
contrast agent to the animal or human.

7. The method of claim 1, wherein said field-of-view is repeatedly imaged at a single
point, line, 2-D area, or 3-D volume.

25 8. The method of claim 1, wherein imaging is sufficiently fast to acquire at least one
complete time point in the data series during a first passage of the contrast agent through the
field-of-view, but before recirculation.

9. The method of claim 1, wherein said analyzing includes applying angiography
techniques based on OCT amplitude/intensity and/or phase to a portion of the OCT data series
30 that was obtained during the first passage of the contrast agent.

10. The method of claim 1, wherein said analyzing includes applying angiography techniques based on OCT amplitude/intensity and/or phase to a portion of the OCT data series that was obtained after the first passage of the contrast agent.

11. The method of claim 1, wherein said analyzing includes using a tracer kinetics analysis technique to characterize the movement of the contrast agent through the tissue sample.

12. The method of claim 1, wherein said analyzing includes computing a baseline measurement value before the contrast agent arrives at the field-of-view.

13. The method of claim 1, wherein the contrast agent is one of: (1) Intralipid with a 10%-30% concentration, (2) a substance that increases or decreases scattering within the sample, (3) a substance that is polarization sensitive, (4) a substance that causes the OCT signal to deviate from baseline due to passage of the tracer, or (5) a mixture of contrast agents for OCT and one or more other imaging modalities for multi-modal imaging.

14. The method of claim 1, wherein the OCT technique involves using low-coherence optical interferometry that uses near infrared light to capture images with micrometer resolution from the tissue sample.

15. A system, comprising:

means for administering a contrast agent to an animal or human, thereby causing the contrast agent to move through a field-of-view in a tissue sample in the animal or human;

means for repeatedly imaging the field-of-view in the tissue sample using optical coherence tomography (OCT) to obtain an OCT data series;

means for analyzing the movement of the contrast agent through the tissue sample based on the OCT data series.

16. The system of claim 15, wherein the field-of-view in the tissue sample is repeatedly imaged as the contrast agent moves through the field-of-view in the tissue sample.

17. The system of claim 15, wherein the parameter is one of: (1) a transit time distribution associated with the movement of the contrast agent through the tissue sample, (2) a flow rate associated with the movement of the contrast agent through the tissue sample, or (3) a volume associated with the contrast agent that moves through the tissue sample.

18. The system of claim 15, wherein the contrast agent is introduced into the bloodstream of the animal or human.

19. The system of claim 15, wherein the contrast agent is endogenously derived from the animal or human.

20. The system of claim 15, wherein said repeatedly imaging the field-of-view in the tissue sample commences either prior to or immediately following the administration of the contrast agent to the animal or human.

21. The system of claim 15, wherein said field-of-view is repeatedly imaged at a single point, line, 2-D area, or 3-D volume.

22. The system of claim 15, wherein imaging is sufficiently fast to acquire at least one complete time point in the data series during a first passage of the contrast agent through the field-of-view, but before recirculation.

23. The system of claim 15, wherein said analyzing includes applying angiography techniques based on OCT amplitude/intensity and/or phase to a portion of the OCT data series that was obtained during the first passage of the contrast agent.

24. The system of claim 15, wherein said analyzing includes applying angiography techniques based on OCT amplitude/intensity and/or phase to a portion of the OCT data series that was obtained after the first passage of the contrast agent.

25. The system of claim 15, wherein said analyzing includes using a tracer kinetics analysis technique to characterize the movement of the contrast agent through the tissue sample.

26. The system of claim 15, wherein said analyzing includes computing a baseline measurement value before the contrast agent arrives at the field-of-view.

27. The system of claim 15, wherein the contrast agent is one of: (1) Intralipid with a 10%-30% concentration, (2) a substance that increases or decreases scattering within the sample, (3) a substance that is polarization sensitive, (4) a substance that causes the OCT signal to deviate from baseline due to passage of the tracer, or (5) a mixture of contrast agents for OCT and one or more other imaging modalities for multi-modal imaging.

28. The system of claim 15, wherein the OCT technique involves using low-coherence optical interferometry that uses near infrared light to capture images with micrometer resolution from the tissue sample.

29. A method, comprising:

extracting a first tissue sample from an animal or human;

processing the first tissue sample to obtain a processed tissue sample that is fully biocompatible and non-immunogenic with respect to the animal or human;

5 administering the processed tissue sample to the animal or human, thereby causing the processed tissue sample to move through a field-of-view in a second tissue sample in the animal or human;

obtaining one or more of images by imaging the field-of-view in the second tissue sample at one or more time instances; and

10 analyzing the movement of the processed tissue sample through the second tissue sample by analyzing the one or more images.

30. The method of claim 29, wherein said analyzing includes analyzing a contrast between multiple images, wherein the contrast between the multiple images is created by the movement of the processed tissue sample through the field-of-view in the second tissue sample.

15 31. The method of claim 29, wherein the one or more images are obtained by using optical coherence tomography (OCT).

32. The method of claim 29, wherein the one or more images are obtained by using near-infrared spectroscopy (NIRS).

20 33. The method of claim 29, wherein the one or more images are obtained by using diffuse correlation spectroscopy (DCS).

34. The method of claim 29, wherein the one or more images are obtained as the processed tissue sample moves through the field-of-view in the second tissue sample.

25 35. The method of claim 29, wherein the parameter is one of: (1) a transit time distribution associated with the movement of the processed tissue sample through the second tissue sample, (2) a flow rate associated with the movement of the processed tissue sample through the second tissue sample, or (3) a volume associated with the processed tissue sample that moves through the second tissue sample.

30 36. The method of claim 29, wherein said analyzing includes using a tracer kinetics analysis technique to characterize the movement of the processed tissue sample through the second tissue sample.

37. The method of claim 29, wherein said analyzing includes computing a baseline measurement value before the processed tissue sample arrives at the field-of-view.

38. A system, comprising:

5 means for extracting a first tissue sample from an animal or human;

means for processing the first tissue sample to obtain a processed tissue sample that is fully biocompatible and non-immunogenic with respect to the animal or human;

10 means for administering the processed tissue sample to the animal or human, thereby causing the processed tissue sample to move through a field-of-view in a second tissue sample in the animal or human;

means for obtaining one or more of images by imaging the field-of-view in the second tissue sample at one or more time instances; and

means for analyzing the movement of the processed tissue sample through the second tissue sample by analyzing the one or more images.

15 39. The system of claim 38, wherein said analyzing includes analyzing a contrast between multiple images, wherein the contrast between the multiple images is created by the movement of the processed tissue sample through the field-of-view in the second tissue sample.

40. The system of claim 38, wherein the one or more images are obtained by using optical coherence tomography (OCT).

20 41. The system of claim 38, wherein the one or more images are obtained by using near-infrared spectroscopy (NIRS).

42. The system of claim 38, wherein the one or more images are obtained by using diffuse correlation spectroscopy (DCS).

25 43. The system of claim 38, wherein the one or more images are obtained as the processed tissue sample moves through the field-of-view in the second tissue sample.

30 44. The system of claim 38, wherein the parameter is one of: (1) a transit time distribution associated with the movement of the processed tissue sample through the second tissue sample, (2) a flow rate associated with the movement of the processed tissue sample through the second tissue sample, or (3) a volume associated with the processed tissue sample that moves through the second tissue sample.

45. The system of claim 38, wherein said analyzing includes using a tracer kinetics analysis technique to characterize the movement of the processed tissue sample through the second tissue sample.

46. The method of claim 29, wherein said analyzing includes computing a baseline
5 measurement value before the processed tissue sample arrives at the field-of-view.

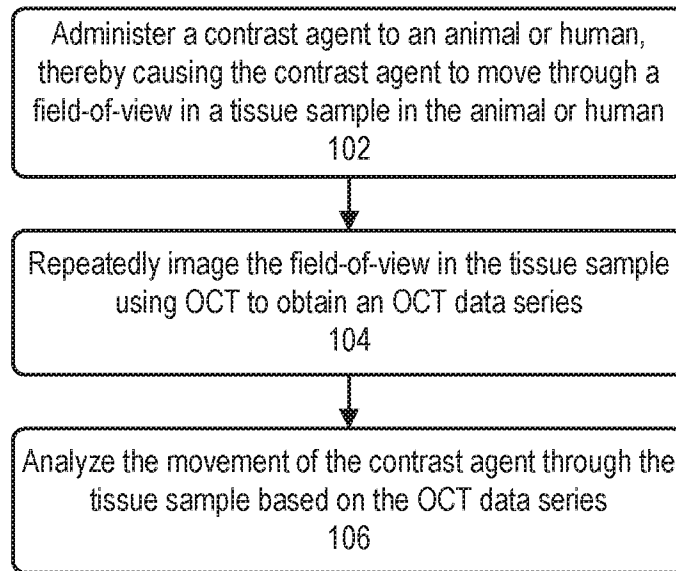


FIG. 1A

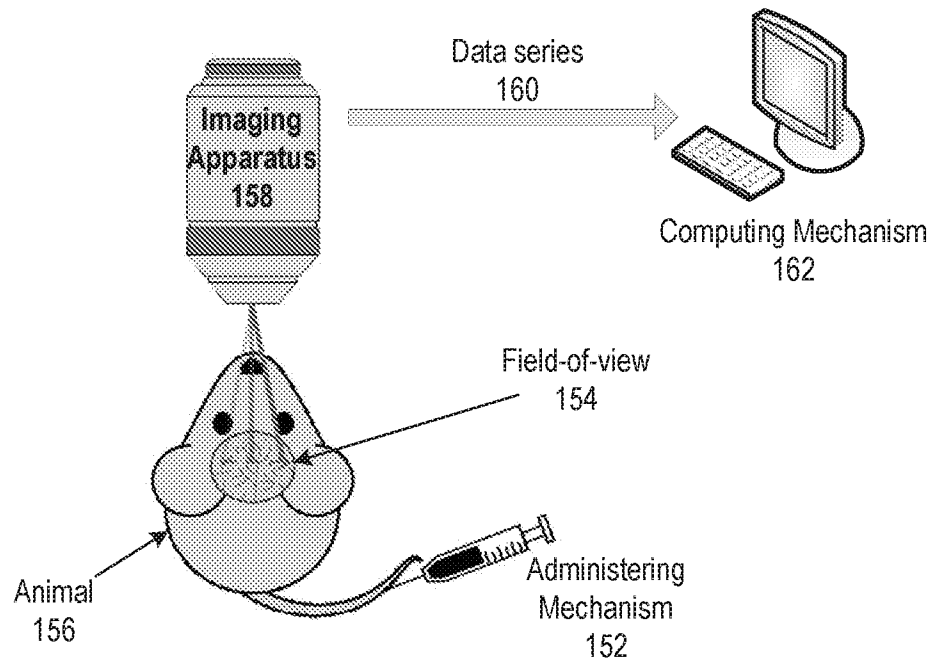


FIG. 1B

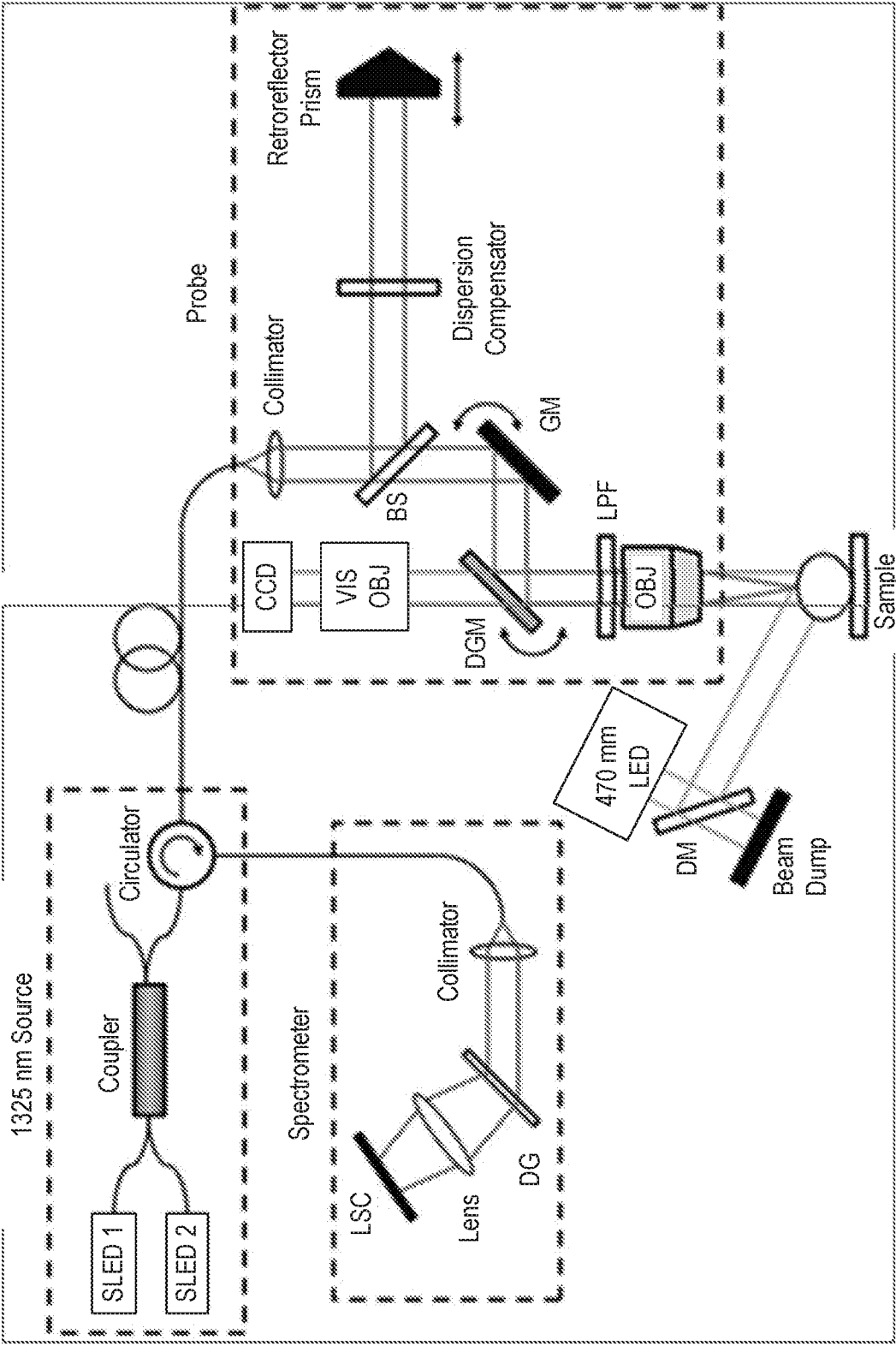


FIG. 2

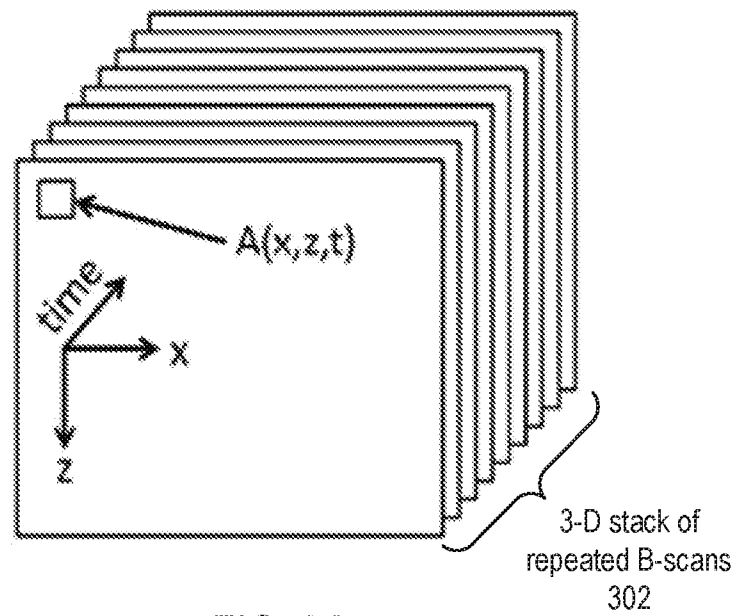


FIG. 3A

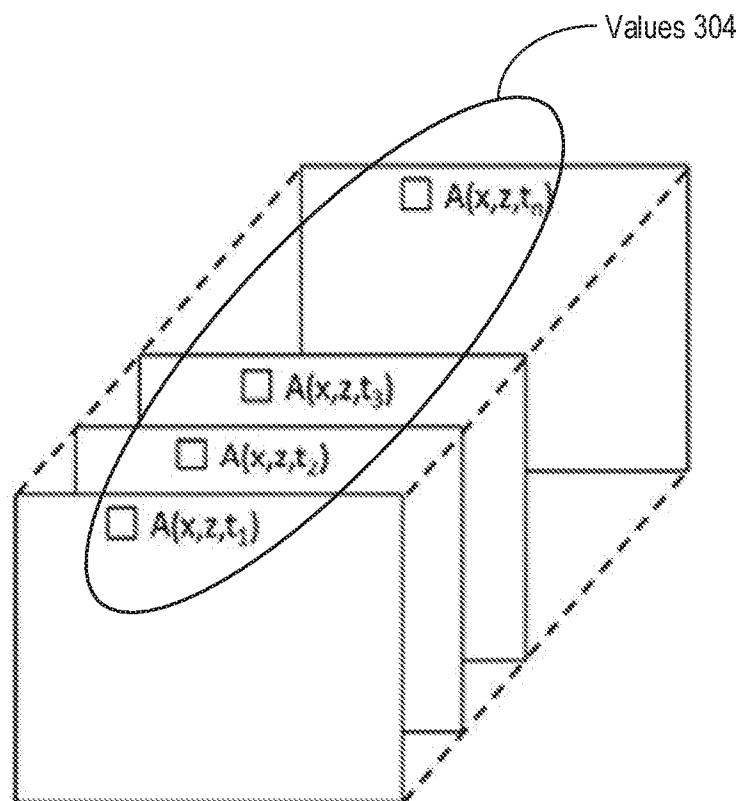


FIG. 3B

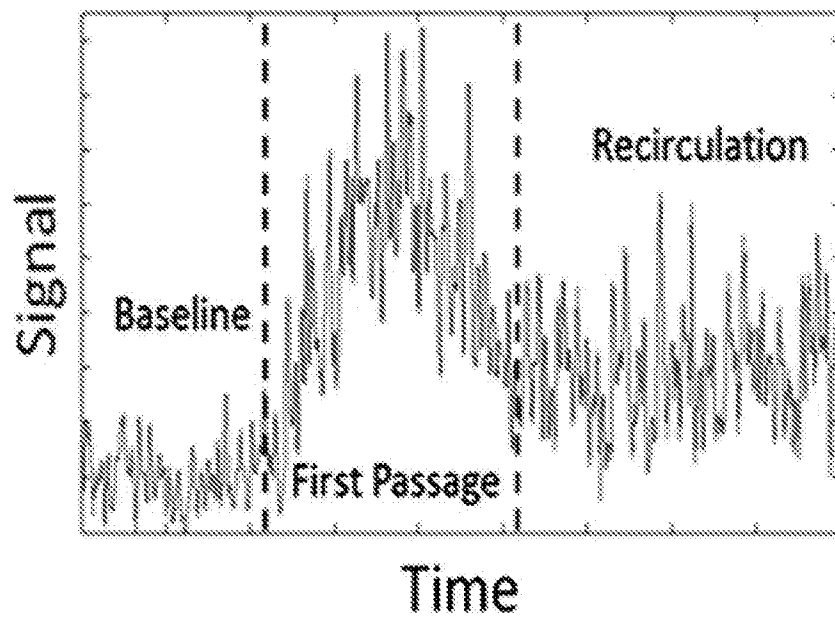


FIG. 3C

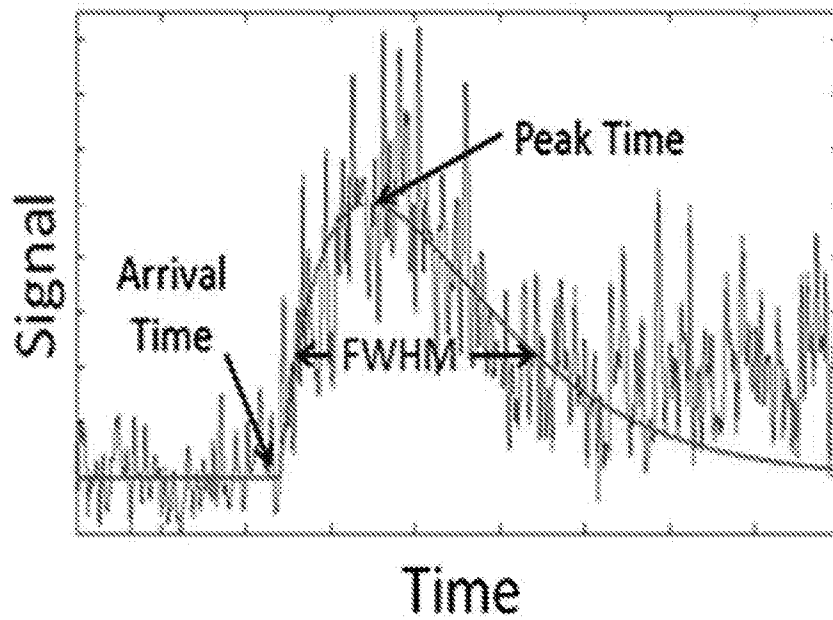


FIG. 3D

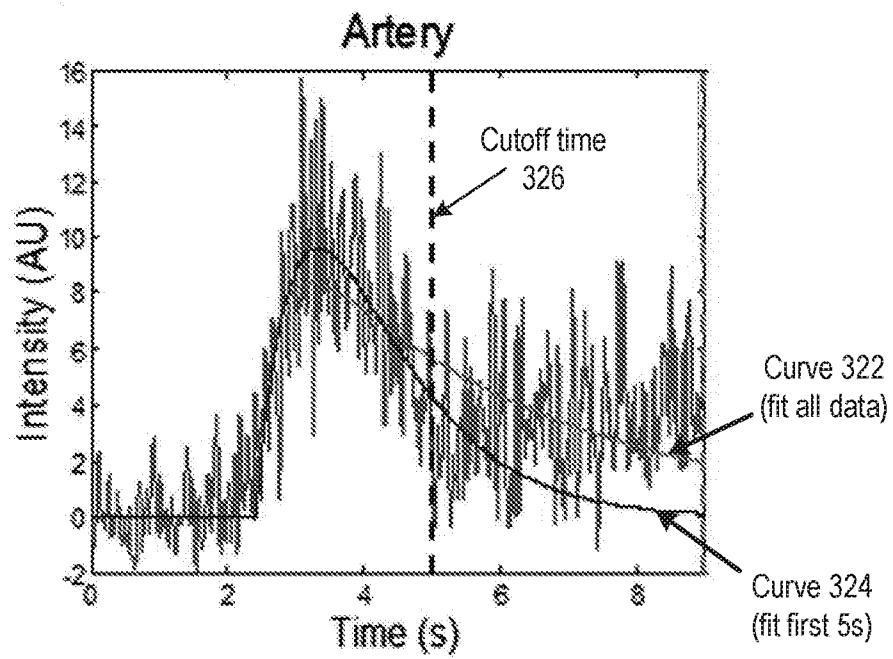


FIG. 3E

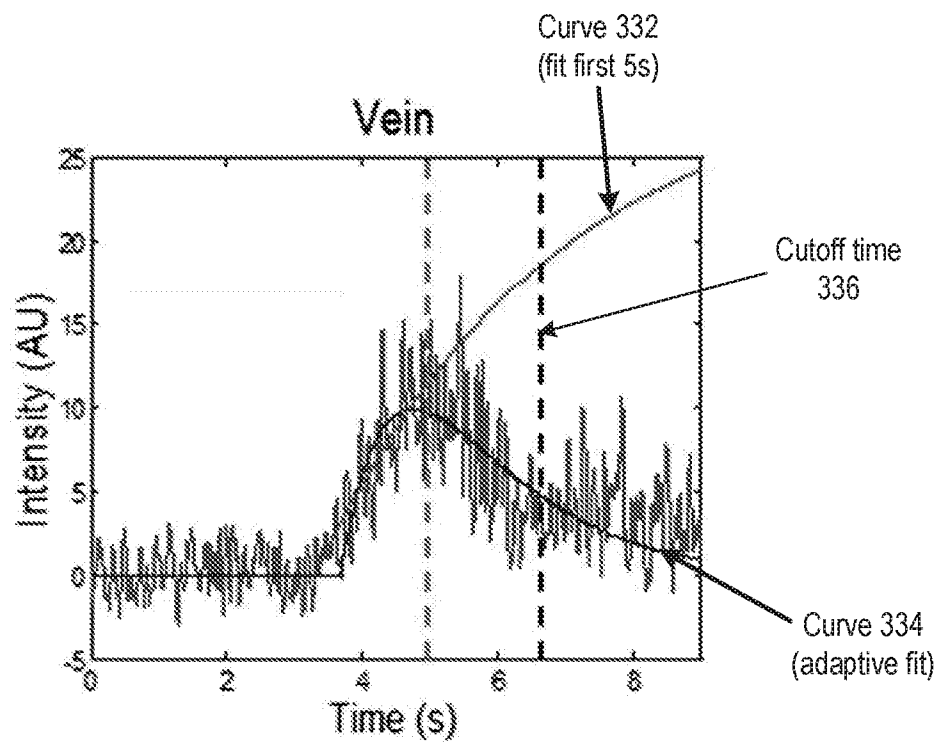
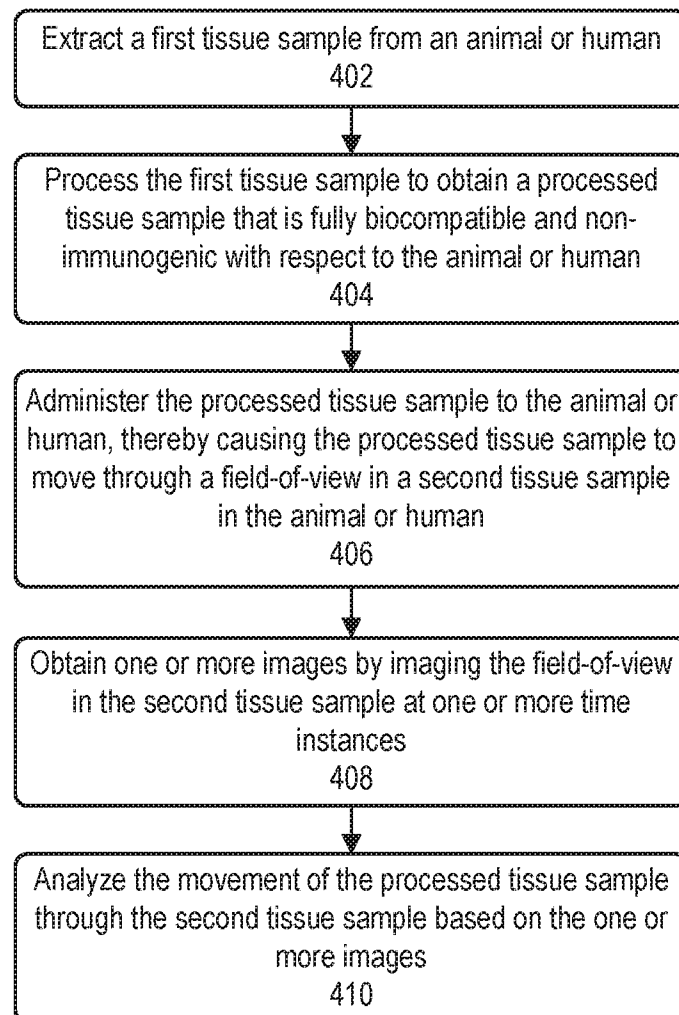


FIG. 3F

**FIG. 4A**

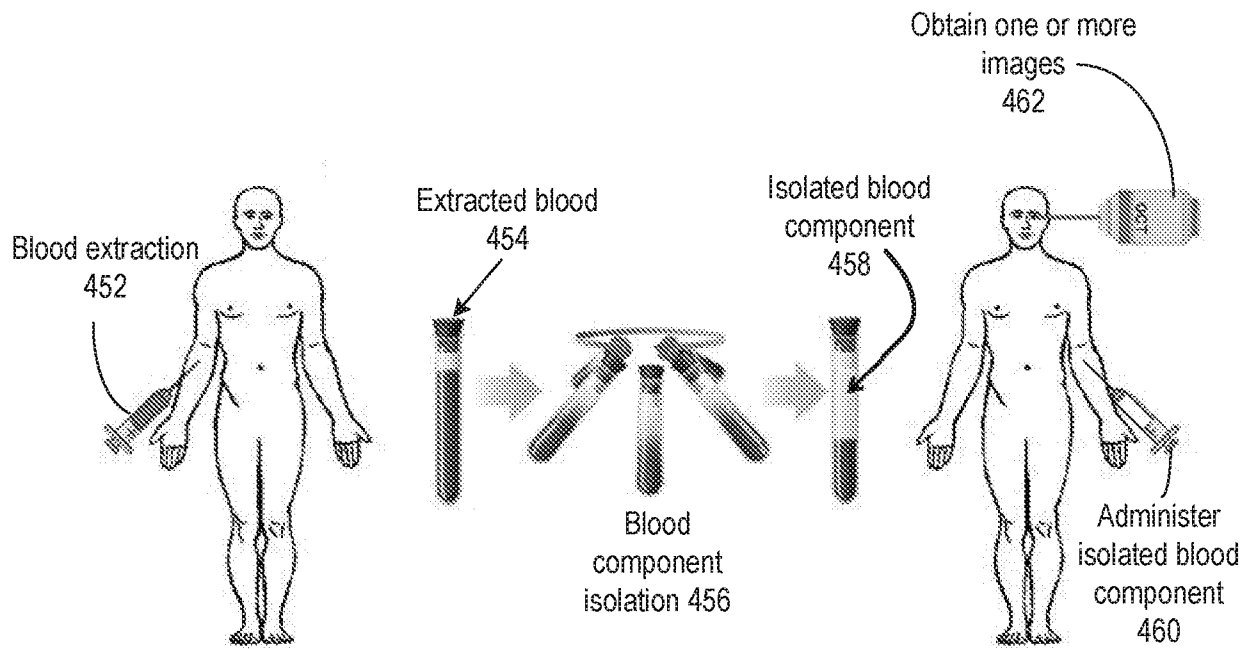


FIG. 4B

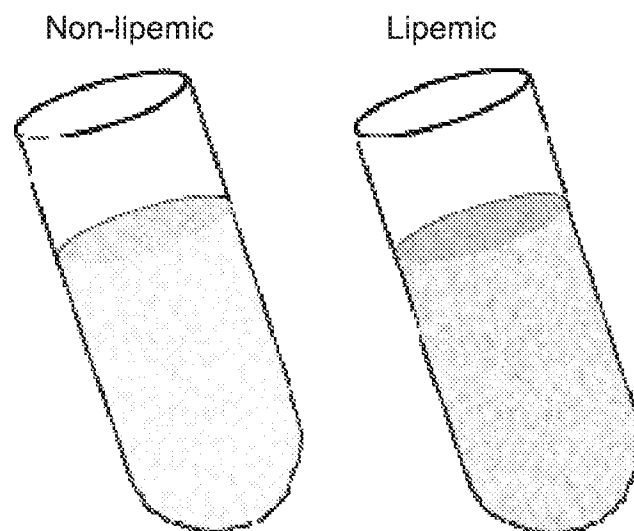


FIG. 5

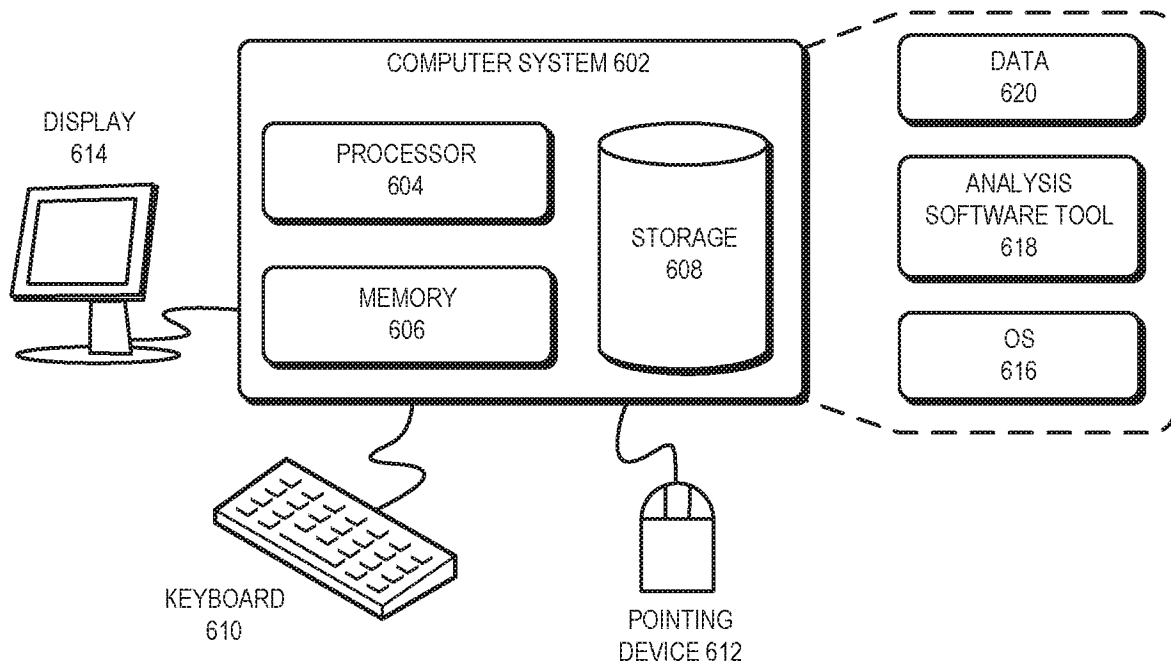


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/32746

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01B 9/00, 9/02 (2016.01)

CPC - G01B 9/02001, 9/02002, 9/02091

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)

IPC(8): G01B 9/00, 9/02 (2016.01)

CPC: G01B 9/02001, 9/02002, 9/02091; USPC: 356/477, 479; 600/476, 478

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 practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google; Google Scholar; Google Patent; EBSCO.

Search terms: opti*, coher*, tomograph*, contrast*, agent*, medi*, movement*, distribut*, dispers*, displac*, inject*, introduc*, bloodstream*, blood_stream*, vein*, artery*, vascular*, angiograph*, tracer*, marker*, kinetic*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2005/0036150 A1 (IZATT, JA, et al.) February 17, 2005; abstract; paragraphs [0004], [0036]-[0038], [0043]-[0049], [0069], [0081]-[0082], [0126]-[0131]; claim 1	1-3, 5-8, 12-17, 19-22, 26-28 ----- 4, 9-11, 18, 23-25
Y	US 2007/0038125 A1 (KLEEN, M, et al.) February 15, 2007; abstract	4, 18
Y	US 2014/0073917 A1 (OREGON HEALTH AND SCIENCE UNIVERSITY) March 13, 2014; paragraph [0112]	9-10, 23-24
Y	US 2010/0030069 A1 (PETER, J) February 04, 2010; paragraphs [0025], [0053]-[0054]	11, 25



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"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

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

16 July 2016 (16.07.2016)

Date of mailing of the international search report

16 SEP 2016

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/32746

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-Continued Within the Next Supplemental Box-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-28

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US16/32746

Continuation of Box No. III - Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-28 are directed toward a method for administering a contrast agent to an animal or human and analyzing the movement of the contrast agent through the tissue sample based on the OCT data series.

Group II: Claims 29-46 are directed toward a method for extracting a first tissue sample from an animal or human and analyzing the movement of the processed tissue sample through the second tissue sample by analyzing the one or more images.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I includes at least administering a contrast agent to an animal or human, thereby causing the contrast agent to move through a field-of-view in a tissue sample in the animal or human; which are not present in Group II.

The special technical features of Group II include at least, extracting a first tissue sample from an animal or human; processing the first tissue sample to obtain a processed tissue sample that is fully biocompatible and non-immunogenic with respect to the animal or human; administering the processed tissue sample to the animal or human, thereby causing the processed tissue sample to move through a field-of-view in a second tissue sample in the animal or human, which are not present in Group I.

The common technical features of Groups I and II are means for obtaining one or more of images by imaging the field-of-view in the tissue sample at one or more time instances and a means for analyzing movement.

These common technical features are disclosed by US20050036150 A1 to Izatt, et al. (hereinafter 'Izatt'). Izatt discloses a means for obtaining one or more of images by imaging the field-of-view in the tissue sample at one or more time instances and a means for analyzing movement (acquiring a plurality of optical coherence tomography images of a tissue sample over time and determining concentration and displacement of a contrast agent; abstract; paragraph [0038]).

Since the common technical features are previously disclosed by Izatt, the common features are not special and so Groups I and II lack unity.