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(71) Demandeur/Applicant:
EAST CAROLINA UNIVERSITY, US

(72) Inventeurs/Inventors:
CHEN, CHENG, US;
FERGUSON, BRUCE T., JR., US;
PENG, ZHIYONG, US;
JACOBS, KENNETH MICHAEL, US

(74) Agent: AIRD & MCBURNEY LP

(54) Titre : PROCEDES ET SYSTEMES D'IMAGERIE LASER MULTISPECTRALE (MSLI) PERMETTANT UNE IMAGERIE ET UNE QUANTIFICATION D'UNE PERFUSION SANGUINE ET D'UN FLUX SANGUIN

(54) Title: MULTI-SPECTRAL LASER IMAGING (MSLI) METHODS AND SYSTEMS FOR BLOOD FLOW AND PERFUSION IMAGING AND QUANTIFICATION

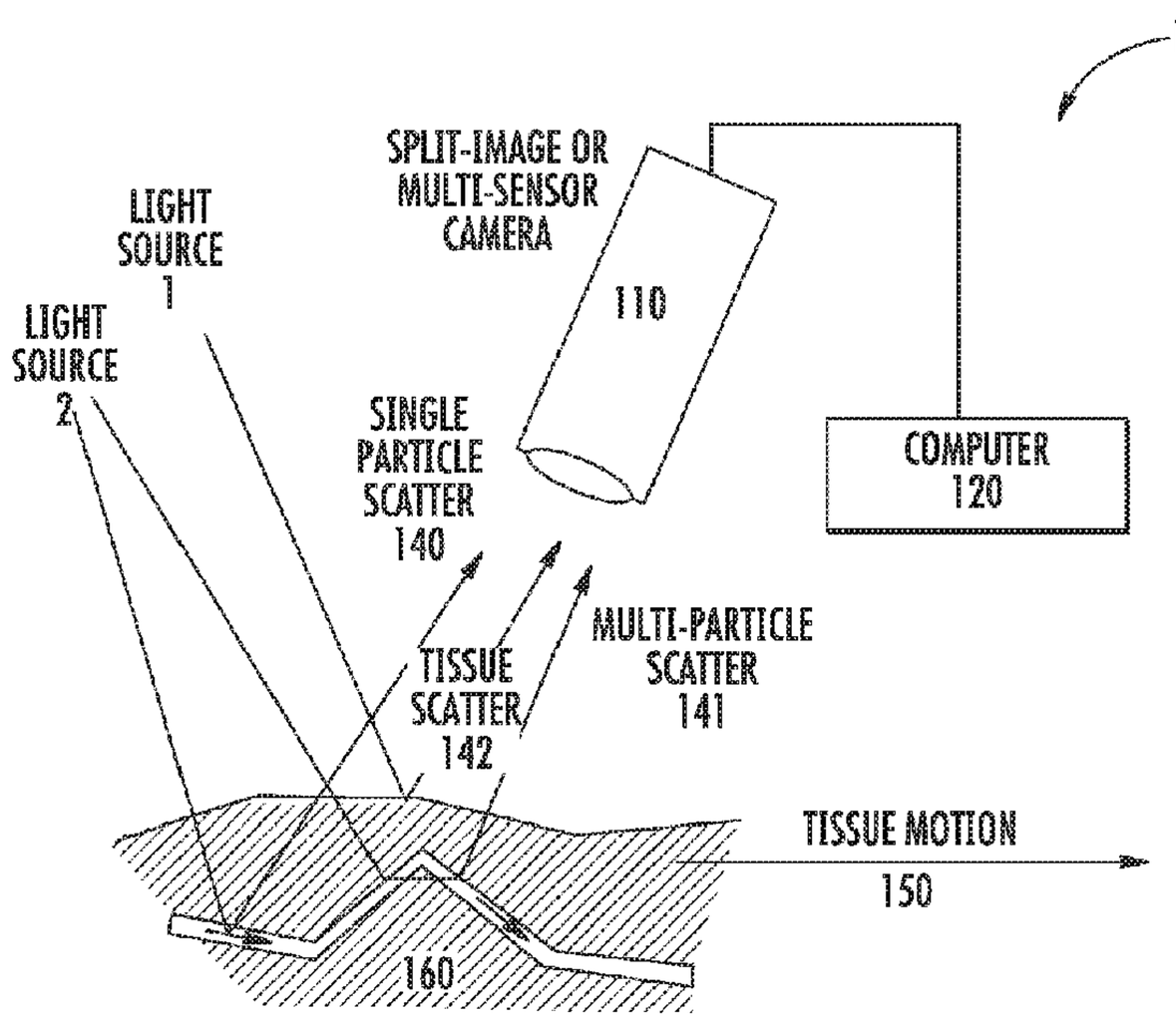


FIG. 1

(57) Abrégé/Abstract:
Some embodiments of the present inventive concept provide a system that uses two wavelengths of differential transmittance through a sample to apply laser speckle or laser Doppler imaging. A first of the two wavelengths is within the visible range that has zero or very shallow penetration. This wavelength captures the anatomical structure of tissue/organ surface and serves as a position marker of the sample but not the subsurface movement of blood flow and perfusion. A second wavelength is in the near Infra-Red (NIR) range, which has much deeper penetration. This wavelength reveals the underlying blood flow physiology and correlates both to the motion of the sample and also the movement of blood flow and perfusion. Thus, true motion of blood flow and perfusion can be derived from the NIR imaging measurement without being affected by the motion artifact of the target.

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(71) **Applicant**: **EAST CAROLINA UNIVERSITY** [US/US]; 2200 Charles Boulevard, Greenville, North Carolina 27858 (US).

(72) **Inventors**: **CHEN, Cheng**; 116F W Victoria Court, Greenville, North Carolina 27834 (US). **FERGUSON, T. Bruce, Jr.**; 8833 Mariner Drive, Raleigh, North Carolina 27615 (US). **PENG, Zhiyong**; 3106 Sherwood Dr., Greenville, North Carolina 27858 (US). **JACOBS, Kenneth Michael**; 2266 Meadowglenn Road, Greenville, North Carolina 27834 (US).

(74) **Agent**: **MYERS BIGEL & SIBLEY, P.A.**; P.O. Box 37428, Raleigh, North Carolina 27627 (US).

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(54) **Title**: MULTI-SPECTRAL LASER IMAGING (MSLI) METHODS AND SYSTEMS FOR BLOOD FLOW AND PERFUSION IMAGING AND QUANTIFICATION

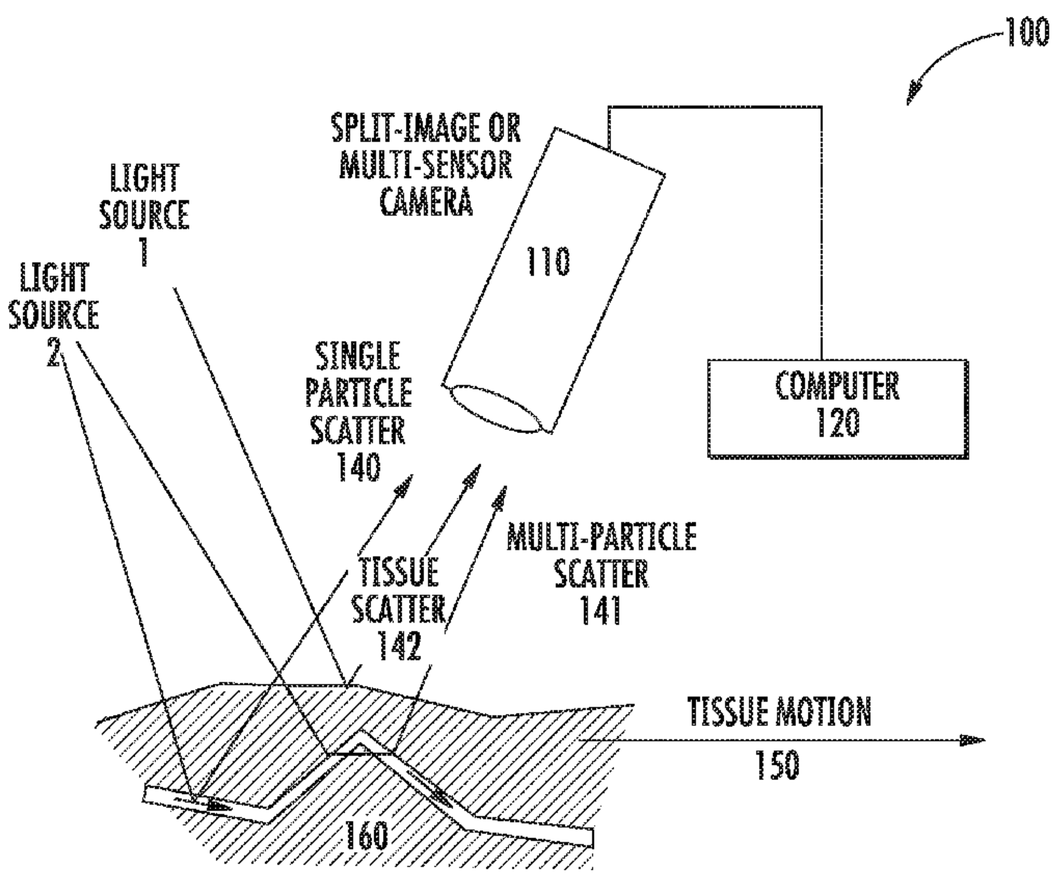


FIG. 1

(57) **Abstract**: Some embodiments of the present inventive concept provide a system that uses two wavelengths of differential transmittance through a sample to apply laser speckle or laser Doppler imaging. A first of the two wavelengths is within the visible range that has zero or very shallow penetration. This wavelength captures the anatomical structure of tissue/organ surface and serves as a position marker of the sample but not the subsurface movement of blood flow and perfusion. A second wavelength is in the near Infra-Red (NIR) range, which has much deeper penetration. This wavelength reveals the underlying blood flow physiology and correlates both to the motion of the sample and also the movement of blood flow and perfusion. Thus, true motion of blood flow and perfusion can be derived from the NIR imaging measurement without being affected by the motion artifact of the target.

WO 2016/153741 A1

MULTI-SPECTRAL LASER IMAGING (MSLI) METHODS AND SYSTEMS FOR BLOOD FLOW AND PERFUSION IMAGING AND QUANTIFICATION

CLAIM OF PRIORITY

[0001] The present application claims the benefit of and priority to U.S. Provisional Application No. 62/136,010, filed March 20, 2015, entitled *Multi-Spectral Laser Imaging (MSLI) Methods and Systems for Blood Flow and Perfusion Imaging and Quantification*, the disclosure of which is hereby incorporated herein by reference as if set forth in its entirety.

RESERVATION OF COPYRIGHT

[0002] A portion of the disclosure of this patent document contains material which is subject to copyright protection. The copyright owner, East Carolina University of Greenville, N.C., has no objection to the reproduction by anyone of the patent document or the patent disclosure, as it appears in the Patent and Trademark Office patent file or records, but otherwise reserves all copyright rights whatsoever.

FIELD

[0003] The present inventive concept relates generally to blood flow and perfusion quantification and, more particularly, to quantification of blood flow and perfusion in terms of distributions of blood velocity and blood flow rate in tissue/organs using imaging techniques, such as Laser Speckle Imaging, Laser Doppler Imaging and the like with multispectral capability.

BACKGROUND

[0004] The measurement results of blood flow and perfusion imaging technologies are typically disrupted by a motion artifact of the target tissue/organ in clinical circumstances. This movement can be micro (*i.e.*, pulsatility of an arteriole due to systole and diastole blood pressure levels), intermediate (*i.e.*, normal peristalsis of the small or large bowel) or macro (*i.e.*, the movement of the heart during the cardiac cycle). This movement can be intrinsic to the imaged tissue (i.e., examples cited above), or extrinsic (i.e., the movement of the heart as a result of the movement of the lungs during ventilation). Thus, in many clinical situations, where accurate quantification of flow and perfusion is desirable, keeping the imaging target

in a stationary status is difficult and, in some clinical scenarios, is not even possible. For example, such as imaging the distributions of blood flow velocity and flow rate for quantifying perfusion in coronary arteries and myocardium of a beating heart. Unfortunately, most conventional laser-based perfusion technologies either assume the target tissue/organ is stationary, which introduces significant inaccuracy or error in the clinical measurement of blood speed or velocity where the target is moving, such as a beating heart, or are simply provide no information for quantification of perfusion in terms of blood flow rate distribution that is critically needed in the clinical situation where the target may or may not be moving.

[0005] Tissues/organs in animals or human respond differently to light of different wavelengths. In general, light of shorter wavelengths can penetrate only the superficial layers of the tissues while light of longer wavelengths can penetrate both superficial layers and sub-surface layers in the spectral region from ultraviolet (UV) to near-infrared (NIR). UV and visible light of wavelengths less than, for example, 550nm is optimal for detailed anatomic visualization in medicine when viewing the surface of tissues and organs. However, unlike NIR light, UV or visible light imaging is usually not inherently capable of revealing the physiological characteristics of tissues/organs in sub-surface layers, in part due to lack of penetration of the tissues/organs. Accordingly, improved methods of visualization and quantification are desired.

SUMMARY

[0006] Some embodiments of the present inventive concept provide multispectral imaging systems including a first light source having a first wavelength configured to image a sample; a second light source, different from the first light source, having a second wavelength, different from the first wavelength, configured to image the sample; a camera configured to receive information, for example, scattered light related to the first and second light sources from the sample, wherein the first wavelength is configured to reflect off a surface of the sample into the camera and the second wavelength is configured to penetrate the sample and provide information related to the sample to the camera; and a processor configured to combine the information related to the first and second light sources provided by the camera to image an anatomical structure of the sample, image physiology of blood flow and perfusion of the sample and/or synthesize the anatomical structure and the physiology of blood flow and perfusion of the sample in terms of blood flow rate distribution.

[0007] In further embodiments, the first and second wavelengths have different wavelengths in a range from 350 nm to 1100 nm.

[0008] In still further embodiments, the first wavelength may be in an ultraviolet (UV) or visible spectrum and the second wavelength may be in a visible or near-infrared spectrum.

[0009] In some embodiments, the sample may be at least one of tissue and an organ.

[00010] In further embodiments, the processor may be further configured to reconstruct a color image using one or more monochromatic cameras in real time.

[00011] In still further embodiments, the processor may be further configured to acquire scattered light in a visible or near-infrared (NIR) spectrum to provide deeper tissue information.

[00012] In some embodiments, an output of the system may provide a unique clarity of visualization.

[00013] In further embodiments, the processor may be further configured to quantitatively analyze the anatomical structure and physiology of blood flow and perfusion of the sample in terms of blood flow rate distribution.

[00014] In still further embodiments, the processor may be further configured to separate motion of the tissues/organs from motion of blood flow and perfusion in the imaged tissues/organs.

[00015] In some embodiments, the processor may be further configured to remove motion artifacts of the imaged sample, for example, tissues/organs, caused by physiologic and/or pathophysiologic movement of the imaged sample in order to improve accuracy of quantification of blood flow and perfusion.

[00016] In further embodiments, the processor may be further configured to remove motion artifact of the images sample caused by movement of an imaging platform/camera in order to improve accuracy of quantification of blood flow and perfusion.

[00017] In still further embodiments, the processor may be further configured to improve quantification accuracy in laser-based blood flow and perfusion measuring technologies by removing motion artifacts.

[00018] In some embodiments, the perfusion measuring technologies may include laser speckle imaging (LSI), laser Doppler imaging (LDI), Florescence imaging, reflectance imaging and/or LSI plus Fluorescence.

[00019] In further embodiments, the processor may be further configured to improve quantification accuracy in laser-based blood flow and perfusion measuring technologies by removing static background caused by a difference of the optical characteristics of an inhomogeneous scattering media.

[00020] In still further embodiments, the processor may be further configured to display anatomical structure and the physiology of blood flow and perfusion of an imaged sample, for example, an imaged tissue/organ simultaneously in real time.

[00021] In some embodiments, the processor may be further configured to image the anatomical structure and blood flow physiology at different depths in the sample.

[00022] In further embodiments, the first wavelength may be configured to extend from between 350nm to 550nm to between 300nm to 600nm into the sample and the second wavelength may be configured to penetrate the sample between 550nm to 1100nm to between 500nm to 1500nm.

[00023] Still further embodiments provide related methods and computer program products.

BRIEF DESCRIPTION OF THE DRAWINGS

[00024] Figure 1 is a block diagram illustrating a system implementing dual wavelength imaging in accordance with some embodiments of the present inventive concept.

[00025] Figure 2 is a more detailed block diagram illustrating various components of a multi-wavelength imaging system in accordance with some embodiments of the present inventive concept.

[00026] Figure 3 is a block diagram of a data processing system according to some embodiments of the present inventive concept(s).

[00027] Figure 4 is a more detailed block diagram of the data processing system illustrated in Figure 3 in accordance with some embodiments of the present inventive concept(s).

[00028] Figures 5A and 5B are a visible light image (5A) and a near infra-red light image (5B) of a hand.

[00029] Figures 6A and 6B are images illustrating the perfusion measurement using only near infra-red light (6A) and dual wavelength illumination (6B) of a stationary hand.

[00030] Figures 7A and 7B are images illustrating the perfusion measurement using only near infra-red light (7A) and dual wavelength illumination 7B) of a shaking hand.

[00031] Figures 8A and 8B are images illustrating the perfusion measurement using only near infra-red light (8A) and dual wavelength illumination (8B) of a stationary hand with blood supply temporarily occluded by squeezing the wrist of the imaged hand using the other hand.

[00032] Figures 9A and 9B illustrated perfusion measurement using only near infra-red light (9A) and dual wavelength illumination (9B) of a large bowel of a pig.

[00033] Figures 10A-10D are images illustrating a visible light image of a piece of small bowel of a pig as to define anatomical structure (10A); a near infra-red light image of the same piece of small bowel as to define the transparency map (10B); blood flow speed distribution map of the same piece of small bowel calculated by 11 frames of the NIR raw images using LSI (10C); and a combined visual effect using A, B, C using an algorithm in accordance with some embodiments of the present inventive concept to reveal both anatomical structure and blood flow physiology (10D).

[00034] Figures 11A-11C are images illustrating a visible light image of a piece of small bowel of a pig as to define anatomical structure by the brightness of the 8 bit grayscale image (11A); blood flow speed distribution map of the same piece of small bowel calculated by 11 frames of the NIR raw images using LSI (11B); and a combined visual effect using A and B using an algorithm in accordance with some embodiments of the present inventive concept to reveal both anatomical structure and blood flow physiology (11C).

[00035] Figures 12A-12D are images illustrating Panel A, an NIR 785nm image of a small bowel (12A); Panel B a Green 532nm image of the same small bowel (12B); Panel C, a reconstructed image of the same small bowel (12C); and Panel D, an image of the same small bowel taken by a regular camera (12D).

[00036] Figures 13A-13D are images illustrating Panel A, an NIR 785nm image of a pig heart (13A); Panel B, Green 532nm image of the same pig heart (13B); Panel C, a reconstructed image of the same pig heart (13C); and Panel D, an image of the same pig heart taken by a regular camera (13D).

[00037] Figures 14A-14E illustrate an image using a visible wavelength (532nm) (14A); an image using near infra-red wavelength (785nm) (14B); a reconstructed image (in gray scale) with the visible and infrared wavelengths (14C); a regular image with room light illumination (14D); and an image showing blood flow and perfusion image (14E).

[00038] Figures 15A-19B illustrate images that compensate for issues during clinical imaging procedures in accordance with some embodiments of the present inventive concept.

DETAILED DESCRIPTION

[00039] Embodiments of the present inventive concept will now be described more fully hereinafter with reference to the accompanying figures, in which preferred embodiments of the inventive concept are shown. This inventive concept may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. Like numbers refer to like elements throughout. In the figures, layers, regions,

elements or components may be exaggerated for clarity. Broken lines illustrate optional features or operations unless specified otherwise.

[00040] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the inventive concept. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" and/or "comprising," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items. As used herein, phrases such as "between X and Y" and "between about X and Y" should be interpreted to include X and Y. As used herein, phrases such as "between about X and Y" mean "between about X and about Y." As used herein, phrases such as "from about X to Y" mean "from about X to about Y."

[00041] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this inventive concept belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the specification and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. Well-known functions or constructions may not be described in detail for brevity and/or clarity.

[00042] It will be understood that when an element is referred to as being "on", "attached" to, "connected" to, "coupled" with, "contacting", etc., another element, it can be directly on, attached to, connected to, coupled with or contacting the other element or intervening elements may also be present. In contrast, when an element is referred to as being, for example, "directly on", "directly attached" to, "directly connected" to, "directly coupled" with or "directly contacting" another element, there are no intervening elements present. It will also be appreciated by those of skill in the art that references to a structure or feature that is disposed "adjacent" another feature may have portions that overlap or underlie the adjacent feature.

[00043] It will be understood that, although the terms first, second, etc. may be used herein to describe various elements, components, regions, layers and/or sections, these elements, components, regions, layers and/or sections should not be limited by these terms. These

terms are only used to distinguish one element, component, region, layer or section from another element, component, region, layer or section. Thus, a first element, component, region, layer or section discussed below could be termed a second element, component, region, layer or section without departing from the teachings of the inventive concept. The sequence of operations (or steps) is not limited to the order presented in the claims or figures unless specifically indicated otherwise.

[00044] Spatially relative terms, such as "under", "below", "lower", "over", "upper" and the like, may be used herein for ease of description to describe one element or feature's relationship to another element(s) or feature(s) as illustrated in the figures. It will be understood that the spatially relative terms are intended to encompass different orientations of the device in use or operation in addition to the orientation depicted in the figures. For example, if a device in the figures is inverted, elements described as "under" or "beneath" other elements or features would then be oriented "over" the other elements or features. Thus, the exemplary term "under" can encompass both an orientation of over and under. The device may be otherwise oriented (rotated 90 degrees or at other orientations) and the spatially relative descriptors used herein interpreted accordingly. Similarly, the terms "upwardly", "downwardly", "vertical", "horizontal" and the like are used herein for the purpose of explanation only unless specifically indicated otherwise.

[00045] As will be appreciated by one of skill in the art, embodiments of the present inventive concept may be embodied as a method, system, data processing system, or computer program product. Accordingly, the present inventive concept may take the form of an embodiment combining software and hardware aspects, all generally referred to herein as a "circuit" or "module." Furthermore, the present inventive concept may take the form of a computer program product on a non-transitory computer usable storage medium having computer usable program code embodied in the medium. Any suitable computer readable medium may be utilized including hard disks, CD ROMs, optical storage devices, or other electronic storage devices.

[00046] Computer program code for carrying out operations of the present inventive concept may be written in an object oriented programming language such as Matlab, Mathematica, Java, Smalltalk, C or C++. However, the computer program code for carrying out operations of the present inventive concept may also be written in conventional procedural programming languages, such as the "C" programming language or in a visually oriented programming environment, such as Visual Basic.

[00047] It will be understood that some embodiments of the present inventive concept implemented in Matlab may provide improved processing speeds in accordance with some embodiments of the present inventive concept.

[00048] Certain of the program code may execute entirely on one or more of a user's computer, partly on the user's computer, as a standalone software package, partly on the user's computer and partly on a remote computer or entirely on the remote computer. In the latter scenario, the remote computer may be connected to the user's computer through a local area network (LAN) or a wide area network (WAN), or the connection may be made to an external computer (for example, through the Internet using an Internet Service Provider).

[00049] The inventive concept is described in part below with reference to flowchart illustrations and/or block diagrams of methods, devices, systems, computer program products and data and/or system architecture structures according to embodiments of the inventive concept. It will be understood that each block of the illustrations, and/or combinations of blocks, can be implemented by computer program instructions. These computer program instructions may be provided to a processor of a general-purpose computer, special purpose computer, or other programmable data processing apparatus to produce a machine, such that the instructions, which execute via the processor of the computer or other programmable data processing apparatus, create means for implementing the functions/acts specified in the block or blocks.

[00050] These computer program instructions may also be stored in a computer readable memory or storage that can direct a computer or other programmable data processing apparatus to function in a particular manner, such that the instructions stored in the computer-readable memory or storage produce an article of manufacture including instruction means which implement the function/act specified in the block or blocks.

[00051] The computer program instructions may also be loaded onto a computer or other programmable data processing apparatus to cause a series of operational steps to be performed on the computer or other programmable apparatus to produce a computer implemented process such that the instructions which execute on the computer or other programmable apparatus provide steps for implementing the functions/acts specified in the block or blocks.

[00052] The present inventive concept relates generally to blood flow and perfusion quantification and, more particularly, to quantification of blood flow and perfusion in tissue/organs in terms of distributions of blood velocity and blood flow rate using imaging techniques, such as Laser Speckle Imaging (LSI), Laser Doppler Imaging (LDI), Florescence

imaging, reflectance imaging and the like with multispectral capability. Some embodiments of the inventive concept use two or more wavelengths in the range from 350 nm to 1100 nm to measure/quantify the blood velocity and blood flow rate distributions for quantification of perfusion, remove motion artifact and enhance visualization for presentation and real-time evaluation and assessment of the synthesized anatomical-physiological result. As used here, "Multispectral Laser Imaging (MSLI)" refers to imaging techniques using two or more wavelengths in accordance with some embodiments of the present inventive concept.

[00053] In particular, some embodiments of the present inventive concept provide a system that uses two wavelengths of differential transmittance through a sample to apply laser speckle or laser Doppler imaging. A first of the two wavelengths may be relatively small within the UV or visible range that has, such as blue light 450–495 nm. Light at this wavelength has very shallow penetration and images the anatomical structure of tissue/organ surface and serves as a position marker of the sample but not the subsurface movement of blood flow and perfusion. A second wavelength may be relatively large in the visible or near Infra-Red (NIR) range. Light at this wavelength has much larger penetration depth and reveals the underlying blood flow physiology and correlates both to the motion of the sample and also the movement of blood flow and perfusion. Using the imaging measurement of the visible light as a baseline, the true motion of blood flow and perfusion can be derived from the NIR imaging measurement without being affected by the motion artifact of the target. Furthermore, the anatomical structure information captured by visible light and the physiological characteristics measured by NIR light is combined as will be discussed herein.

[00054] As discussed in the background of the present application, using only visible or NIR spectrums may result in various issues with the final images produced. Accordingly, some embodiments of the present inventive concept combine different wavelengths of visible and NIR spectrum (350 nm – 1100 nm) into an imaging system, such as LSI, LDI, Fluorescence, Reflectance or LSI plus Fluorescence and the like. The combination, as discussed herein, may reveal much more information of the tissue/organ than using one single wavelength. In particular, MSLI in accordance with some embodiments discussed herein can (1) account for and remove the motion artifact present in imaging clinical biologic structures, which creates blood flow and perfusion quantification inaccuracies; (2) improve visualization over current technologies by exact synthesis of both anatomic structure and the physiology of blood flow and perfusion simultaneously in real time; (3) through a combination of (1) and (2), improve the accuracy of quantification of blood flow and

perfusion in clinical applications as will be discussed herein with respect to Figures 1 through 19B.

[00055] In some embodiments, in addition to using multiple wavelengths over the visible and NIR spectrum (350-1100 nm), embodiments of the present inventive concept can, for example, combine two or more laser imaging techniques such as near infra-red fluorescence (NIRF) and Laser Speckle Imaging (LSI), or NIRF and Laser Doppler Imaging (LDI), into one system as will also be discussed below with respect to the Figures.

[00056] Referring first to Figure 1, a block diagram illustrating a simplistic system implementing dual wavelength imaging in accordance with some embodiments of the present inventive concept will be discussed. As illustrated in Figure 1, the system 100 includes at least two light sources, first 130 and second 131 light sources, respectively, a sample 160, a camera 110 and a communications device (computer 120). In some embodiments of the present inventive concept, the first light source delivers visible light 130 and the second light source delivers NIR 131 light. As discussed above, the coherent short wavelength 130 (visible source) does not penetrate deep into the sample 160 (tissue/organ), but provides detail of the surface of the sample 160 in the tissue scatter (142). In contrast, the coherent NIR source 131 penetrates deep into the sample 160 and may provide single (140) or multi particle (141) scatter. The reflections 140, 141, 142 off the sample 160 are captured by a camera 110, which may be, for example, a split-image or multi-sensor camera. In particular, in some embodiments the camera may be a multi-sensor camera, rather than a single camera with one sensor chip. The multi-sensor camera has multiple sensors and each sensor is configured to image one wavelength or wavelength range.

[00057] The information can be processed by the communications device 120, which combines the visible and NIR wavelength images to provide improved blood flow and profusion data in accordance with some embodiments of the present inventive concept. As will be understood, the data provided by embodiments discussed herein account for movement 150 of the sample (tissue/organ) 160 and provide a much improved image thereof.

[00058] Referring now to Figure 2, a more detailed block diagram illustrating various components of a multi-wavelength imaging system in accordance with some embodiments of the present inventive concept will be discussed. As illustrated in Figure 2, the system 205 includes at least two laser light sources, visible 230 and NIR 231, a connecting fiber 233, components of an imaging system 237, a sample 260, a beam splitter 280, a camera 210 and a communications device (computer system 220). In operation, when the NIR laser delivers NIR light to a living sample 260, such as a tissue/organ, a portion of the NIR light will go

through single or multiple scattering of both stationary and moving particles inside the sample and reflect back. When the visible laser 230 delivers non-penetrating visible light, such as light having 430nm, to a living sample 260, such as a tissue/organ, most of the light will be reflected back by the surface within less than 100μm depth. For the NIR laser 230, approximately ninety five percent of the light will be returned from a top 700μm of the sample 260, which is enough penetration to pass through coronary artery walls at, for example, a 300μm depth, and generate information from moving particles, such as red blood cells, as well as from stationary tissue.

[00059] The reflected visible light contains the surface movement information of the sample 260 and, thus, reflects the motion artifact. The reflected NIR light contains the surface and subsurface movement information of the sample 260 and, thus, reflects both motion artifact and movement of the blood flow. As illustrated in Figure 2, the light produced by the lasers 230 and 231 may be provided to a fiber 233, which may have multiple fiber legs and may include a plurality of splitting fibers 235 as illustrated. However, embodiments of the present inventive concept are not limited to the configuration illustrated in Figure 2. For example, more or less fibers may be used without departing from a scope of the present inventive concept. Furthermore, the light on the fibers may pass through various elements of an imaging system 237 before reaching the sample 260. For example, the light may traverse polarizers, collimators, expanders, diffusers and the like before reaching the sample 260 without departing from the scope of the present inventive concept.

[00060] The incident light 270 illuminates the sample 260 and the reflected light 275 is provided to a beamsplitter 280. In some embodiments of the present inventive concept, the beamsplitter 280 may be a dichroic beam splitting system that separates the NIR 283 and visible light 285. The separated light 283 and 285 may pass through polarizers, filters and the like 287 before being delivered to the camera 210. As discussed above, the camera 210 can be, for example, a split-image or multi-sensor camera without departing from the scope of the present inventive concept. As stated, the multi-sensor camera has multiple sensors each configured to image a wavelength or wavelength range.

[00061] The NIR 283 and visible 285 images are redirected to the camera 210 and a split image is created on one camera sensor or on separate camera sensors that have been synchronized and aligned. As discussed above, different wavelengths have different penetration levels in the tissue/organ. Using multi-spectrum image design as discussed herein, the anatomical structure and blood flow physiology at different depths in the tissue/organ can be revealed as will be discussed below with respect to various figures.

[00062] As illustrated in Figures 1 and 2, systems in accordance with embodiments of the present inventive concept include communications devices 120, 220, which are used for the various processing necessary to implement embodiments of the present inventive concept. Referring now to Figure 3, a data processing system 300 that may be used in the systems of Figures 1 and 2, for example, in the communications devices 120, 210, in accordance with some embodiments of the inventive concept will be discussed. It will be understood that the data processing system 300 may included in any of the components of the system without departing from the scope of the present inventive concept. For example, the data processing system 300 may be included in the camera 110, 210 or split between various elements of the system without departing from the scope of the present inventive concept.

[00063] Referring now to Figure 3, an exemplary embodiment of a data processing system 300 suitable for use in the systems of Figures 1 and 2 includes a user interface 344 such as a keyboard, keypad, touchpad or the like, I/O data ports 346 and a memory 336 that communicates with a processor 338. The I/O data ports 346 can be used to transfer information between the data processing system 300 and another computer system or a network. These components may be conventional components, such as those used in many conventional data processing systems, which may be configured to operate as described herein.

[00064] Referring now to Figure 4, a more detailed block diagram of the data processing system 400 in accordance with some embodiments of the present inventive concept will be discussed. The processor 338 communicates with a display 445 via and address/data bus 447, the memory 336 via an address/data bus 448 and the I/O data ports 346 via an address/date bus 449. The processor 338 can be any commercially available or custom microprocessor or ASICs. The memory 336 is representative of the overall hierarchy of memory devices containing the software and data used to implement the functionality of the data processing system 400. The memory 336 can include, but is not limited to, the following types of devices: cache, ROM, PROM, EPROM, EEPROM, flash memory, SRAM, and DRAM.

[00065] As illustrated in Figure 4, the memory 336 may include several categories of software and data used in the data processing system 400: an operating system 452; application programs 454; input/output (I/O) device drivers 458; and data 456. As will be appreciated by those of skill in the art, the operating system 452 may be any operating system suitable for use with a data processing system, such as OS/2, AIX or zOS from International Business Machines Corporation, Armonk, NY, Windows95, Windows98, Windows2000, WindowsXP, or Vista from Microsoft Corporation, Redmond, WA, Unix, Linux, LabView,

or a real-time operating system such as QNX or VxWorks, or the like. The I/O device drivers 458 typically include software routines accessed through the operating system 452 by the application programs 454 to communicate with devices such as the I/O data port(s) 346 and certain memory 336 components. The application programs 454 are illustrative of the programs that implement the various features of the data processing system 400 included in a system in accordance with some embodiments of the present inventive concept and preferably include at least one application that supports operations according to some embodiments of the present inventive concept. Finally, the data 456 represents the static and dynamic data used by the application programs 454, the operating system 452, the I/O device drivers 458, and other software programs that may reside in the memory 336.

[00066] As illustrated in Figure 4, the data 456 according to some embodiments of the present inventive concept may include acquired visible images 460, acquired NIR images/data 461, calculated blood flow/perfusion data 463 and images/video 464. Although the data 456 illustrated in Figure 4 includes four different files 460, 461, 463 and 464, embodiments of the present inventive concept are not limited to this configuration. Two or more files may be combined to make a single file; a single file may be split into two or more files and the like without departing from the scope of the present inventive concept.

[00067] As further illustrated in Figure 4, the application programs 454 may include an image processing module 451 and an image capture module 452 in accordance with some embodiments of the inventive concept. While the present inventive concept is illustrated, for example, with reference to the image processing module 451 and the image capture module 452 being application programs in Figure 4, as will be appreciated by those of skill in the art, other configurations may also be utilized while still benefiting from the teachings of the present inventive concept. For example, the image processing module 451 and the image capture module 452 may also be incorporated into the operating system 452 or other such logical division of the data processing system 400. Thus, the present inventive concept should not be construed as limited to the configuration of Figure 4, but is intended to encompass any configuration capable of carrying out the operations described herein.

[00068] Furthermore, while the image processing module 451 and the image capture module 452 are illustrated in a single data processing system, as will be appreciated by those of skill in the art, such functionality may be distributed across one or more data processing systems. Thus, the present inventive concept should not be construed as limited to the configurations illustrated in Figures 3 and 4, but may be provided by other arrangements and/or divisions of function between data processing systems.

[00069] In certain embodiments, such as an LSI application, the velocity of a target fluid can be calculated using the following equation:

$$v(i,j) = v_0 + \frac{a}{C(i,j)^2} \qquad \text{Eqn. (1)}$$

where $v(i,j)$ is the velocity of target fluid, v_0 is an added term to account for background noise and may be zero after the baseline has been removed; a is a constant related to imaging parameters, laser parameters, time/spatial smoothing parameters for obtaining c and reflects the optical characteristics of the target fluid; c is the laser speckle contrast; and i and j are the row and column pixel index.

[00070] For an LDI application, the velocity of a target fluid can be calculated using the following equation:

$$v(i,j) = \frac{\lambda}{2\sin\theta}\Delta f \qquad \text{Eqn. (2)}$$

where $v(i,j)$ is velocity of target fluid; where λ is the wavelength; Δf is the change in Doppler frequency (Doppler frequency shift); and θ is half of the angle between the two beams. Typically, there is no direct formula to apply for NIRF, and the like.

[00071] However, even when the imaged object is stationary, there is movement present that must be accounted for to accurately determine blood flow in vessels and perfusion in tissue. As recently as 2013, experts in the field of LSI discussed motion artifact as one of the two key questions still to be answered in this field. Therefore, systems and methods that have the capability to identify this motion contribution and account for its magnitude are needed and included in technologies claiming to be able to assess, image, and /or quantify blood flow in vessels and perfusion in tissues experimentally and in vivo.

[00072] Referring now to Figures 5A and 5B, Figure 5A is a visible light image of a hand and Figure 5B is a near infra-red light image of a hand. These images may be used to calculate the motion artifact and the movement of the blood flow and perfusion in accordance with some embodiments of the present inventive concept.

[00073] In particular, to remove the motion artifact of the tissue/organ that is caused by movement of tissue/organ, such as aspiration, spasm, heart beat and the like and/or the camera, Galilean velocity addition can be calculated using the following equation:

$$\mathbf{v}_{12}(\mathbf{r}) = \mathbf{v}_{13}(\mathbf{r}) + \mathbf{v}_{32}(\mathbf{r}) = \mathbf{v}_{13}(\mathbf{r}) - \mathbf{v}_{23}(\mathbf{r}) \qquad \text{Eqn. (3)}$$

where: $\mathbf{v}_{13}(\mathbf{r})$ is the velocity distribution of object of interest (blood flow and perfusion) relative to detector (camera); $\mathbf{v}_{23}(\mathbf{r})$ is the velocity distribution of the host object (the tissue/organ in which the blood vessel is embedded) relative to detector (camera); and $\mathbf{v}_{12}(\mathbf{r})$ is the velocity distribution of an object of interest (blood flow and perfusion) relative to the

host object (the tissue/organ in which the blood vessel is embedded). Thus, embodiments of the present inventive concept may address a need to determine $\mathbf{v}_{12}(\mathbf{r})$ under the condition that the image signals by the all the current LSI or LDI method provides only $\mathbf{v}_{13}(\mathbf{r})$. According to some embodiments of the present inventive concept, the multi spectrum imaging approach, both $\mathbf{v}_{13}(\mathbf{r})$ and $\mathbf{v}_{23}(\mathbf{r})$ can be made available.

[00074] Using LSI as an example, using the Eqn. (1) above, the speckle contrast of coherent NIR laser light $C_{NIR}(i,j)$ is associated with $\mathbf{v}_{13}(\mathbf{r})$, which is the velocity distribution of an object of interest (blood flow and perfusion) relative to detector (camera). $\mathbf{v}_{13}(\mathbf{r})$ is affected by the movement of blood flow and the movement of tissue/organ caused by factors such as aspiration, spasm, heart beat etc. and the movement of the camera. The visible laser light, especially within the 450~495nm wavelength range (blue laser light), has much less penetration in soft tissue/organ compared with the NIR laser light.

[00075] Using Eqn. (1) set out above, the speckle contrast of coherent visible laser light $C_{VIS}(i,j)$ is mainly associated with $\mathbf{v}_{23}(\mathbf{r})$, which is the velocity distribution of the host object (the tissue/organ that the blood vessel is embed) relative to detector (camera). $\mathbf{v}_{23}(\mathbf{r})$ is affected by the movement of tissue/organ caused by factors such as aspiration, spasm, heart beat etc. and the movement of the camera. Using Eqn. (3), $\mathbf{v}_{12}(\mathbf{r})$ can be derived using $\mathbf{v}_{13}(\mathbf{r})$ and $\mathbf{v}_{23}(\mathbf{r})$ thus the velocity distribution of object of interest (blood flow and perfusion) relative to the host object (the tissue/organ that the blood vessel is embed) can be quantified without the effect of the movement of tissue/organ and the movement of the camera.

[00076] The speckle contrast of coherent visible laser light $C_{VIS}(i,j)$ as a baseline can be used to normalize the speckle contrast of coherent NIR laser light $C_{NIR}(i,j)$ based on this mathematic model to reduce the velocity component of the motion artifact. Computer algorithms may be designed to normalize (subtract or divide) $C_{NIR}(i,j)$ using $C_{VIS}(i,j)$ to yield one or multiple stabilized blood flow and perfusion maps in real time. The algorithms may be processed by, for example, a data processor as discussed above with respect to Figures 3-4.

[00077] Referring now to Figures 6A and 6B, images generated using the measurement of the blood flow and perfusion using only NIR and dual wavelength illumination of a stationary hand will be discussed. As illustrated, the measurement of the blood flow and perfusion using only NIR and dual wavelength illumination of a stationary hand are very similar. This is because when the sample/target is stationary, the motion artifact as baseline measured by visible light is close to zero. Thus, the result without removing the baseline (Figure 6A:

using only NIR light) and the result with the baseline removed (Figure 6B: using dual wavelength illumination) are almost identical.

[00078] Referring now to Figures 7A and 7B, images illustrating the measurement of the blood flow and perfusion using only NIR and dual wavelength illumination of a shaking hand will be discussed. As illustrated therein, the measurement of the blood flow and perfusion using only NIR and dual wavelength illumination of a shaking hand are very different. The measurement with only NIR light (Figure 7A) shows much higher perfusion level which is caused by the motion artifact. The measurement with dual wavelength illumination (Figure 7B) is almost identical to the measurement of the stationary hand. This is because when the sample/target is moving the motion artifact as baseline measured by visible light is not zero. Thus, the result without removing the baseline (Figure 7A: using only NIR light) shows more "blood flow and perfusion" than the result with the baseline removed (Figure 7B: using dual wavelength illumination).

[00079] Referring now to Figures 8A and 8B, images illustrating both the perfusion measurement with only NIR and the dual wavelength illumination will be discussed. In particular, Figures 8A and 8B are images illustrating the perfusion measurement using only near infra-red light (8A) and dual wavelength illumination (8B) of a stationary hand with blood supply temporarily occluded by squeezing the wrist of the imaged hand using the other hand. As illustrated, a decrease induced by the temporary occlusion of the blood supply to the hand is clear.

[00080] Different from LSI, LDI uses interference of two coherent light beams: the one from the laser as the light source and the one reflected from the moving object whose frequency is slightly shifted from that of the incident light. LDI determines the speed of one "pixel" or points or a small region of the object where the incident beam is focused on. An image is obtained by scanning the focused beam. Similar to the LSI of Eqn. (1) using Eqn. (2), measurement of $v_{13}(r)$ and $v_{23}(r)$ in LDI can be achieved using a penetrating NIR beam and a non-penetrating visible beam. Again, using Eqn. (3) $v_{12}(r)$ of the fiducial points relative to the host object (the tissue/organ that the blood vessel is embed) can be identified.

[00081] Furthermore, practically, the laser speckle contrast is a mixture of static background and dynamic part. The dynamic part of the speckle contrast is associated with the motion and the static background is caused by the difference of the optical characteristics of the inhomogeneous scattering media. Since among the current LSI technologies, baseline speckle contrast at a no flow situation is not available, other than in a controlled phantom/tubing experiment, the static background of the speckle contrast is a major obstacle

to accurately quantifying blood flow in tissue/organ. Multi-spectrum illumination schemes provide a baseline speckle contrast at no flow situation $C_{VIS}(i,j)$ using visible coherent laser light. The speckle contrast of coherent visible laser light $C_{VIS}(i,j)$ can be used to normalize the speckle contrast of coherent NIR laser light $C_{NIR}(i,j)$ based a mathematic model in accordance with embodiments of the present inventive concept to reduce the static background in the speckle contrast as illustrated in Figures 9A and 9B. Figures 9A and 9B illustrate perfusion measurement using only near infra-red light (9A) and dual wavelength illumination (9B) of a large bowel of a pig. Measurement inaccuracy caused by the static contrast can be seen on the surgical drape 950 in Figure 9A. In Figure 9B, the "fake" blood flow and perfusion is not visible on the surgical drape 950 due to reduction of the static contrast.

[00082] Embodiments of the present inventive concept propose the visualization of both anatomical structure and blood flow physiology of the tissue and organ by one of two approaches. However, it will be understood that embodiments of the present inventive concept are not limited to the approaches discussed herein.

[00083] Referring now to Figure 10A-10D, a first approach using a dual layer design will be discussed. Referring first to Figure 10A (Panel A), an anatomical layer represented by a raw (original) image frame of visible light is illustrated. (Anatomical layer) $Img_{VIS}(i,j)$ is an 8 bit gray scale visible image of the sample/target tissue/organ and i and j are the pixel indexes along the horizontal and vertical direction. In some embodiments, the brightness, contrast and gamma value of this image might be adjusted to achieve better visualization effect.

[00084] Referring now to Figure 10B, a processed image is produced based on one or more raw image frames of near infra-red light to reflect two-dimensional (2D) speed distribution of blood flow and perfusion of the imaged tissue/organ using Laser Speckle or Laser Doppler Imaging technology. (Physiological layer) $Img_{NIR}(i,j)$ is an 8 bit indexed image with its numerical values mapped to a predefined color map. Usually, the color ranges from blue to red (0 to 255) with blue representing no/minimum flow speed and red representing the highest flow speed that the system can detect.

[00085] Referring now to Figure 10C, a transparency map is produced using methods that overlap the anatomical layer or parts of the anatomical layer over a physiological one, which will cause the bottom layer to be invisible (covered) or partially invisible (covered). Methods that overlap the physiological layer or parts of the physiological layer over anatomical one

will cause the bottom layer to be invisible (covered) or partially invisible (covered). A transparency map/matrix is applied in accordance with embodiments of the present inventive concept to ensure the visibility of both layers using the following equation:

$$T(i,j) = \left(\frac{Img(i,j) - Min(Img(i,j))}{Max(Img(i,j)) - Min(Img(i,j))}\right)^{x} \qquad \text{Eqn. (4)}$$

where T (i, j) is the transparency map with Img being a raw (original) image frame of visible or near infra-red light and x being an adjustable parameter >0 and <=2. Basically, each pixel value in $T(i,j)$ is between 0 and 1 with 0 representing no transparency and 1 representing 100% transparency. Parameter x controls the contrast of the transparency map and if x > 1, transparency has a larger dynamic range and if x < 1, the transparency has a smaller dynamic range. Figure 10D represents the combined visual effect using A, B and C in accordance with embodiments of the present inventive concept to reveal both anatomical structure and physiology.

[00086] Referring now to Figures 11A through 11C, a second approach using color and brightness design will be discussed. As illustrated in Figure 11A, an anatomical layer is represented by image brightness: a raw (original) image frame of visible light. $Img_{VIS}(i,j)$ is an 8 bit gray scale visible image of the sample/target tissue/organ and i and j are the pixel indexes along horizontal and vertical direction. The brightness, contrast and gamma value of this image may be adjusted to achieve better visualization effect.

[00087] Referring now to Figure 11B, a physiological layer is represented by image color: a processed image based on one or more raw image frames of near infra-red light to reflect 2D speed distribution of blood flow velocity and perfusion of the imaged tissue/organ using Laser Speckle or Laser Doppler Imaging technology. In a first step, an 8 bit indexed color image is generated with its numerical values mapped to a predefined color map. Usually, the color ranges from blue to red (0 to 255) with blue representing no/minimum flow speed and red representing the highest flow speed that the system can detect. In a second step, the 8 bit indexed color image is converted to a normalized RGB map $RGB_{NIR}(i,j)$ with the color of each pixel being represented by (R, G, B) three values and each value range from 0 ~ 1. It will be understood that since the Figures are in black and white, the corresponding grey scale has been employed herein.

[00088] Referring now to Figure 11C, anatomical and physiological layers are fused together by creating an 8 bit RGB color image as $Img(i,j) = Img_{VIS}(i,j) \times RGB_{NIR}(i,j)$. Note, each color channel (matrix $R_{NIR}(i,j)$, , $G_{NIR}(i,j)$ and $B_{NIR}(i,j)$) is multiplied by the same visible image $Img_{VIS}(i,j)$.

[00089] According to some embodiments of the present inventive concept, multi wavelength imaging design may be used to simultaneously combine different imaging technologies together. For example, as discussed herein, NIR fluorescence technology based on indocyanine green uses 808nm illumination and the fluorescence emission light is 830nm and 808nm reflection light is considered as noise and filtered out. In accordance with some embodiments of the present inventive concept, the 808nm reflection light can be used to achieve LSI or LDI while maintaining the 830nm fluorescence function.

[00090] Referring now to Figures 12A-12D, images illustrating Panel A, an NIR 785nm image of a small bowel (12A); Panel B a Green 532nm image of the same small bowel (12B); Panel C, a reconstructed color image of the same small bowel (12C); and Panel D, an image of the same small bowel taken by a regular camera (12D) will be discussed. In particular, using the multi spectral imaging system in accordance with some embodiments of the present inventive concept, an original color image can be constructed by using each spectrum as one RGB color channel. For example, using an NIR image as a red color channel and a 532nm image as a green color channel, the color image of a small intestine can be generated without using a color camera as illustrated in Figures 12A-12D. It will be understood that since the Figures are black and white, the corresponding grey scale has been employed herein.

[00091] Referring now to Figures 13A-13D, images illustrating Panel A, an NIR 785nm image of a pig heart (13A); Panel B, Green 532nm image of the same pig heart (13B); Panel C, a reconstructed color image of the same pig heart (13C); and Panel D, an image of the same pig heart taken by a regular camera (13D) will be discussed. Figures 13A-13D illustrate using an NIR image as a red color channel and a 532nm image as a green color channel, the color image of a pig heart can be generated without using a color camera. If the information of one color channel is missing, an algorithm is designed to generate this data using the information of the other two color channels. Since the color of a sample (tissue/organ) is mainly red, embodiments of the present inventive concept can generate color that is very close to the original one as long as the information of the red color channel is available as discussed with respect to Figures 10A-10D and 11A-11D. Thus, embodiments of the present inventive concept allow the reconstructed color image to reveal information of deeper tissue/organ if NIR is used as the red color channel as shown in Panel C (Figure 12C) vs. Panel D (Figure 12D).

[00092] As discussed briefly above with respect to the Figures, some embodiments of the present inventive concept use two wavelengths of differential transmittance through target tissue to apply LSI or LDI. In some embodiments, a first wavelength is within in the visible

range having zero or very shallow penetration, such as blue light (450–495 nm). The imaging result of this non-penetrating illumination serves as capturing the anatomical structure of tissue/organ surface and position marker of the target tissue/organ, but not the subsurface movement of blood flow and perfusion. A second of the two wavelengths is Near Infra-Red (NIR), which has much deeper penetration and the imaging result of this NIR illumination reveals the underlying blood flow physiology, which correlates both to the motion of the target tissue/organ and also the movement of blood flow and perfusion.

[00093] Using the imaging measurement of the visible light as a baseline, the true motion of blood flow and perfusion can be derived from the NIR imaging measurement without being affected by the motion artifact of the target. Furthermore, the anatomical structure information captured by visible light and the physiological characteristics measured by NIR light may be synthesized together according to some embodiments of the present inventive concept. The synthesized imaging product according to embodiments discussed herein provides a previously unattainable clarity of visualization and accuracy of quantification of blood flow and perfusion across the spectrum of clinical applications of laser imaging technologies.

[00094] Thus, embodiments of the present inventive concept provide improved image quality and real time data acquisition (several seconds vs. minutes for all other technologies) and analysis. This real time aspect of the present inventive concept makes this technology a real option for sustained adoption of the technology by a surgeon/provider. Embodiments of the present inventive concept accurately depict and quantify blood flow and perfusion.

[00095] Further embodiments of the present inventive concept are directed to color image reconstruction using multi-wavelength imaging techniques discussed herein. It will be understood that the images are present in a gray scale as the patent application publishes in black and white. In particular, using a dual wavelength imaging technique as discussed herein, two images may be acquired simultaneously. One is near infra-red image $IR(x, y)$ and the other is a visible image $VIS(x, y)$. X and Y represent the index of the horizontal and vertical pixel. To reconstruct a red green blue (RGB) color image, red, green and blue channels are calculated separately as follows:

$$R(x,y) = (2^N - 1) \times a_1 \times \left(\frac{NIR(x,y) - \min(NIR(x,y))}{\max(NIR(x,y) - \min(NIR(x,y)}\right)^{b_1} \quad \text{Eqn. (5)}$$

$$G(x,y) = (2^N - 1) \times a_2 \times \left(\frac{VIS(x,y) - \min(VIS(x,y))}{\max(VIS(x,y) - \min(VIS(x,y)}\right)^{b_2} \quad \text{Eqn. (6)}$$

$$B(x,y) = (2^N - 1) \times a_3 \times \left(\frac{VIS(x,y) - \min(VIS(x,y))}{\max(VIS(x,y) - \min(VIS(x,y)}\right)^{b_3} \quad \text{Eqn. (7)}$$

$$\frac{NIR(x,y)-\min(NIR(x,y))}{\max(NIR(x,y)-\min(NIR(x,y)} \quad \text{Eqn. (8)}$$

where R(x,y), G(x,y), B(x,y) are the red, green and blue channels, respectively, of the RGB color image; N is the bit of the color map, for example, 8 bit or 16 bit; a and b are the adjusting parameters for each channel; min is the function to get the minimum value; max is the function to get the maximum value; and Eqn. (8) serves as a normalization of the original image of one specific wavelength. Furthermore, the brightness, contrast and gamma value of the original image of one specific wavelength might be adjusted before applying the equations above.

[00096] The multi-wavelength color image recreation technique in accordance with some embodiments of the present inventive concept may reduce the need for an extra color camera in the device; can create a color image with a minimum of two wavelengths; and compared with traditional color images, the color image produced in accordance with embodiments discussed herein visualizes a larger depth of penetration due to use of near infra-red wavelength.

[00097] Referring now to Figures 14A through 14E, various images of a segment of a large bowel of a pig imaged using the multi-wavelength imaging device in accordance with some embodiments of the present inventive concept will be discussed. Figure 14A is an image of the bowel of the pig obtained using a visible wavelength (532nm). Figure 14B is an image of the bowel of the pig using a near infra-red wavelength (785nm). Figure 14C is an image of the bowel of the pig reconstructed with the wavelengths of Figures 14A and 14B. Figure 14D is a regular color image (shown in gray scale) of the bowel with room light illumination. Figure 14E is a blood flow and perfusion image of the bowel in accordance with some embodiments of the present inventive concept.

[00098] Referring now to Figures 15A to 19B, details with respect to real time image quality test protocols will be discussed. Real time image quality test protocols are developed based on customized algorithms using image registration and image metadata to examine the following issues during a clinical imaging procedure:

- Movement of target: Figures 15A and 15B illustrate images of a stationary hand (15A) and a moving hand (15B) detected by a customized image registration algorithm.
- Movement of a field of view or the Camera: Figures 16A and 16B illustrate imaging of a hand image captured by stationary camera (16A) and a hand captured by moving camera (16B) detected by customized image registration algorithm.

- Blocked field of view: Figures 17A and 17B illustrate an image of a hand (17A) and an image of a hand that is partially blocked by a twister (17B) and this blocked field of view is detected by a customized image registration algorithm.
- Intrusion of headlight of surgeon/physician: Figures 18A and 18B illustrate an image of a hand (18A) and an image of a hand with a head light shining on it (18B) and this extra light within the FOV is detected by a customized algorithm using metadata in the image.
- Ambient light condition: Figures 19A and 19B illustrate an image of a hand with a room light off (19A) and an image of a hand image with the room light on (19B) and this is detected by customized algorithm using metadata in the image.

[00099] The goal of this process is to reduce the likelihood, or possibly eliminate, low quality images caused by incorrect image acquisition to improve the visualization and increase accuracy of the quantification of the blood flow and perfusion imaging in accordance with some embodiments of the present inventive concept.

[000100] As discussed above, the data obtained using the imaging methods discussed above can only be used to derive distribution of blood flow speed u. In clinics, the information on distribution of blood flow rate given by the product of blood flow velocity u and the cross section area of blood vessel A is needed. To obtain the distribution of u(r) where r is the three dimensional coordinate, the Navier-Stokes equation has to be solved, which is given by Equations (9) and (10) set out below:

$$\rho \cdot (\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u}\nabla \cdot \mathbf{u}) = -\nabla p + \mu \cdot \nabla^2 \mathbf{u} + \mathbf{F} \qquad \text{Eqn. (9)}$$

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}) = 0 \qquad \text{Eqn. (10)}$$

where ρ is the density (kg/m^3), **u** is the flow velocity vector (m/s), p is the pressure (m/s), **F** is the volume force vector (N/m^3) and m is the viscosity. Solving the Navier-Stokes equations produces a velocity field, *i.e.* a distribution of fluid velocity in space and time. Once this velocity field is obtained, other quantities of interest, such as flow rate and drag force, can be calculated. These calculated quantities can be compared to the experimental data obtained using the methods discussed above to validate the data.

[000101] Computational procedures for a non-invasive measurement of blood flow rate distribution in principal vessels in tissues/organs will now be discussed with respect to some embodiments of the present inventive concept. Procedures begin by illuminating a tissue region of interest with a coherent light source, such as a laser with sufficiently long wavelength for relatively large penetration depth between, for example, 550 nm to about 1100 nm as the second wavelength. Using methods discussed above, scattered light at the second wavelength is acquired to determine spatial distribution of blood flow speed in the principal vessels and perfusion distribution in tissue in the region of interest. A velocity field of $\mathbf{u}(\mathbf{r})$ for the region of interest is calculated numerically. In some embodiments, the velocity field is calculated using Equations (9) and (10) set out above. Blood flow speed in the region of interest based on the calculated velocity field is calculated. The calculated blood flow speed in the region of interest is compared to the blood flow speed determined using the acquired image data at the second wavelength from the region of interest to verify results.

[000102] In the drawings and specification, there have been disclosed example embodiments of the inventive concept. Although specific terms are employed, they are used in a generic and descriptive sense only and not for purposes of limitation, the scope of the inventive concept being defined by the following claims.

THAT WHICH IS CLAIMED IS:

1. A multispectral imaging system, the system comprising:

a first light source having a first wavelength configured to image a sample;

a second light source, different from the first light source, having a second wavelength, different from the first wavelength, configured to image the sample;

a camera configured to receive information related to the first and second light sources from the sample, wherein the light at first wavelength is configured to image a surface of the sample into the camera and the light at second wavelength is configured to penetrate the sample and provide information related to the sample to the camera; and

a processor configured to combine the information related to the first and second light sources provided by the camera to image an anatomical structure of the sample, image physiology of blood flow and perfusion of the sample and/or synthesize the anatomical structure and the physiology of blood flow and perfusion of the sample in terms of a blood flow rate distribution.

2. The system of Claim 1, wherein the first and second wavelengths have different wavelengths in a range from 350 nm to 1100 nm.

3. The system of Claim 2, wherein the first wavelength is in one of an ultraviolet (UV) and a visible spectrum and the second wavelength is in one of a visible and near infrared spectrum.

4. The system of Claim 1, wherein the sample comprises at least one of tissue and an organ.

5. The system of Claim 1, wherein the processor is further configured to reconstruct a color image using at least one monochromatic camera in real time.

6. The system of Claim 1, wherein the processor is further configured to use light of wavelength in one of a visible and a near infrared (NIR) spectrum to replace a red color spectrum to provide deeper tissue information.

7. The system of Claim 1, wherein an output of the system provides a unique clarity of visualization

8. The system of Claim 1, wherein the processor is further configured to quantitatively analyze the anatomical structure and physiology of blood flow and perfusion of the sample in terms of a blood flow rate distribution.

9. The system of Claim 1, wherein the processor is further configured to separate motion of the sample from motion of blood flow and perfusion in the imaged sample.

10. The system of Claim 1, wherein the processor is further configured to remove motion artifacts of the imaged sample caused by physiologic and/or pathophysiologic movement of the imaged sample in order to improve accuracy of quantification of blood flow and perfusion.

11. The system of Claim 1, wherein the processor is further configured to remove motion artifact of the imaged sample caused by movement of an imaging platform/camera in order to improve accuracy of quantification of blood flow and perfusion.

12. The system of Claim 1, wherein the processor is further configured to improve quantification accuracy in laser-based blood flow and perfusion measuring technologies by removing motion artifacts.

13. The system of Claim 12, wherein the perfusion measuring technologies comprise laser speckle imaging (LSI), laser Doppler imaging (LDI), Florescence imaging, reflectance imaging and/or LSI plus Fluorescence.

14. The system of Claim 12, wherein the processor is further configured to improve quantification accuracy in laser-based blood flow and perfusion measuring technologies by removing static background caused by a difference of the optical characteristics of an inhomogeneous scattering media.

15. The system of Claim 1, wherein the processor is further configured to display anatomical structure and the physiology of blood flow and perfusion of a sample simultaneously in real time.

16. The system of Claim 1, wherein the processor is further configured to image the anatomical structure and blood flow physiology at different depths in the sample.

17. The system of Claim 1, wherein the first wavelength is configured to extend from between 350nm to 550nm to between 300nm to 600nm into the sample and the second wavelength is configured to penetrate the sample from 550nm to 1100nm to between 500nm to 1500nm.

18. A method for multispectral imaging in a multispectral imaging system, the method comprising:

imaging a sample using a first light source having a first wavelength;

imaging the sample using a second light source, different from the first light source, having a second wavelength, different from the first wavelength;

receiving information related to the first and second light sources from the sample at a camera, wherein the light at first wavelength is configured to reflect off a surface of the sample into the camera and the light at second wavelength is configured to penetrate the sample and provide information related to the sample to the camera; and

combining the information related to the first and second light sources provided by the camera at a processor to image an anatomical structure of the sample, image physiology of blood flow and perfusion of the sample and/or synthesize the anatomical structure and the physiology of blood flow and perfusion of the sample in terms of a blood flow rate distribution.

19. The method of Claim 18, wherein the first wavelength is in one of an ultraviolet (UV) and a visible spectrum and the second wavelength is in one of a visible and a near infrared spectrum.

20. A computer program product for multispectral imaging in a multispectral imaging system, the computer program product comprising:

a non-transitory computer readable storage medium having computer readable program code embodied in the medium, the computer readable program code comprising:

computer readable program code to image a sample using a first light source having a first wavelength;

computer readable program code to image the sample using a second light source, different from the first light source, having a second wavelength, different from the first wavelength;

computer readable program code to receive information related to the first and second light sources from the sample at a camera, wherein the light at first wavelength is configured to reflect off a surface of the sample into the camera and the light at second wavelength is configured to penetrate the sample and provide information related to the sample to the camera; and

computer readable program code to combine the information related to the first and second light sources provided by the camera at a processor to image an anatomical structure of the sample, image physiology of blood flow and perfusion of the sample and/or synthesize the anatomical structure and the physiology of blood flow and perfusion of the sample in terms of blood flow rate distribution.

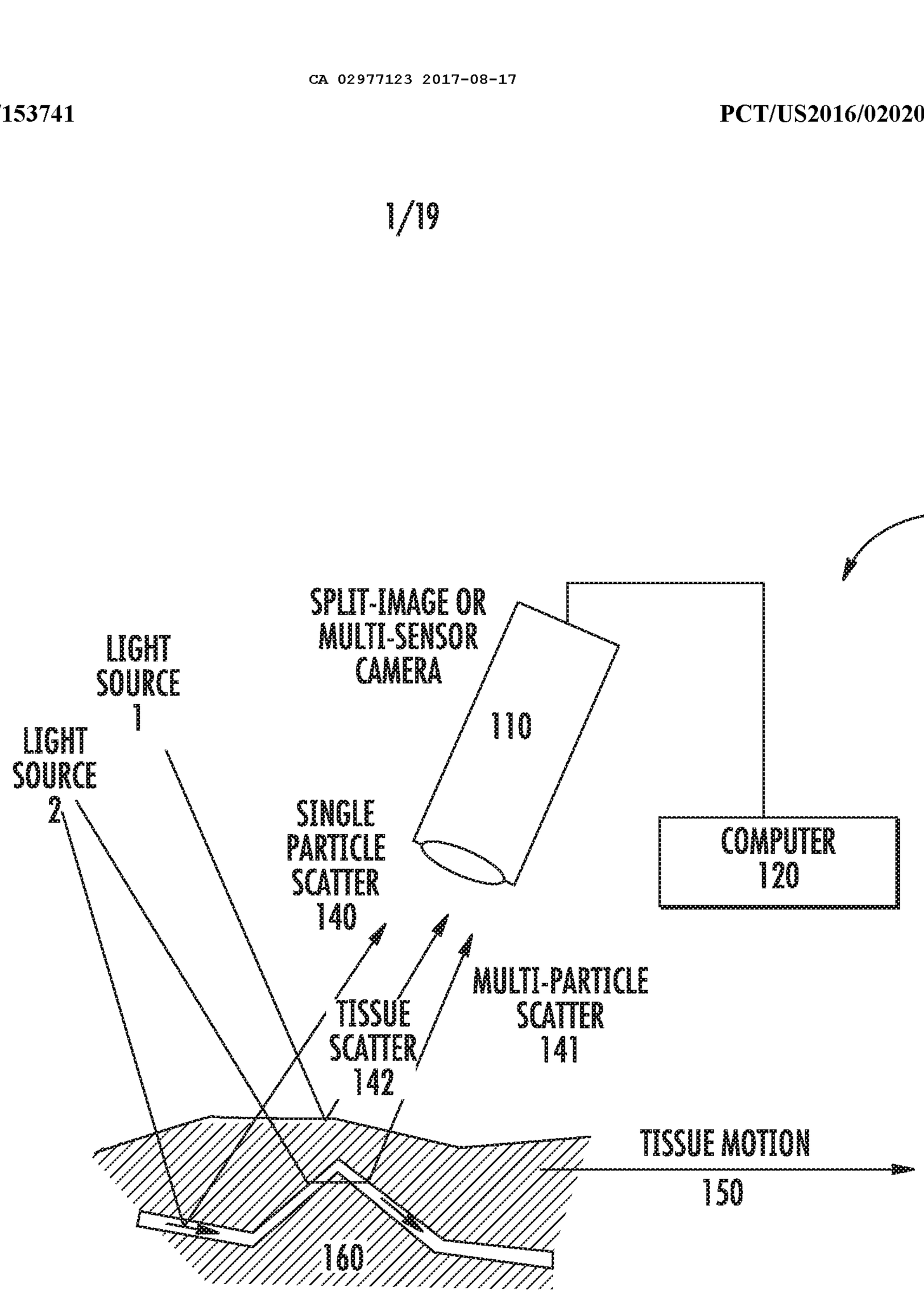

100
SPLIT-IMAGE OR MULTI-SENSOR CAMERA
110
LIGHT SOURCE 1
LIGHT SOURCE 2
SINGLE PARTICLE SCATTER 140
COMPUTER 120
MULTI-PARTICLE SCATTER 141
TISSUE SCATTER 142
TISSUE MOTION
150
160

FIG. 1

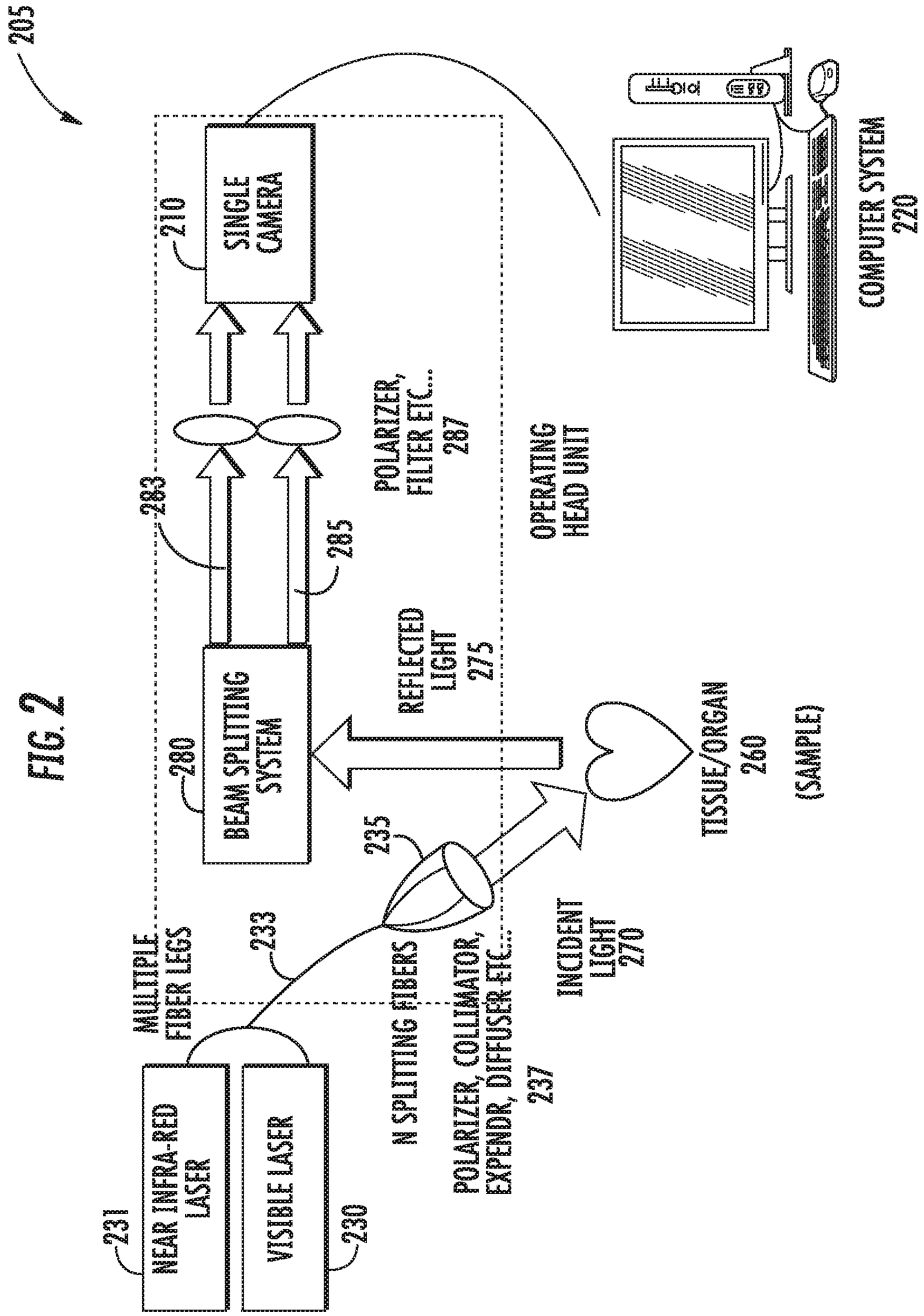

205
SINGLE CAMERA
210
283
285
POLARIZER, FILTER ETC... 287
REFLECTED LIGHT 275
OPERATING HEAD UNIT
COMPUTER SYSTEM 220
MULTIPLE FIBER LEGS
233
BEAM SPLITTING SYSTEM
280
235
TISSUE/ORGAN 260 (SAMPLE)
N SPLITTING FIBERS
POLARIZER, COLLIMATOR, EXPENDR, DIFFUSER ETC... 237
INCIDENT LIGHT 270
NEAR INFRA-RED LASER
231
VISIBLE LASER
230

FIG. 2

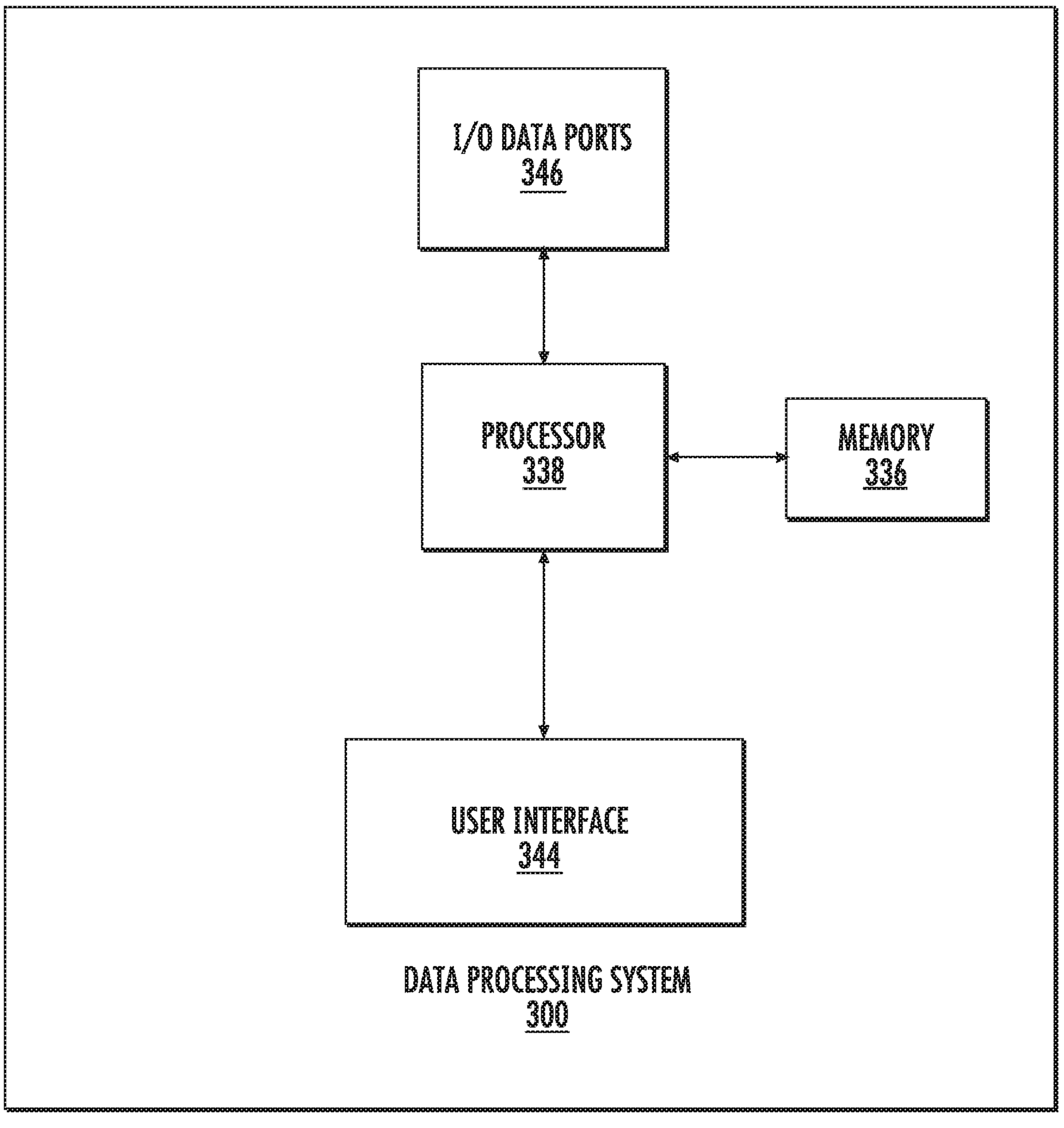

I/O DATA PORTS
346
PROCESSOR
338
MEMORY
336
USER INTERFACE
344
DATA PROCESSING SYSTEM
300

FIG. 3

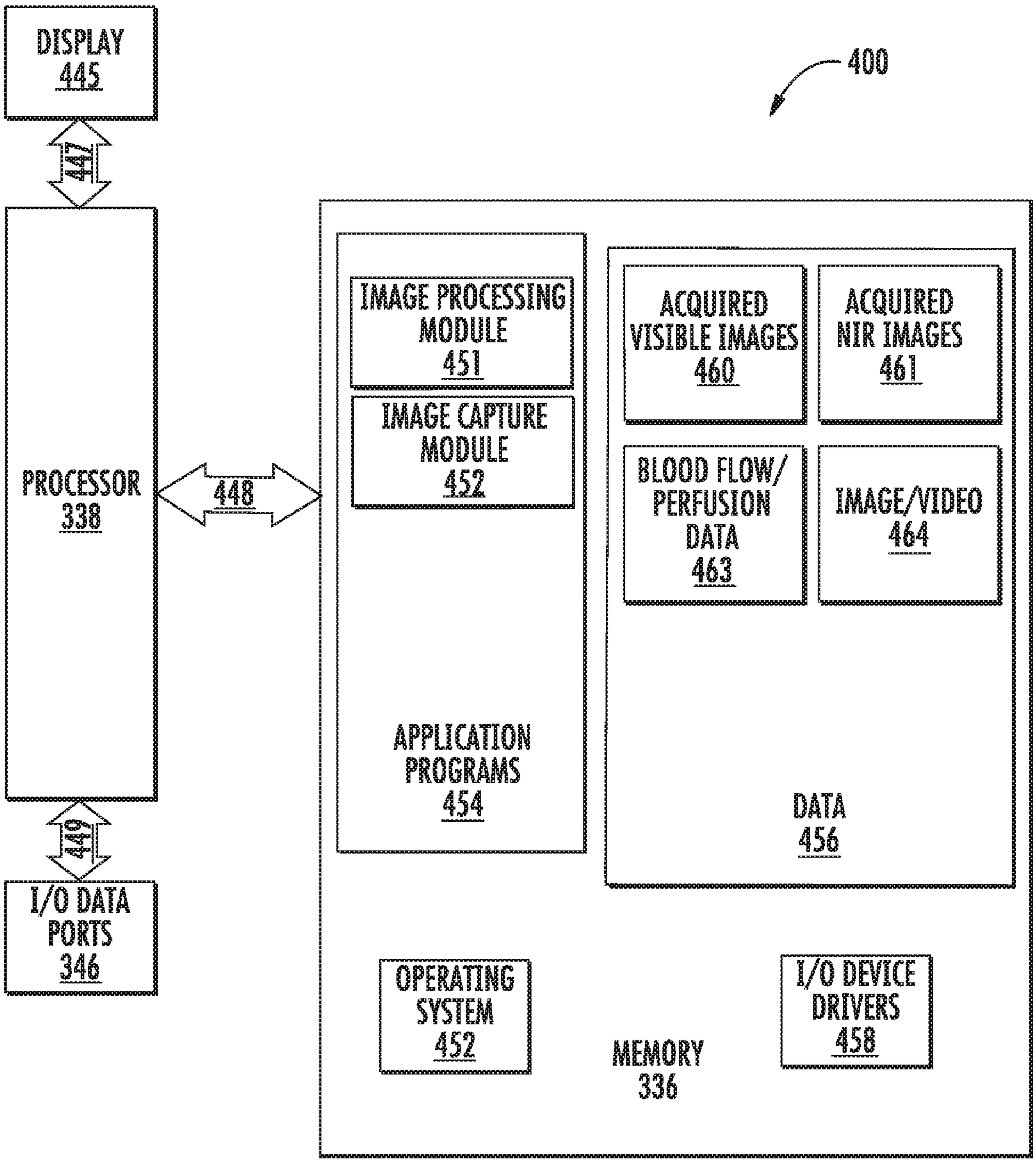

400
DISPLAY
445
447
PROCESSOR
338
448
I/O DATA
PORTS
346
449
IMAGE PROCESSING
MODULE
451
IMAGE CAPTURE
MODULE
452
APPLICATION
PROGRAMS
454
OPERATING
SYSTEM
452
MEMORY
336
ACQUIRED
VISIBLE IMAGES
460
ACQUIRED
NIR IMAGES
461
BLOOD FLOW/
PERFUSION
DATA
463
IMAGE/VIDEO
464
DATA
456
I/O DEVICE
DRIVERS
458

FIG. 4

FIG. 5A

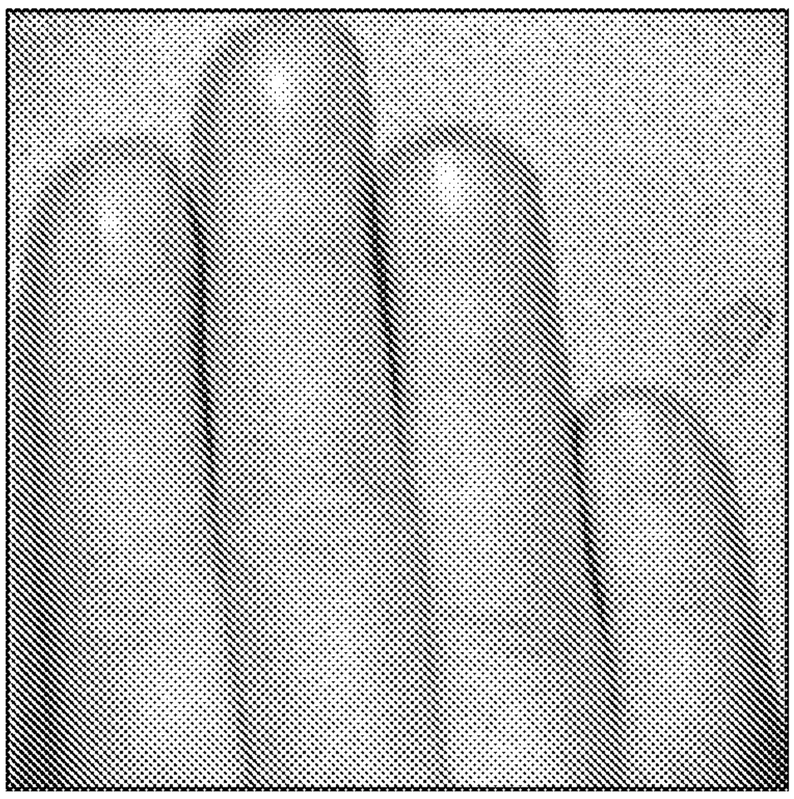


FIG. 5B

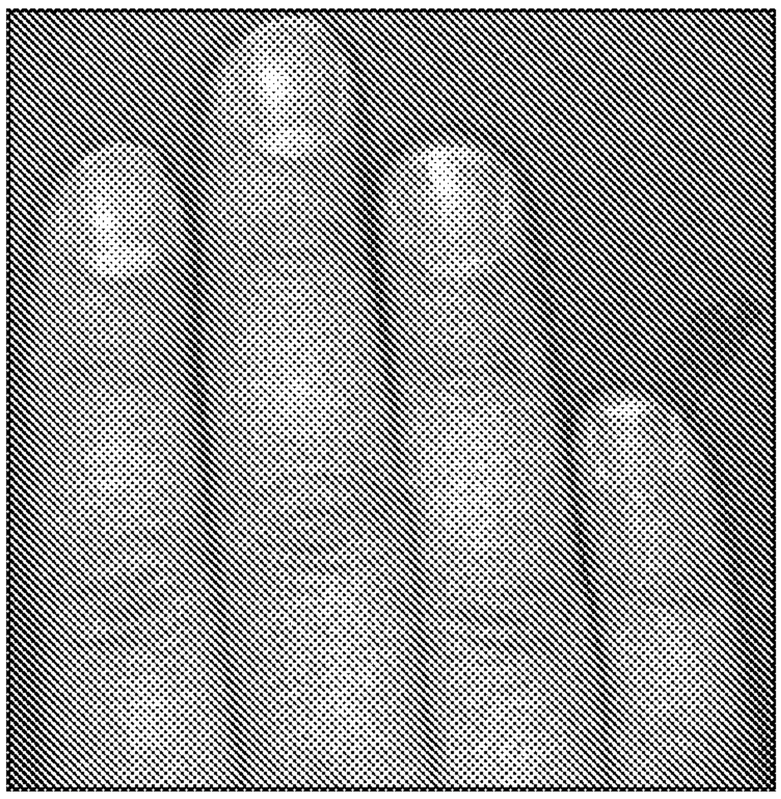


FIG. 6A

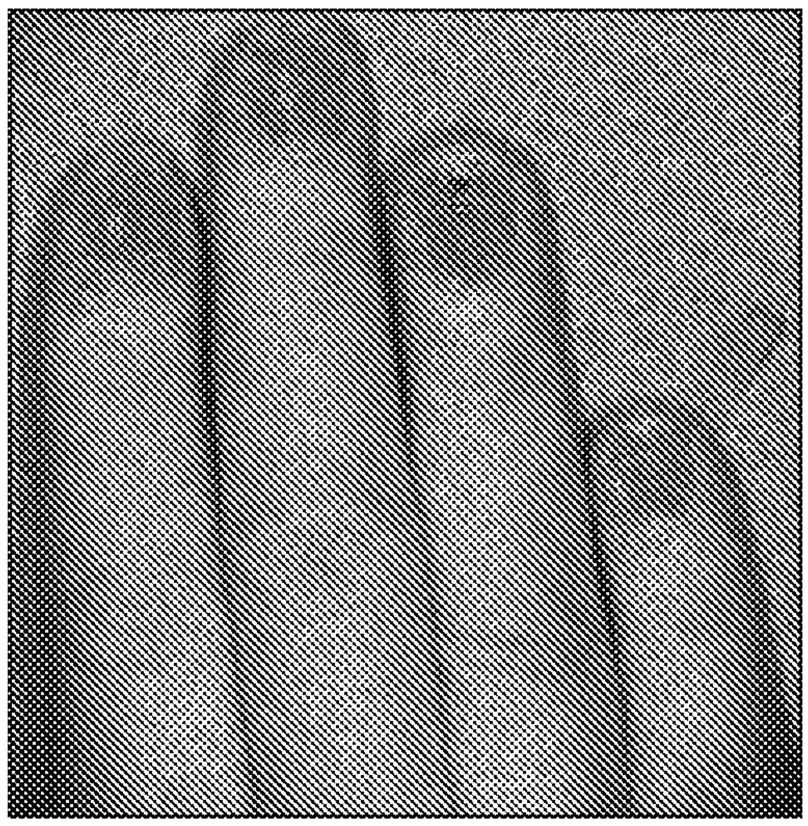


FIG. 6B

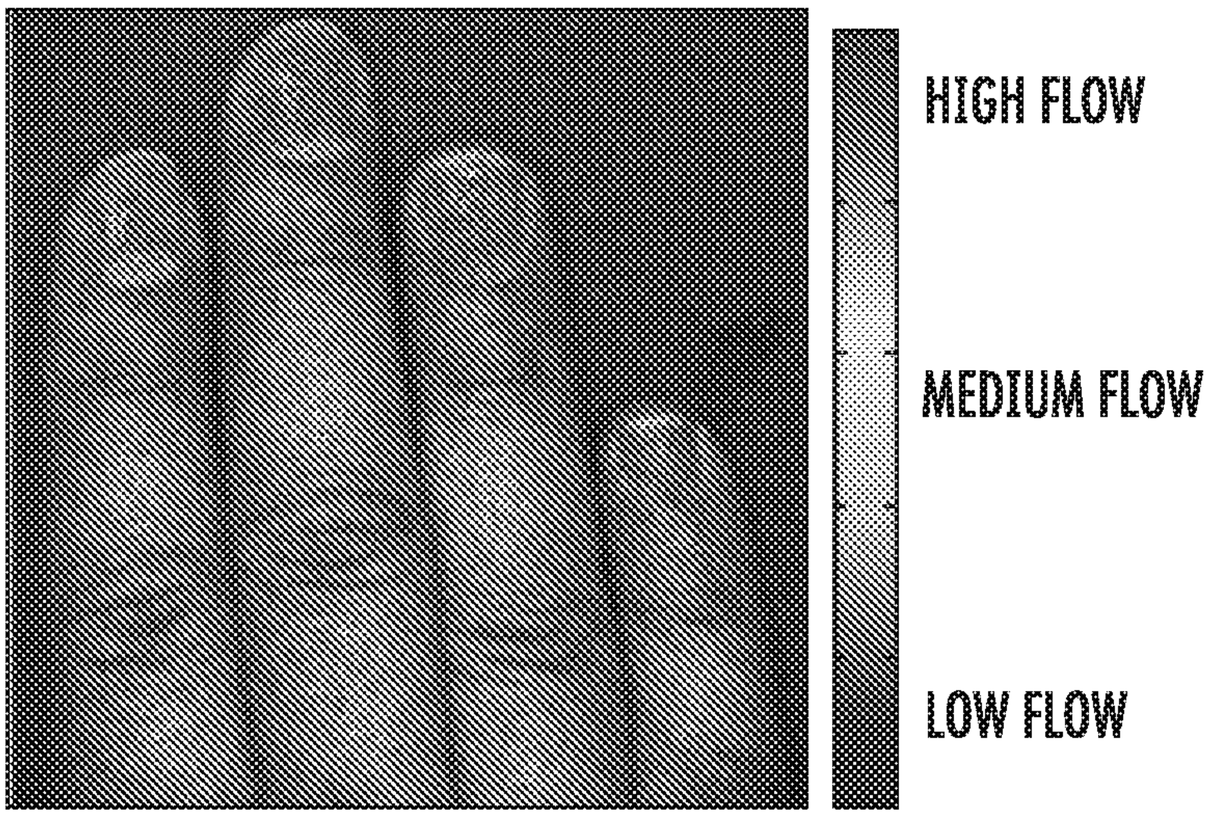


FIG. 7A

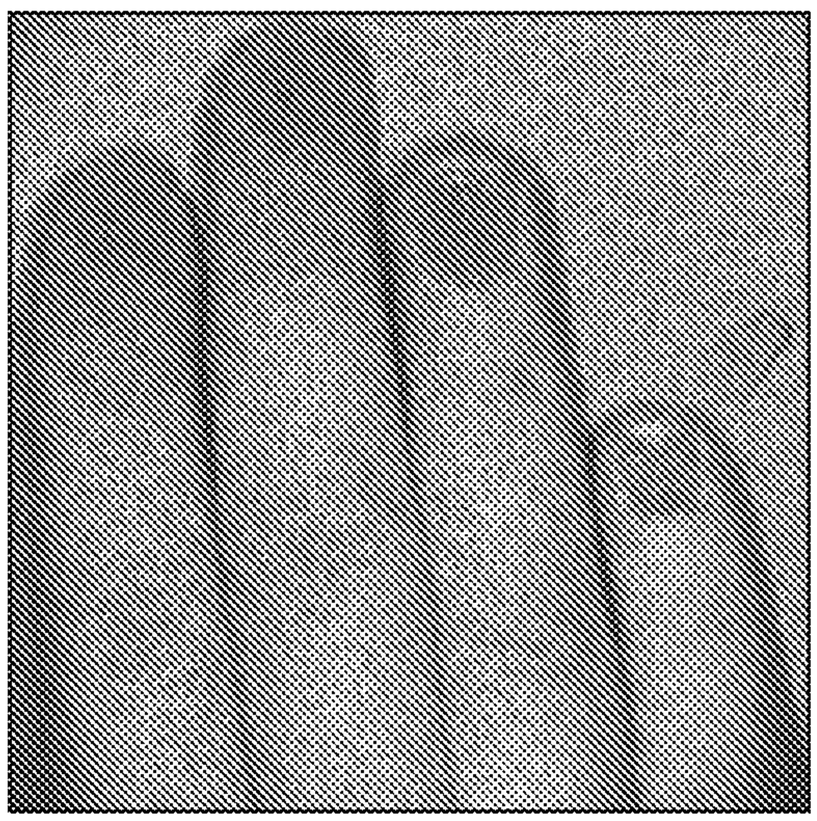


FIG. 7B

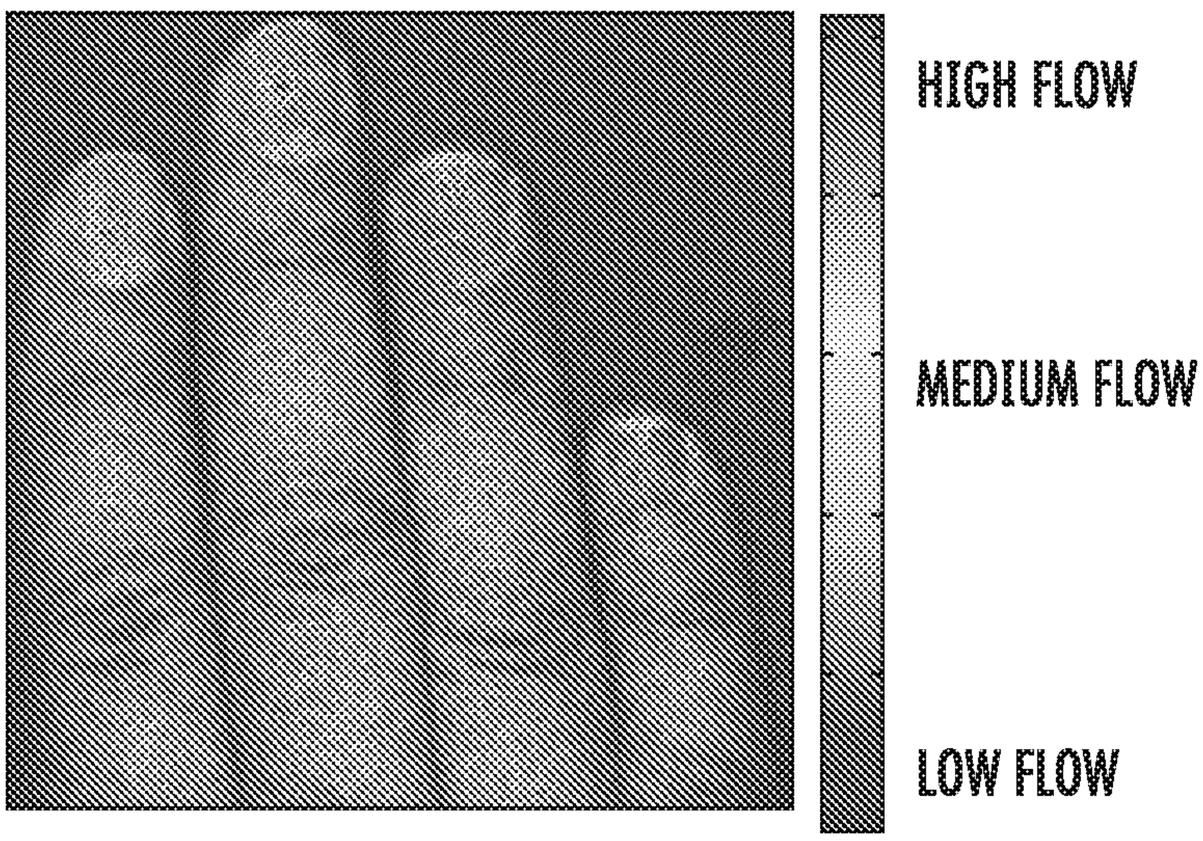


FIG. 8A

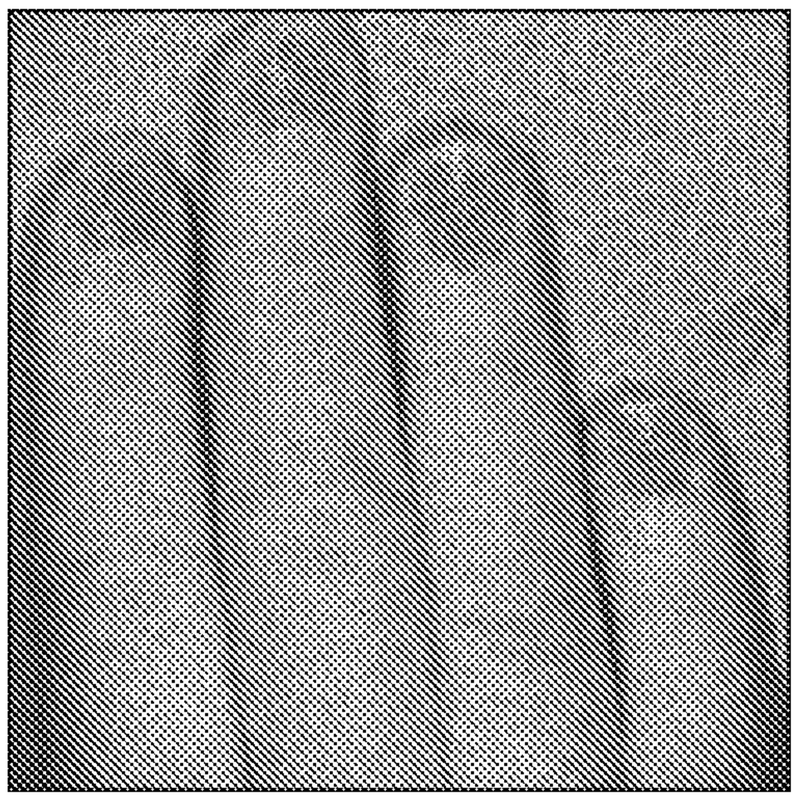


FIG. 8B

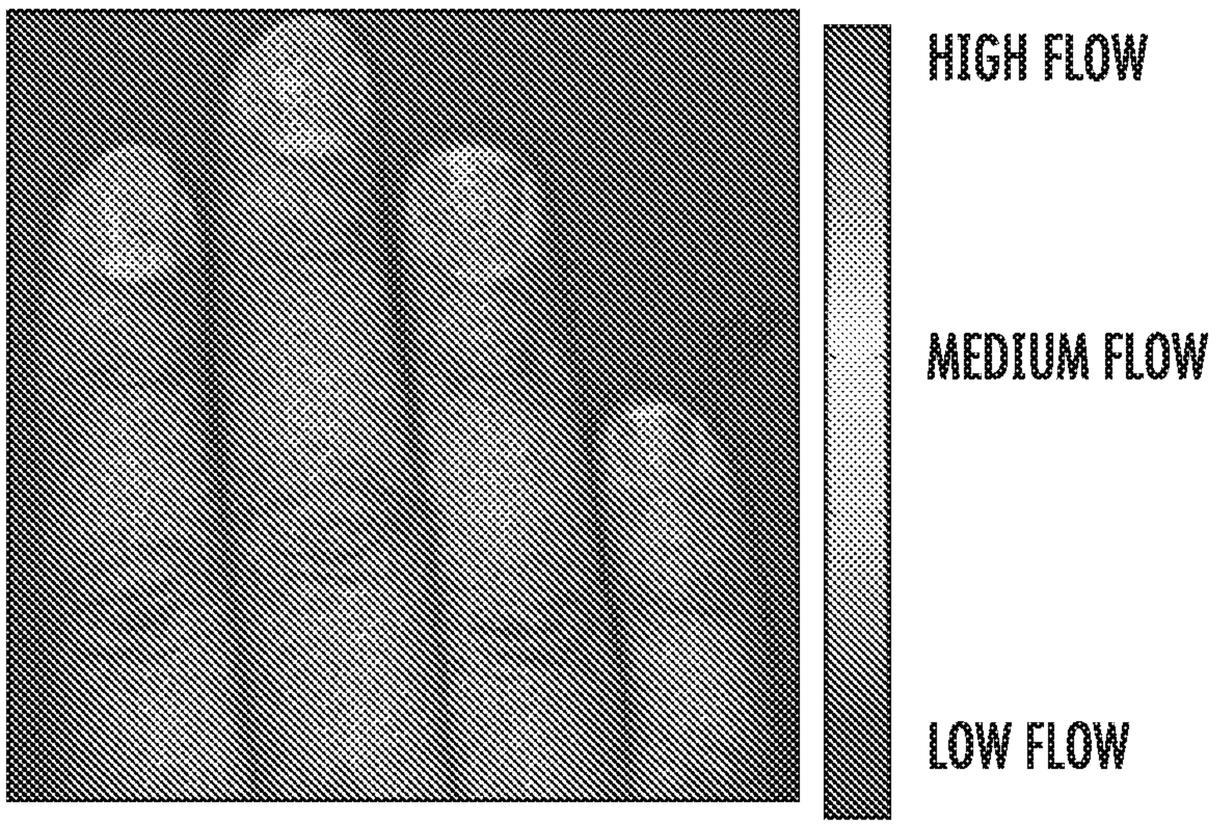


FIG. 9A



FIG. 9B

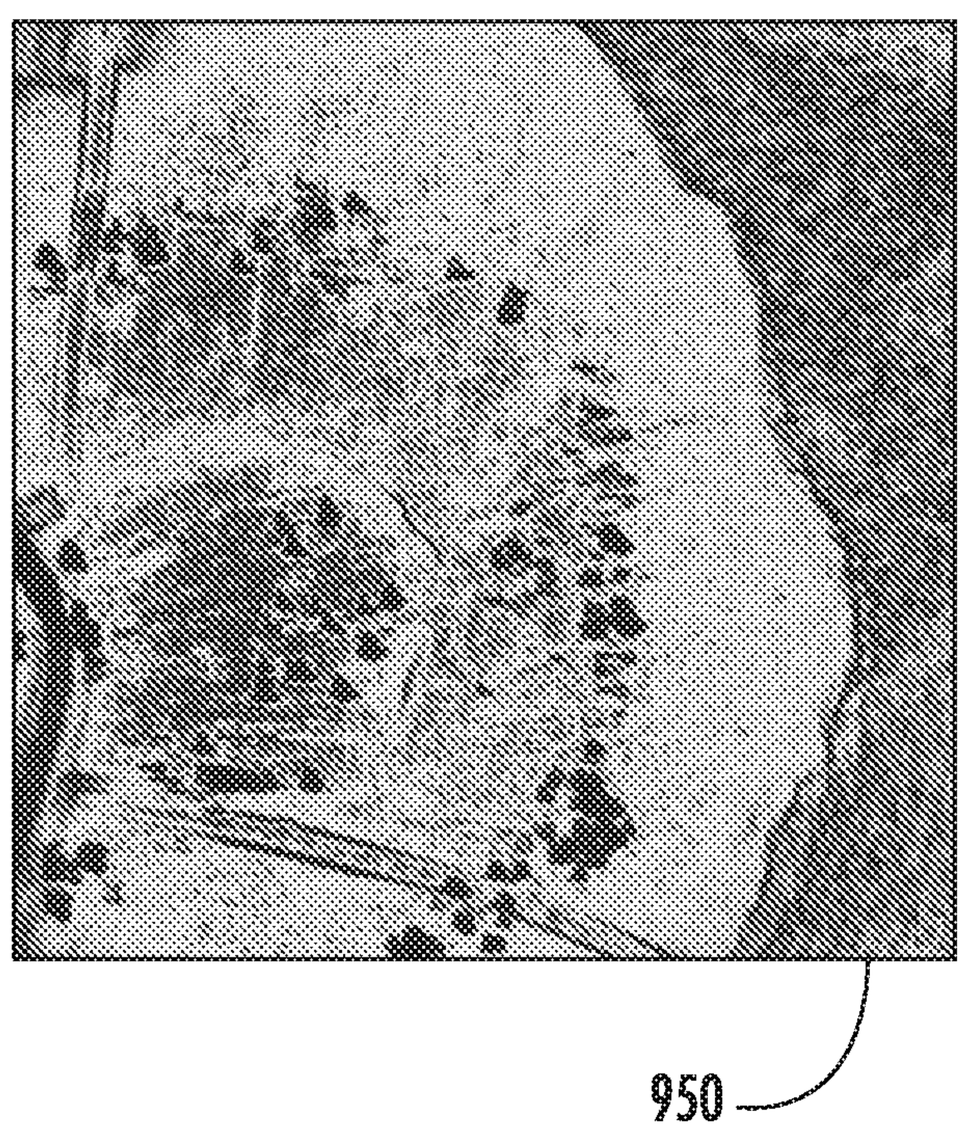


FIG. 10A

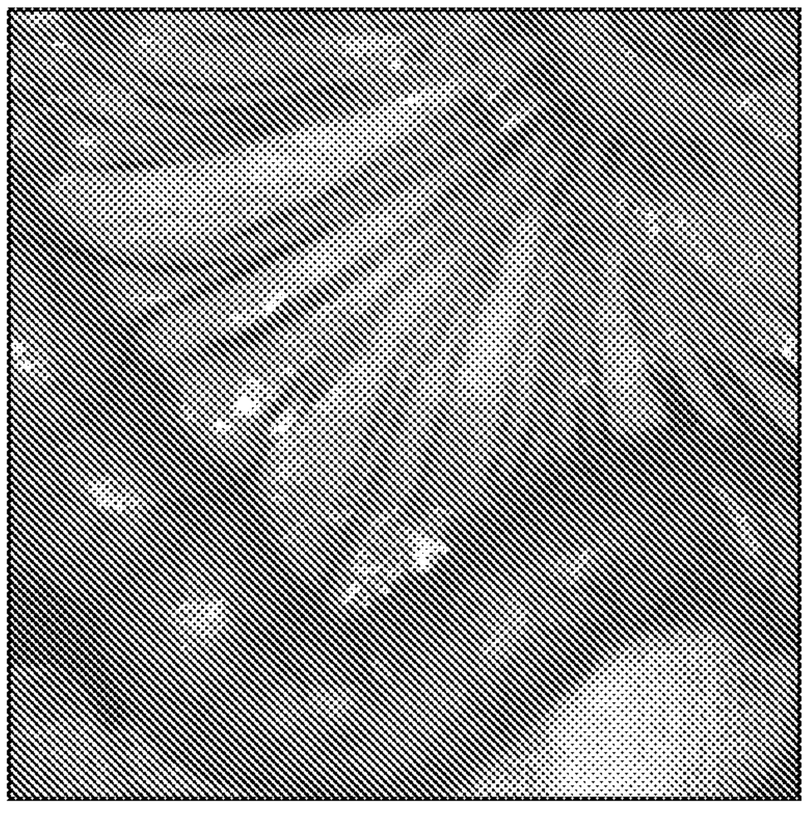


FIG. 10B

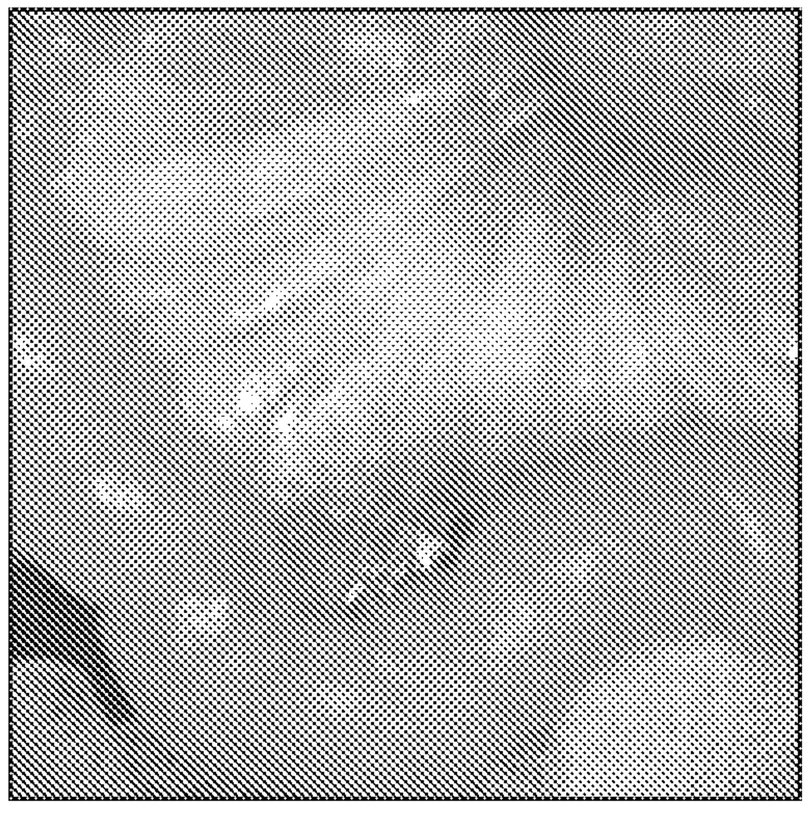


FIG. 10C

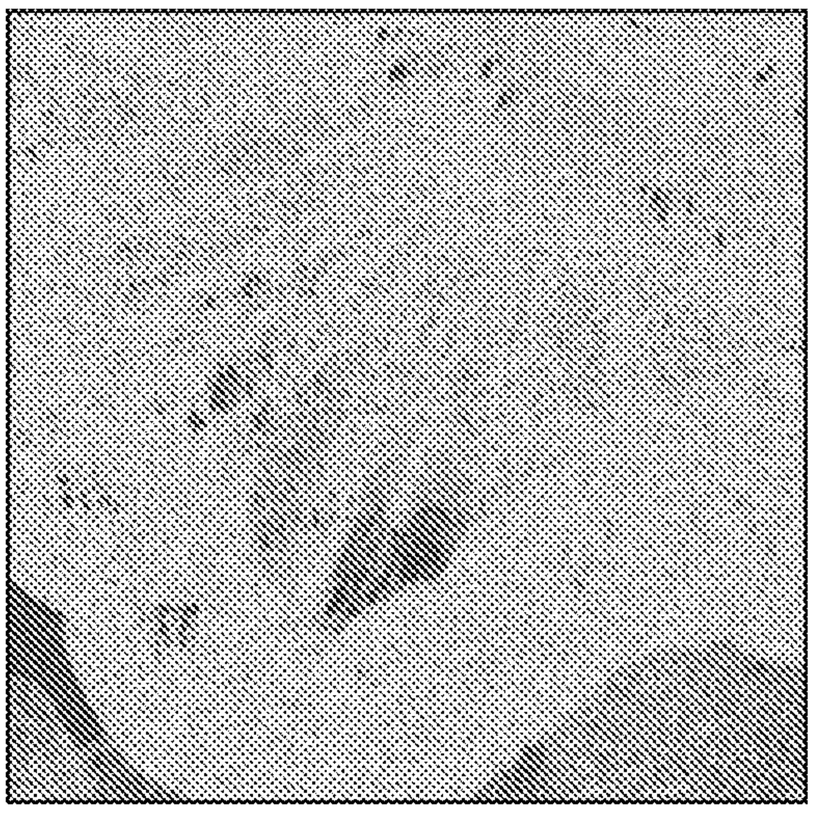


FIG. 10D

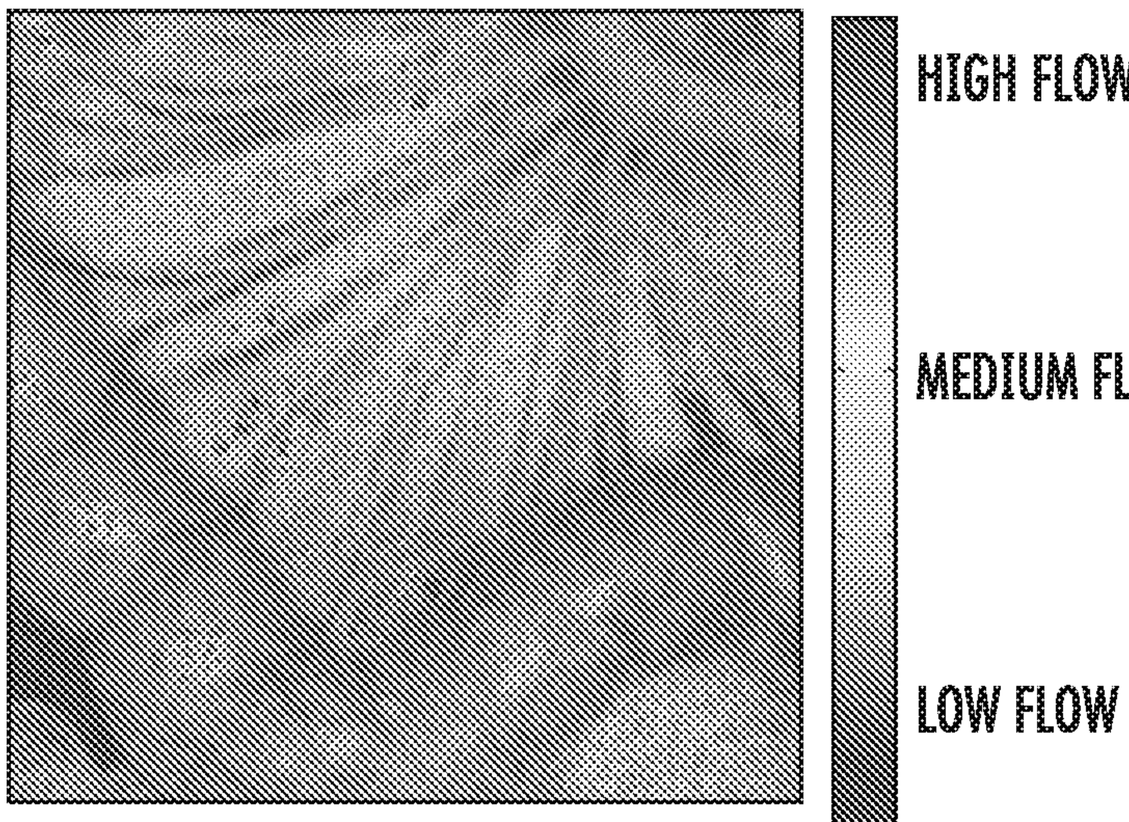
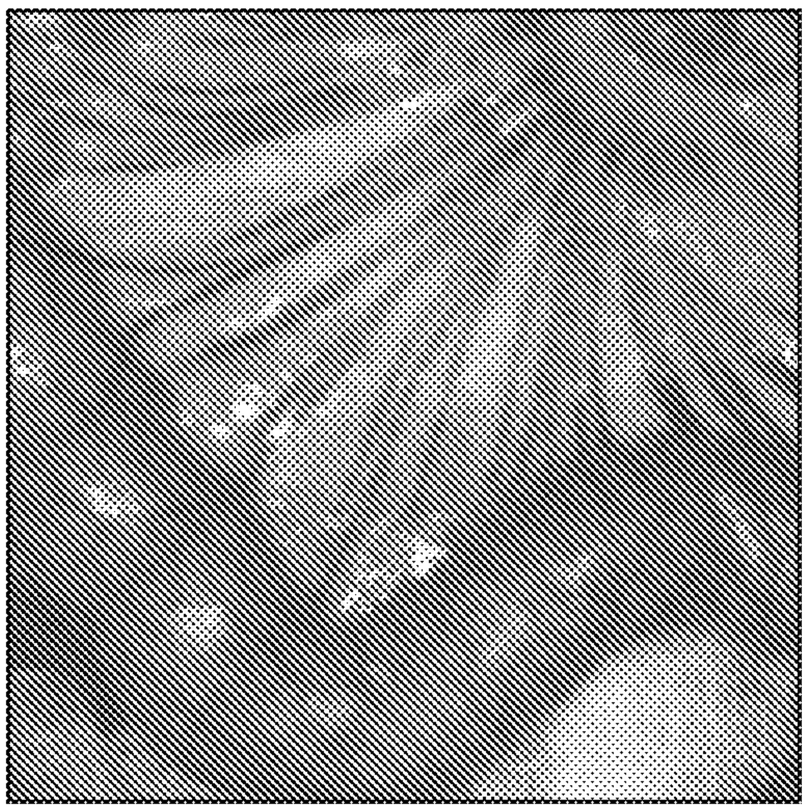


FIG. 11A

PANEL A



PANEL B

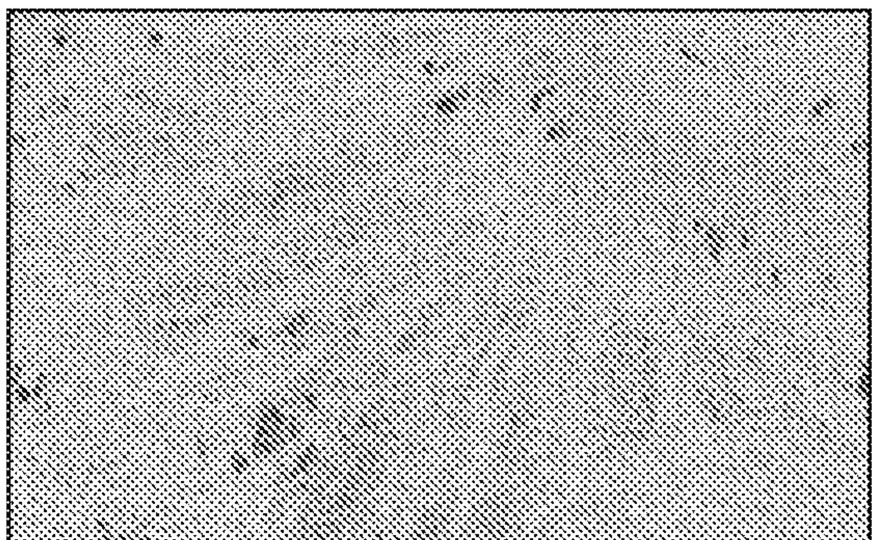


FIG. 11B

PANEL C (A ADJUSTS BRIGHTNESS AND B ADJUSTS COLOR)

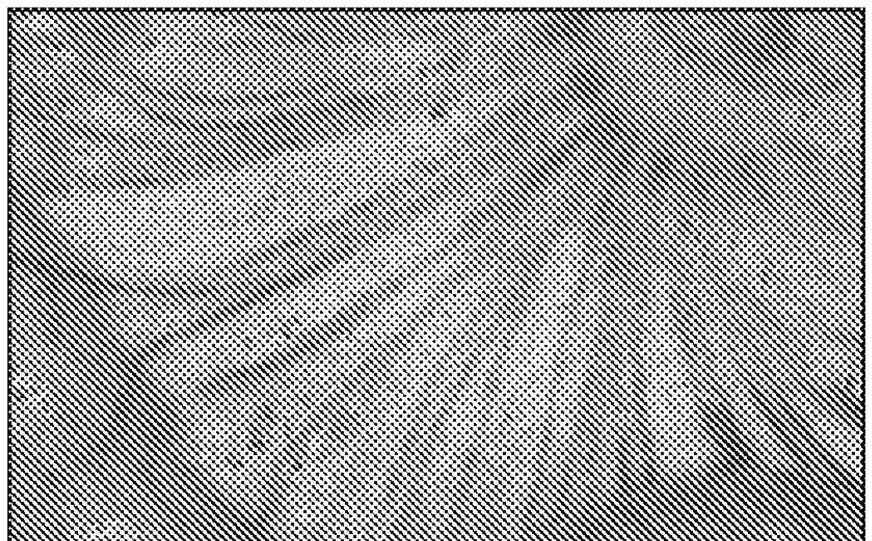


FIG. 11C

FIG. 12A

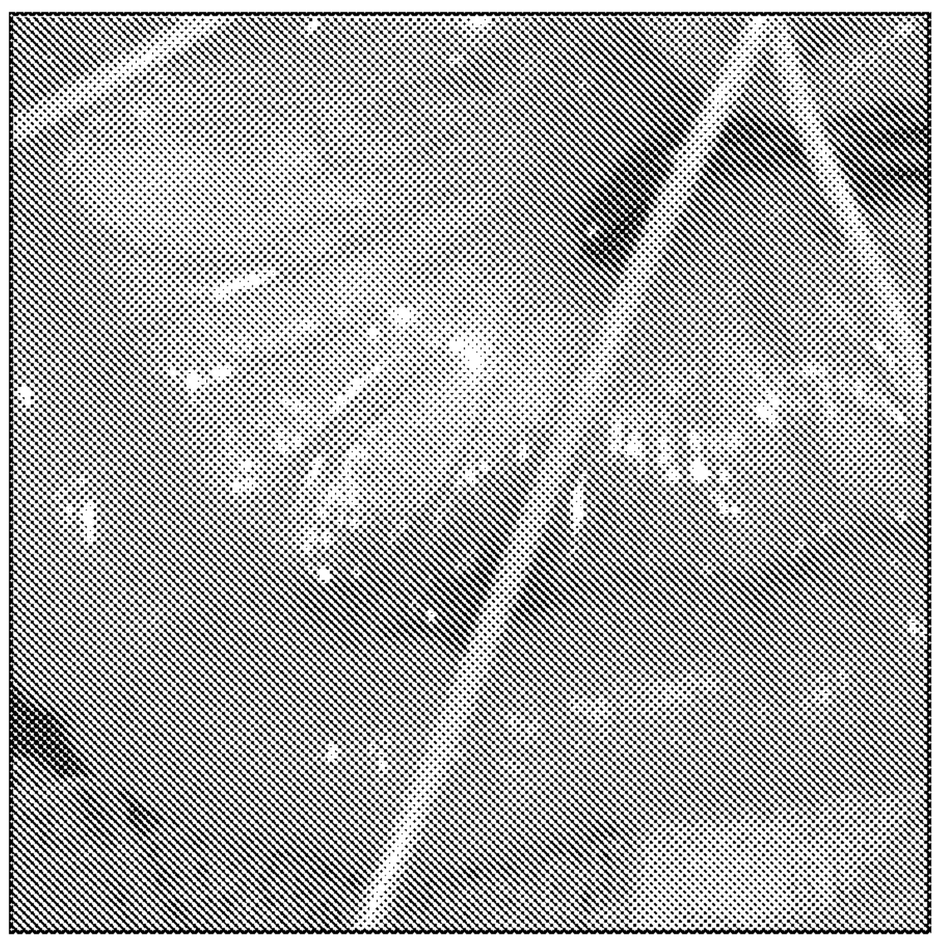
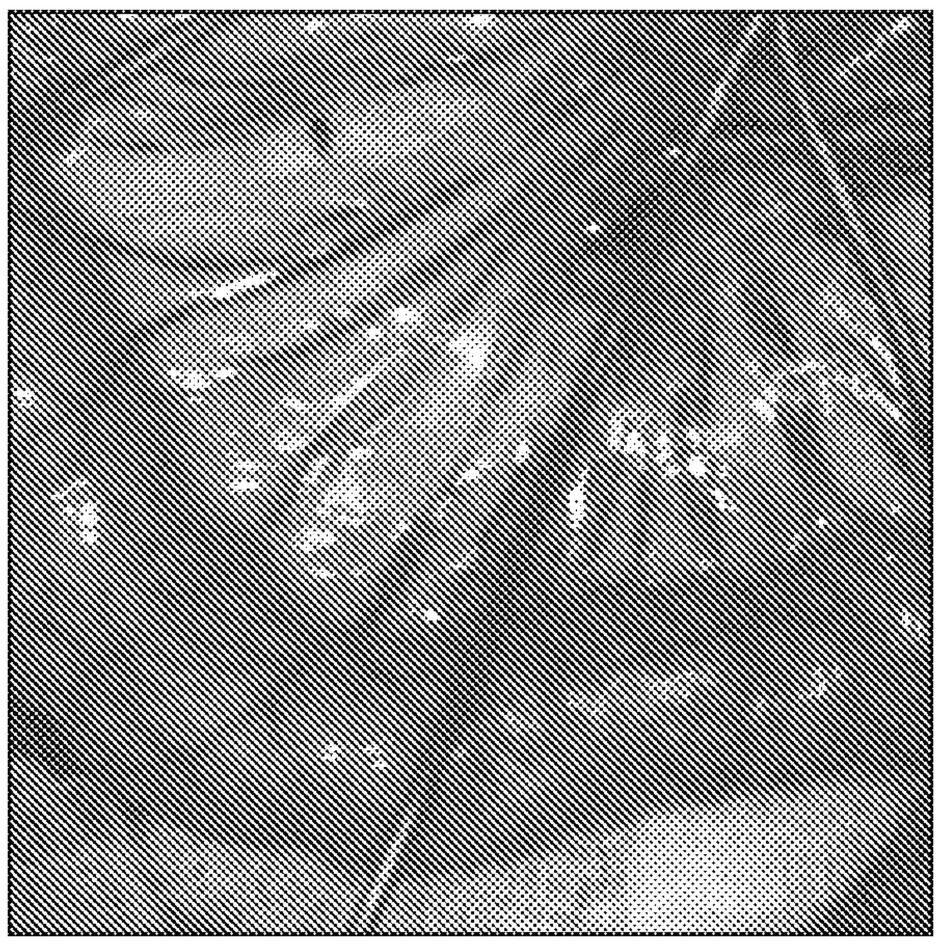


FIG. 12B



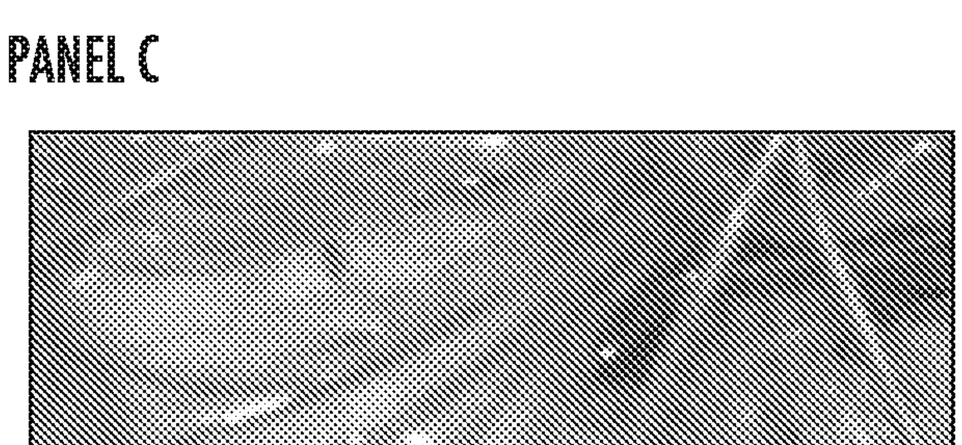


FIG. 12C

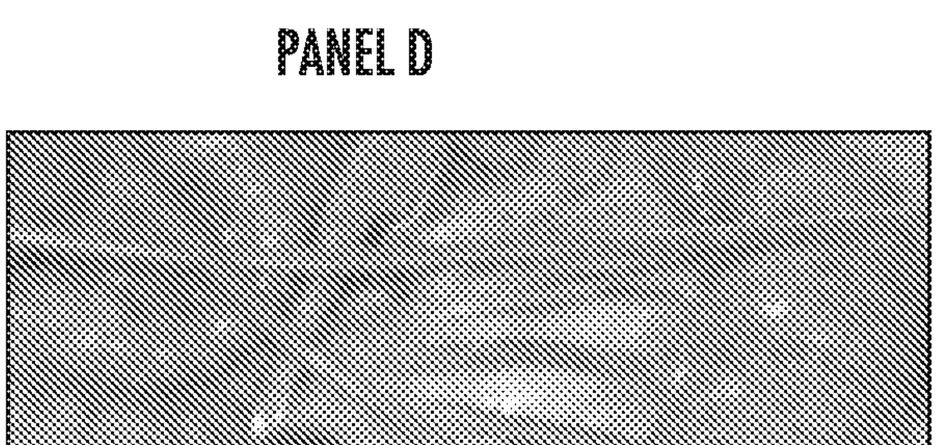


FIG. 12D

FIG. 13A

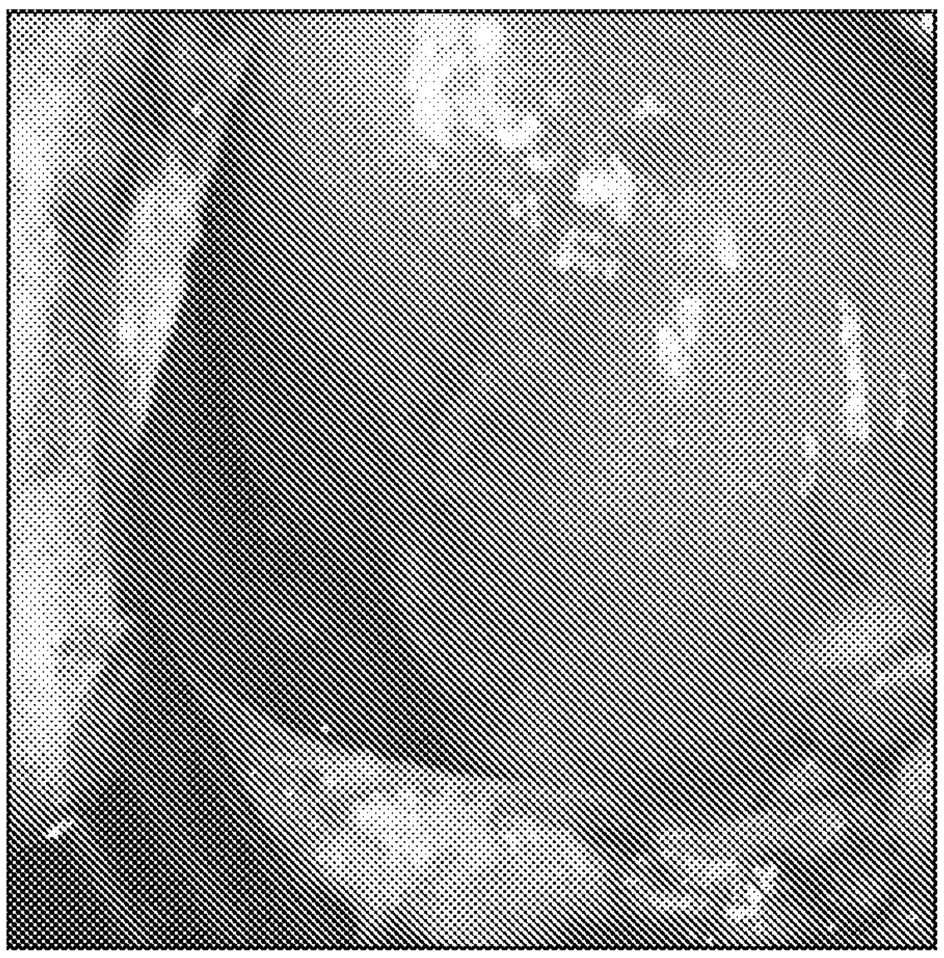
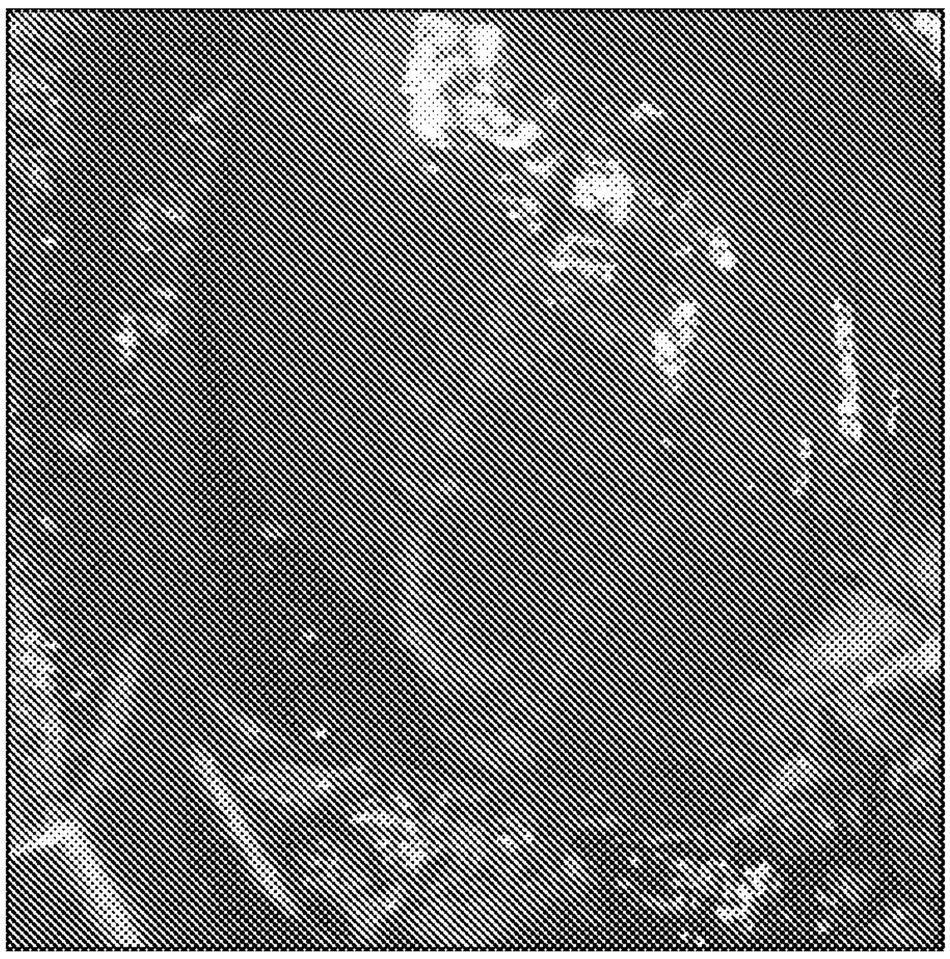


FIG. 13B



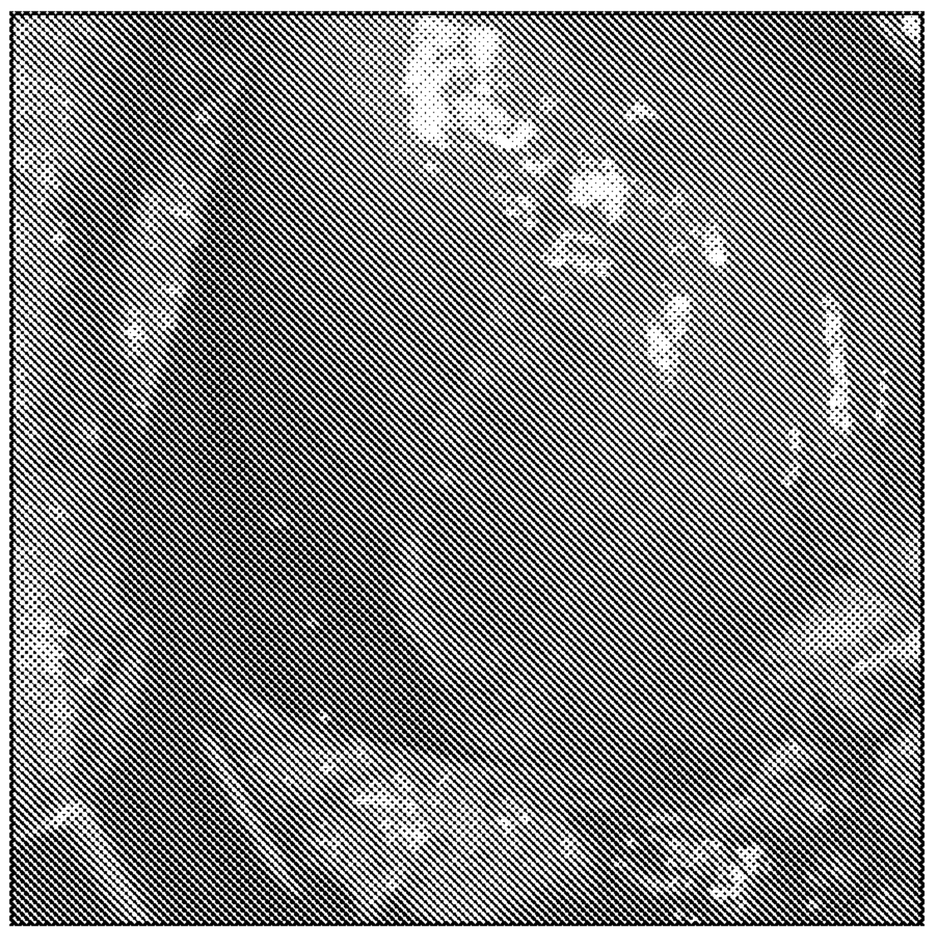


FIG. 13C

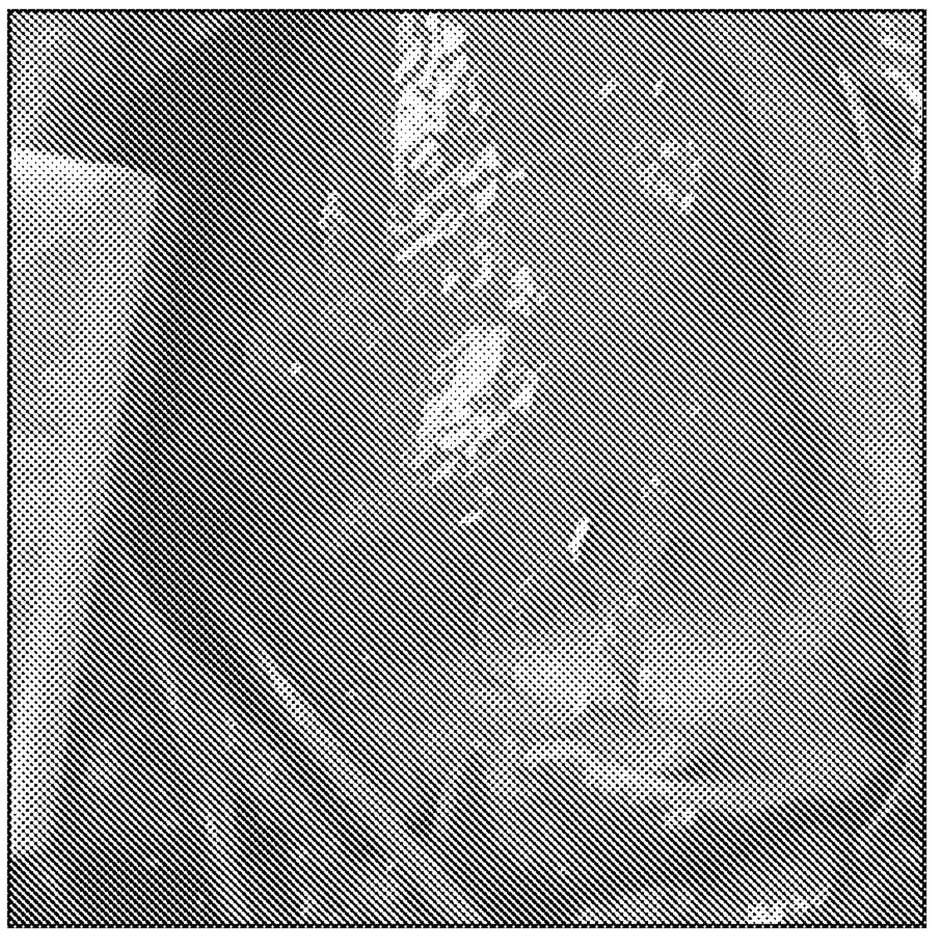


FIG. 13D

FIG. 14A

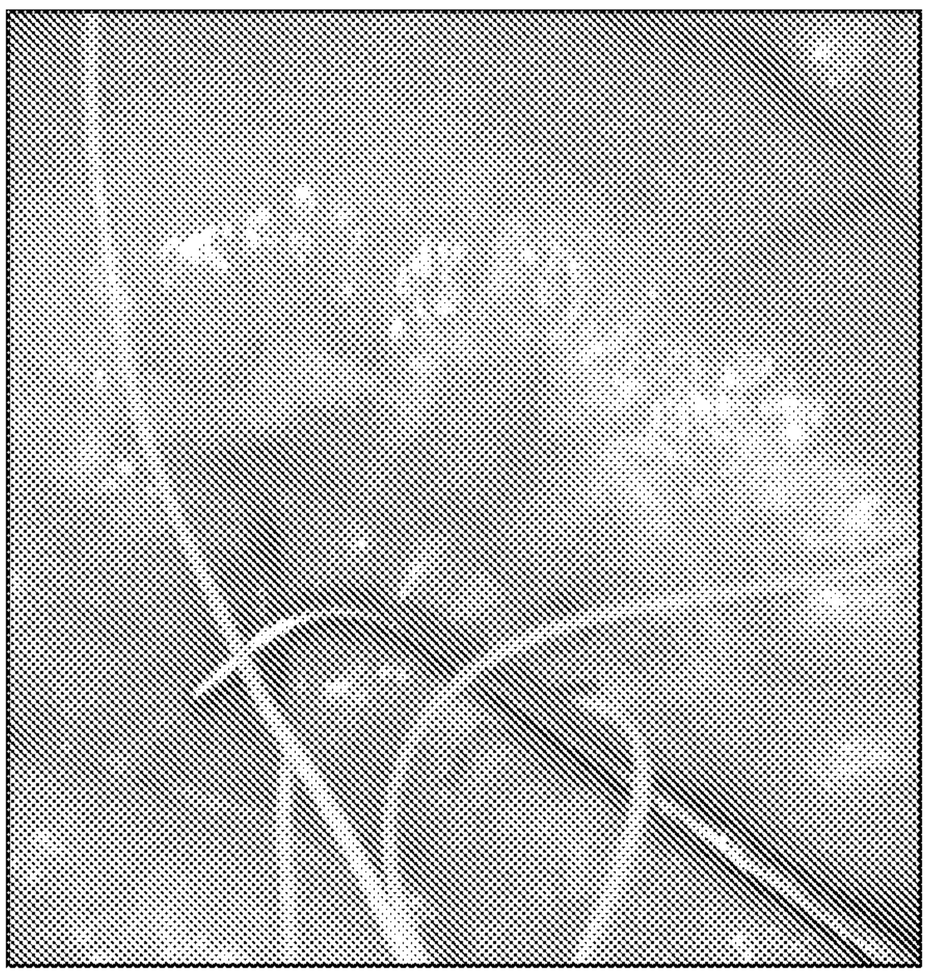


FIG. 14B

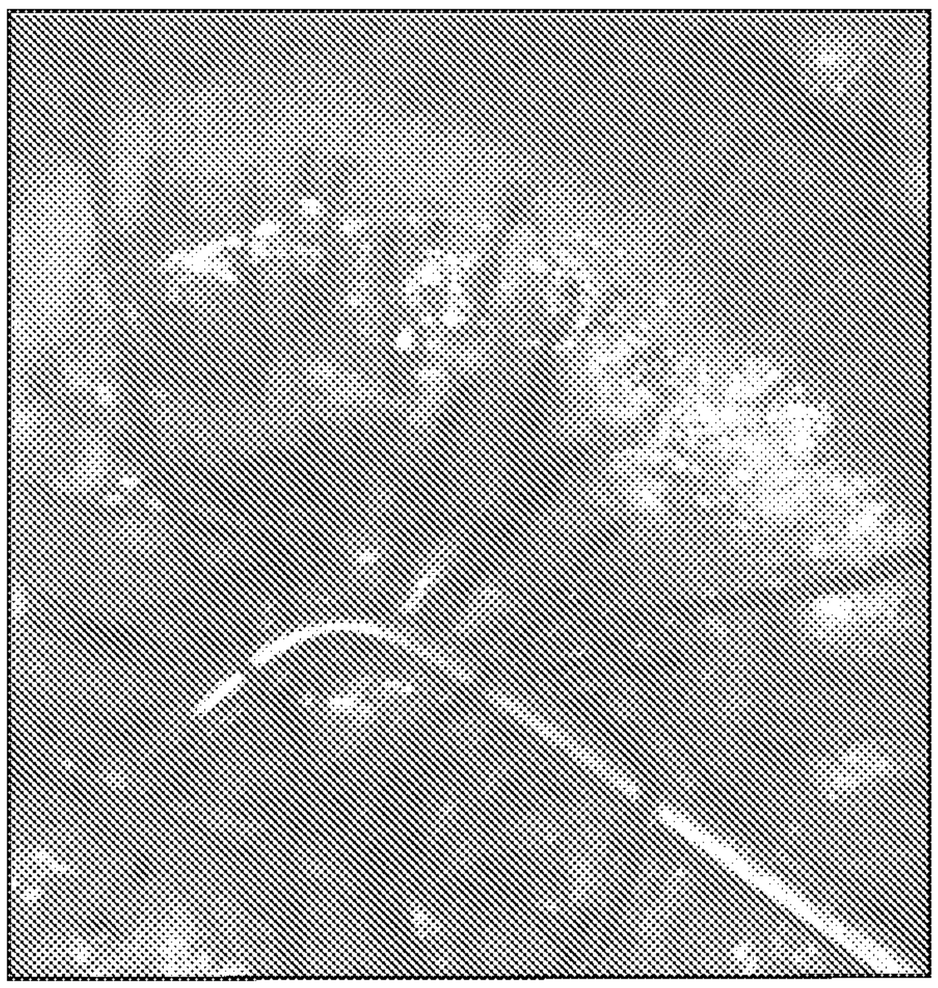


FIG. 14C

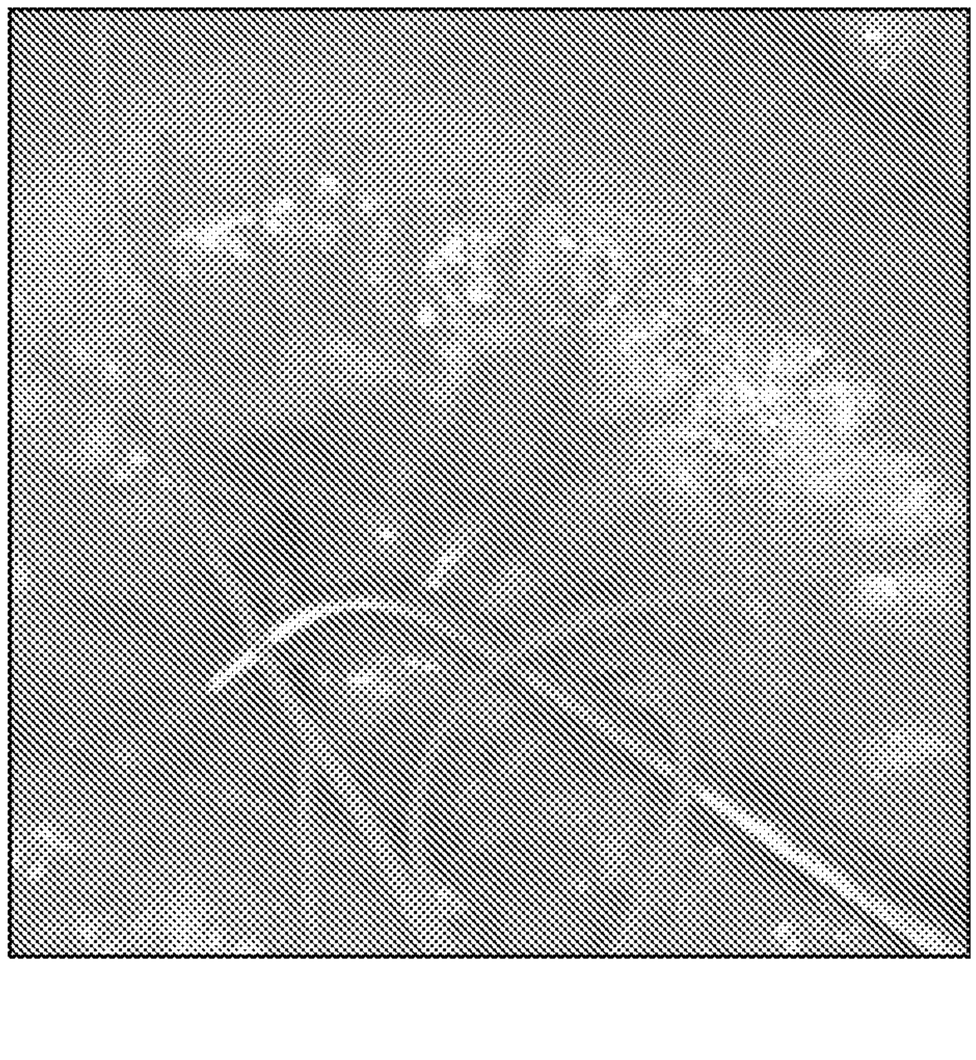


FIG. 14D

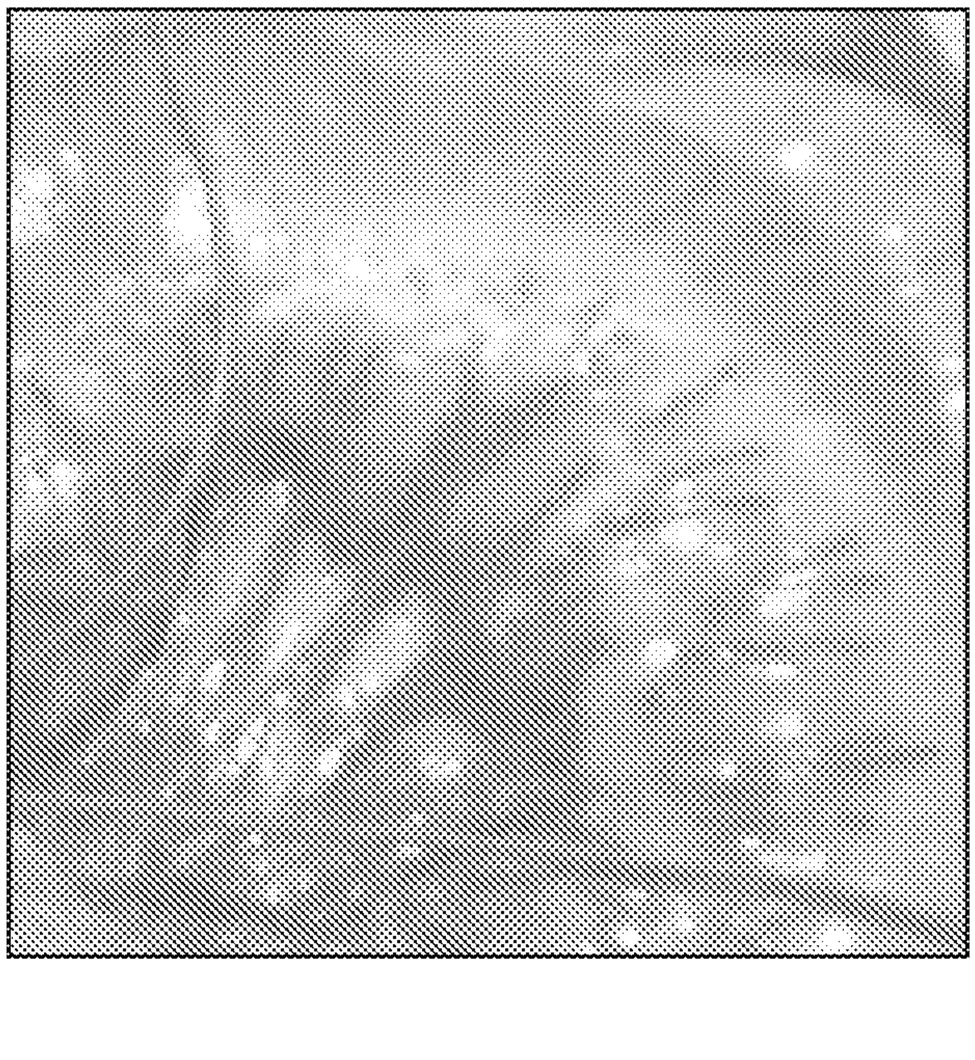


FIG. 14E

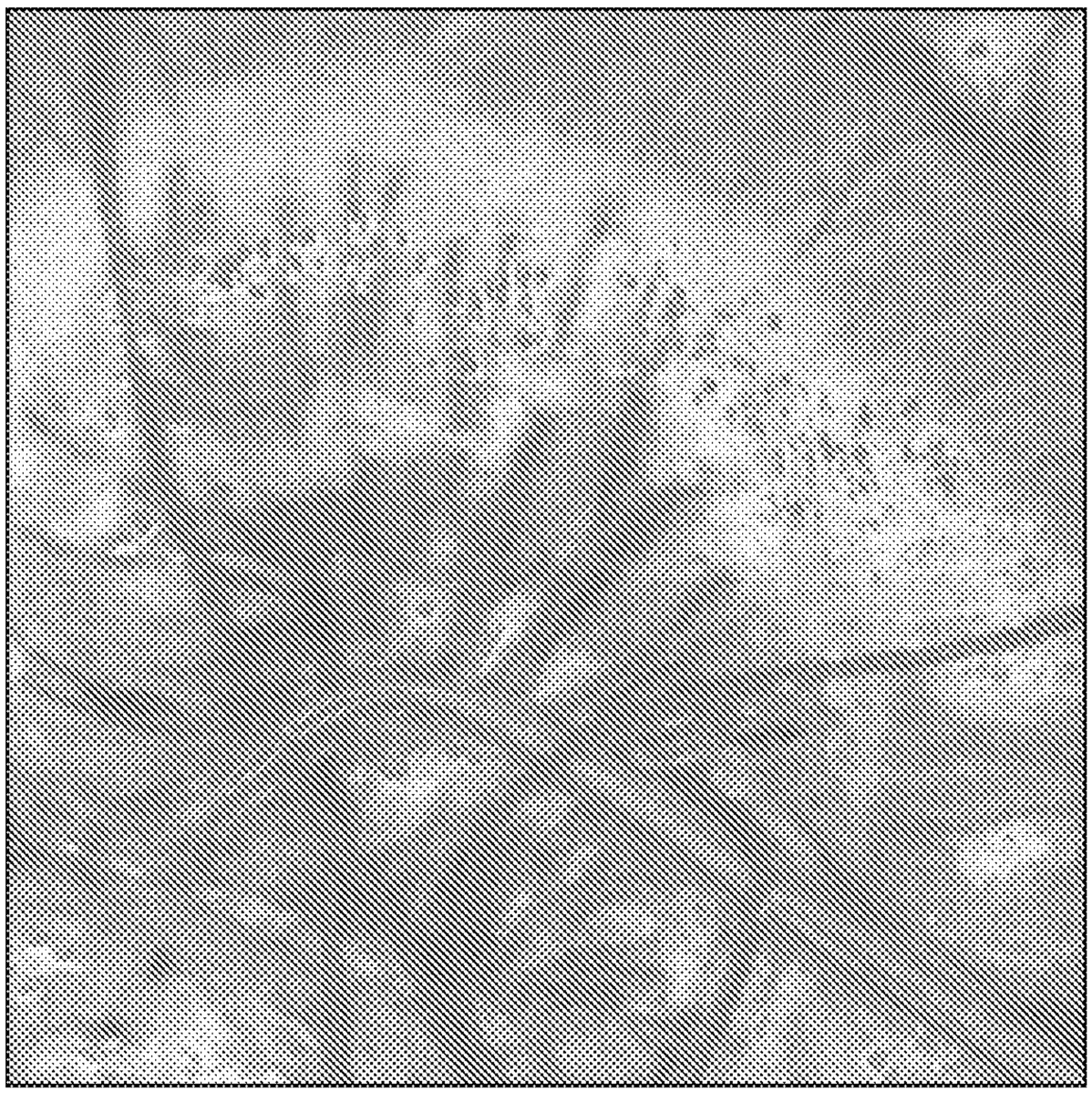


FIG. 15A

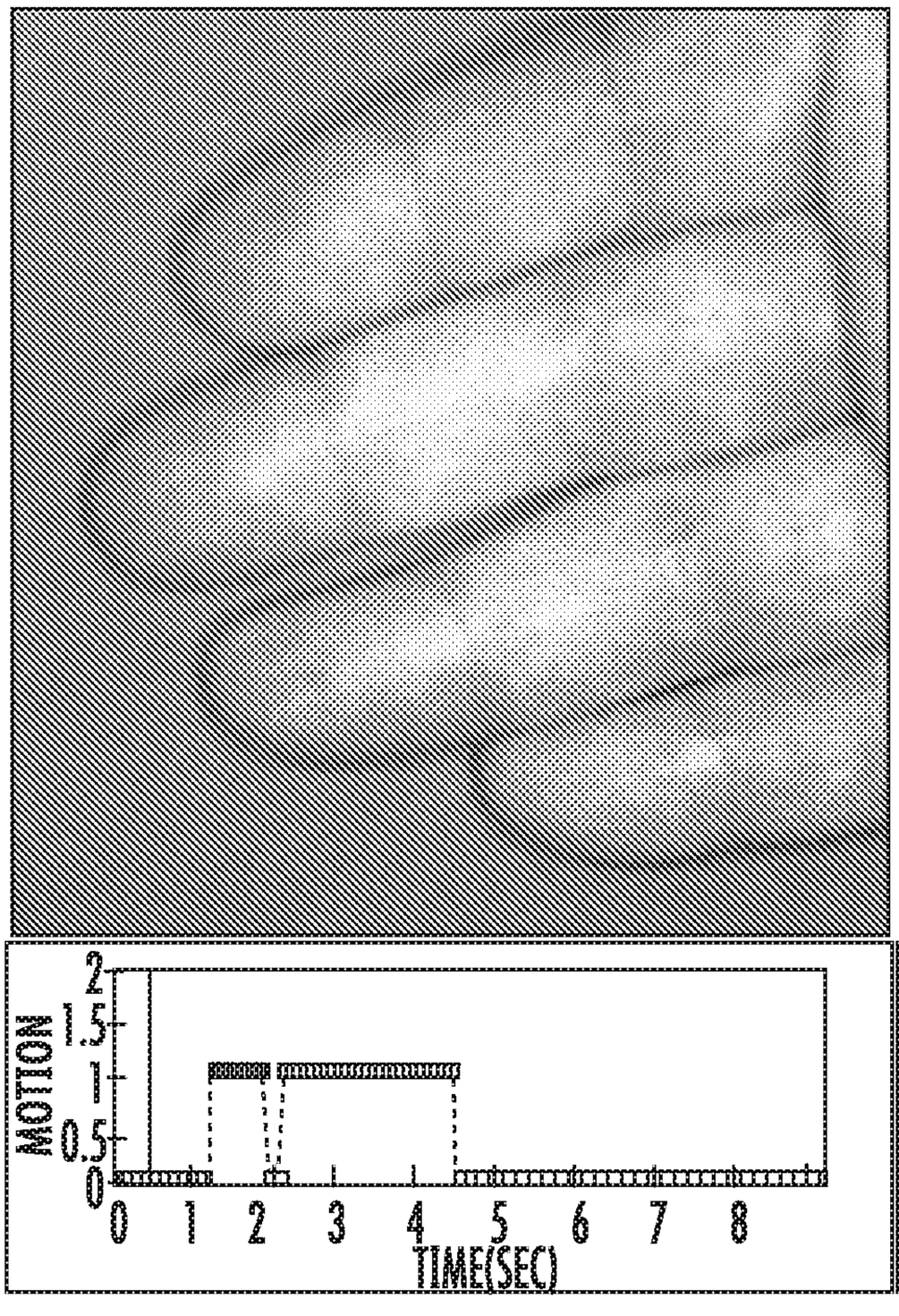
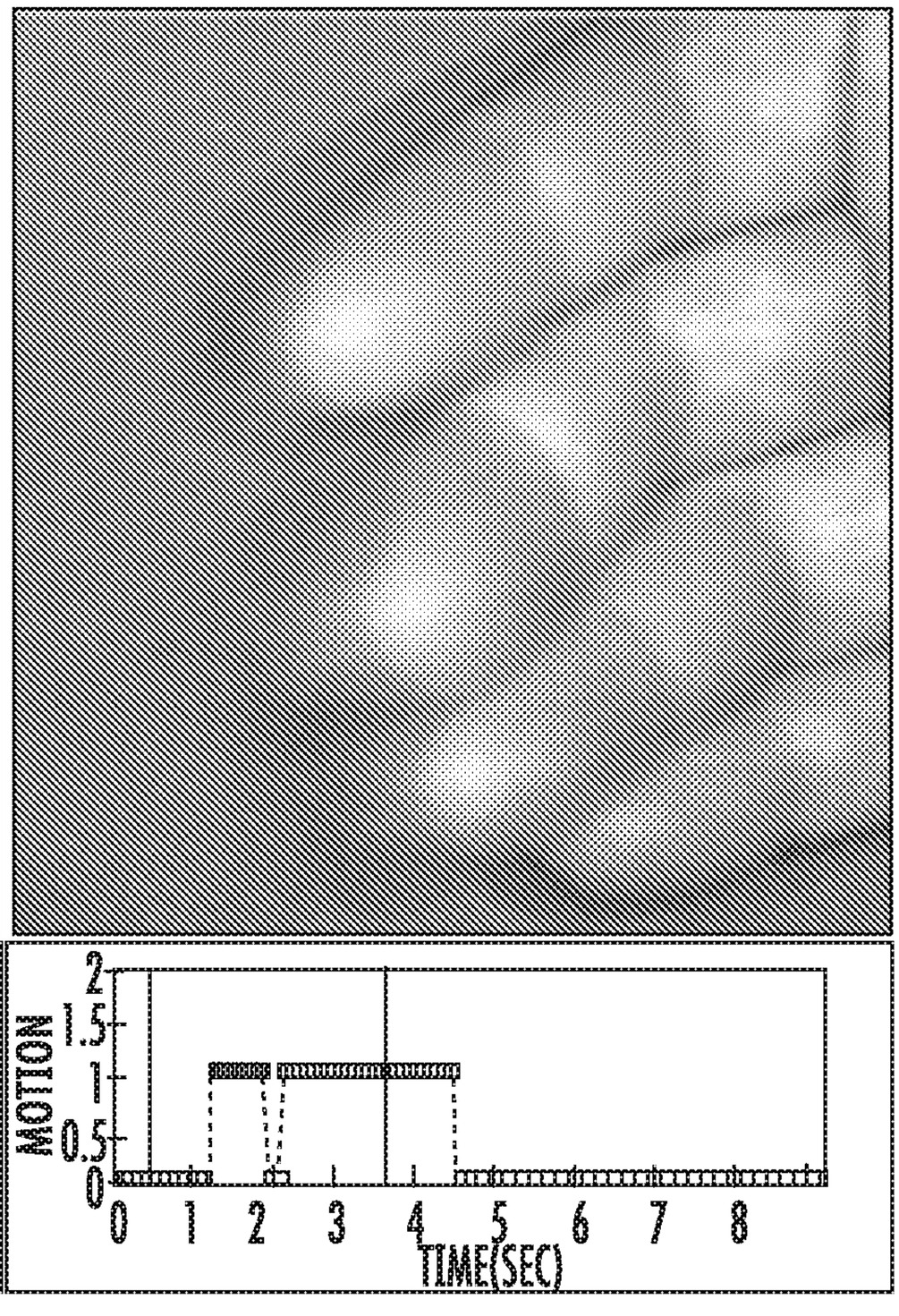


FIG. 15B



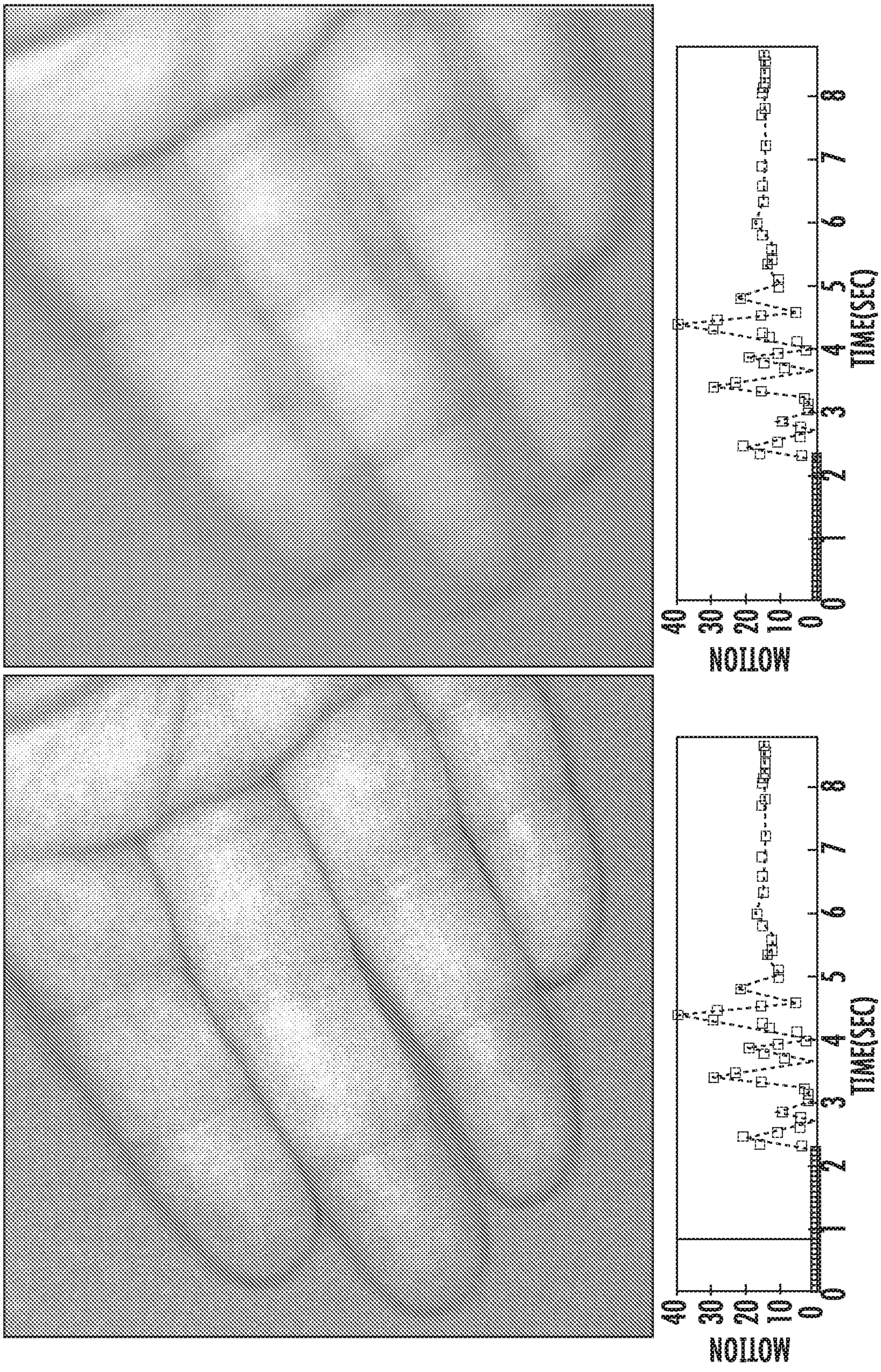


FIG. 16B

FIG. 16A

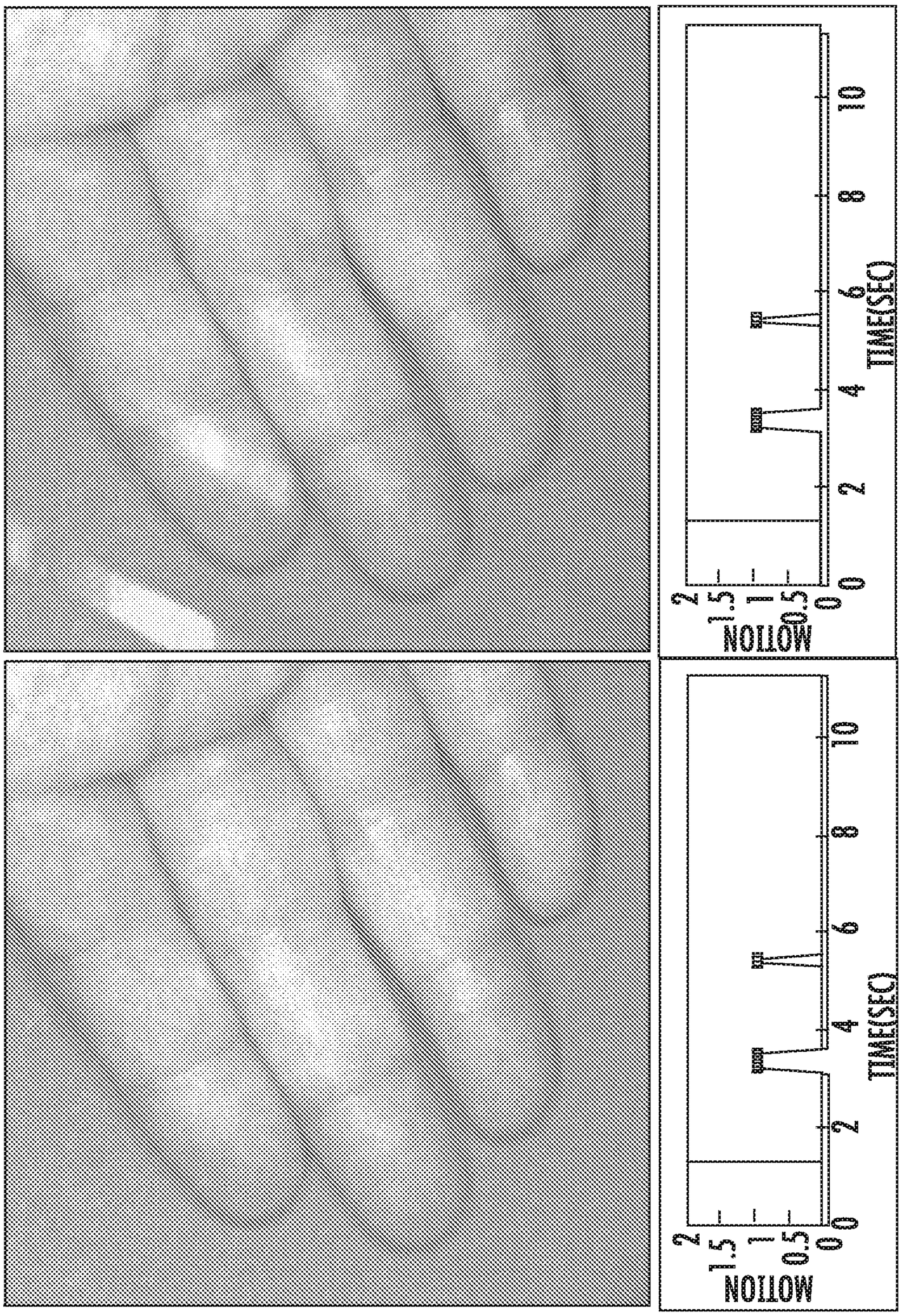


FIG. 17A

FIG. 17B

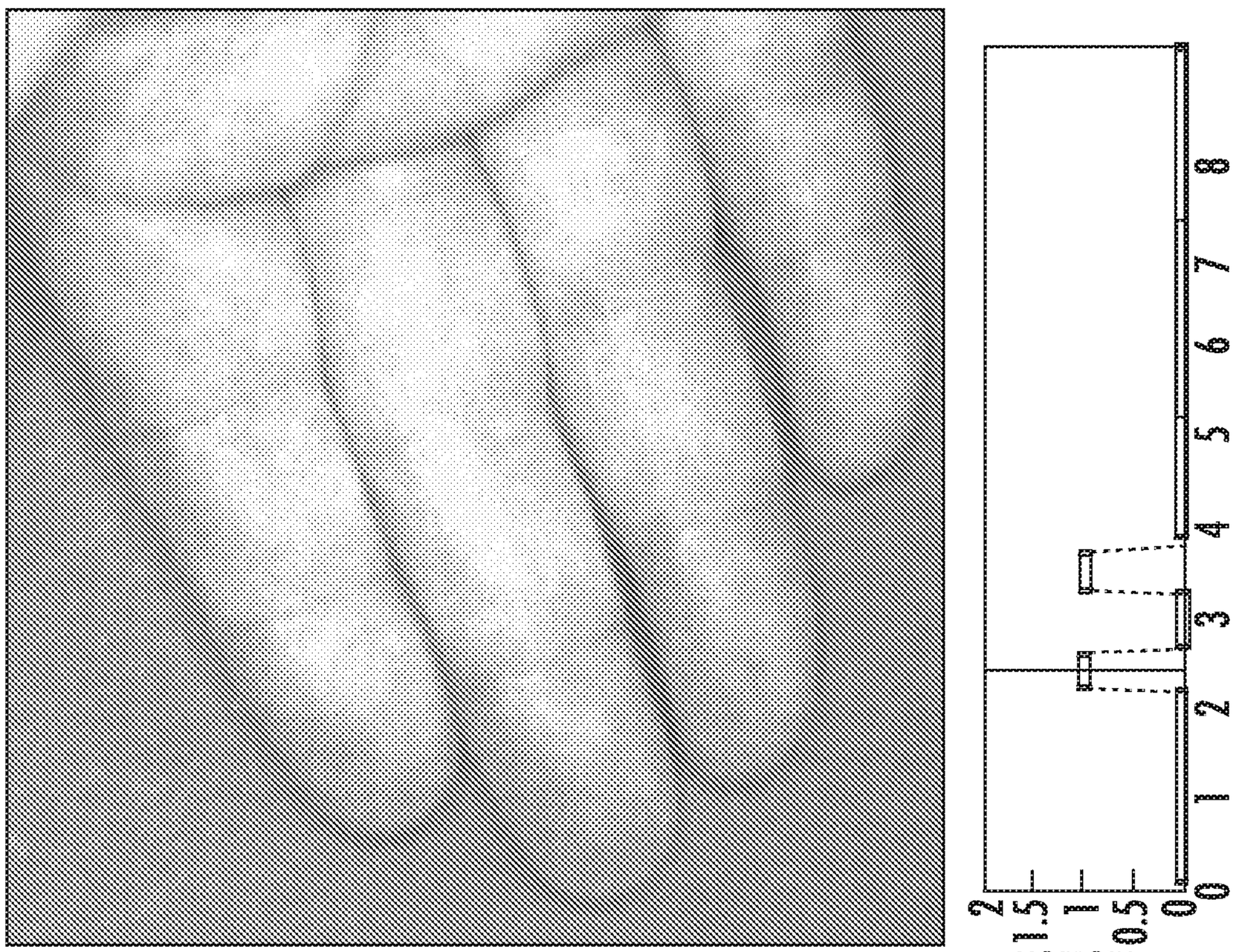


FIG. 18B

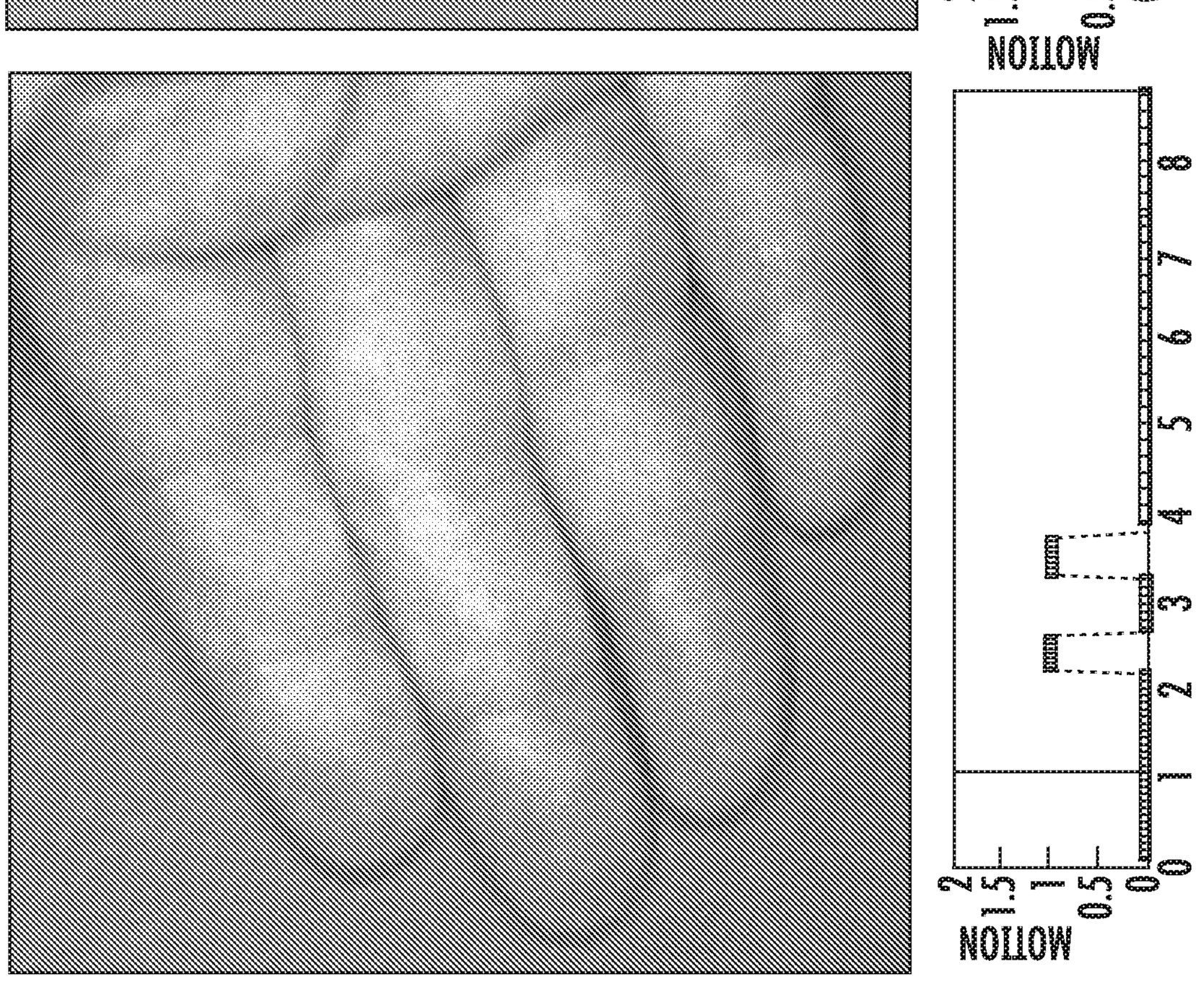


FIG. 18A

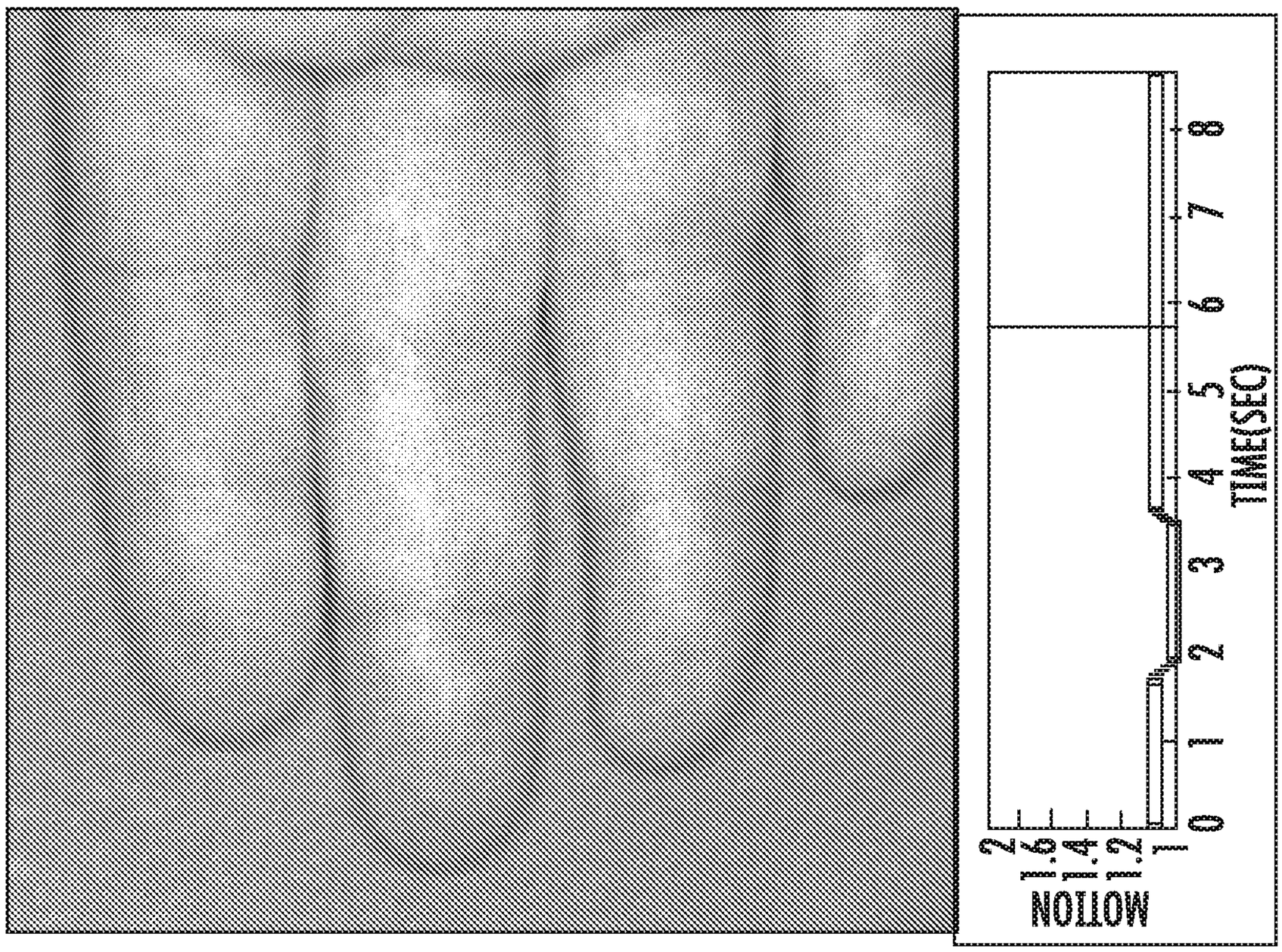


FIG. 19B

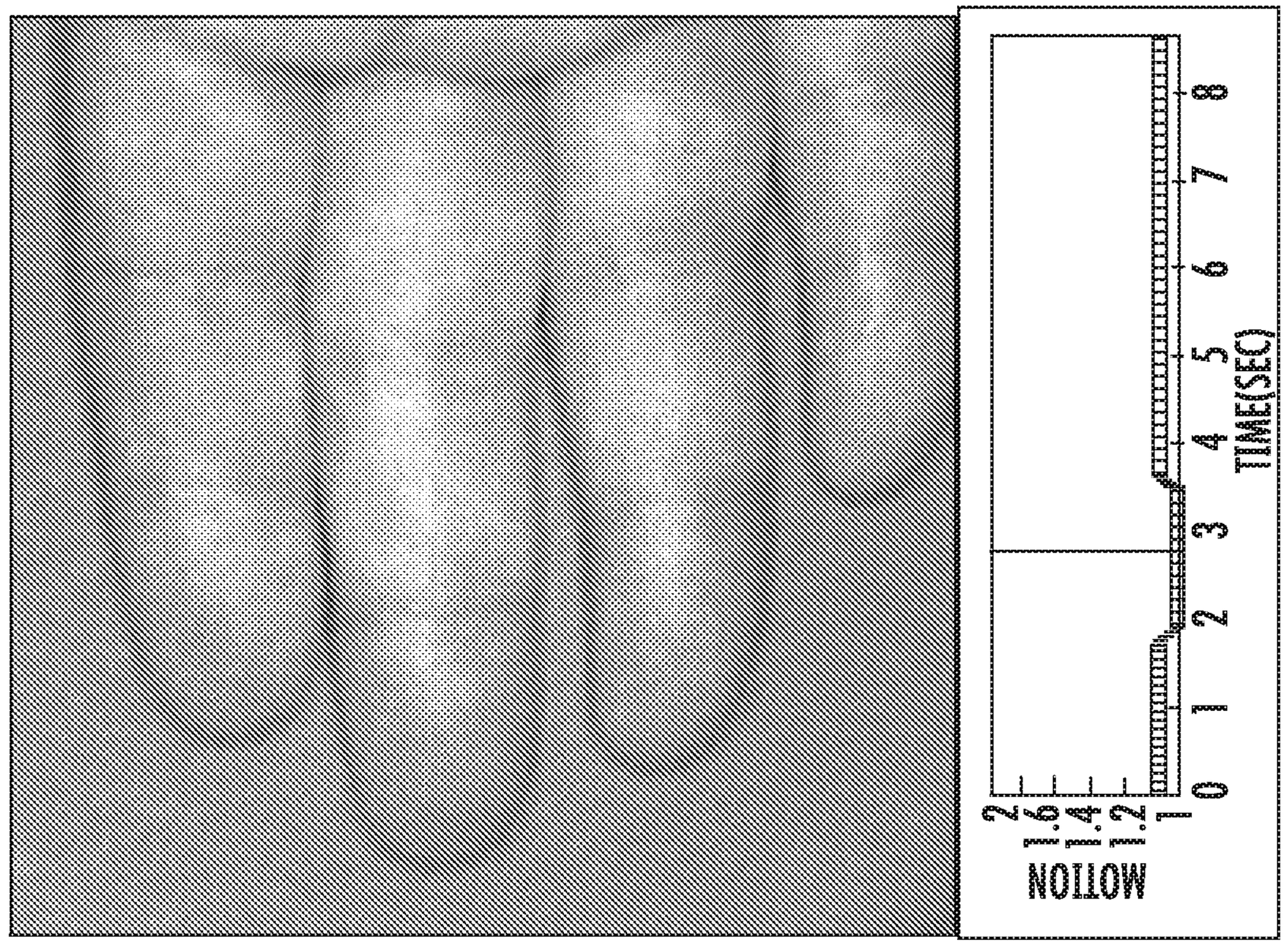


FIG. 19A

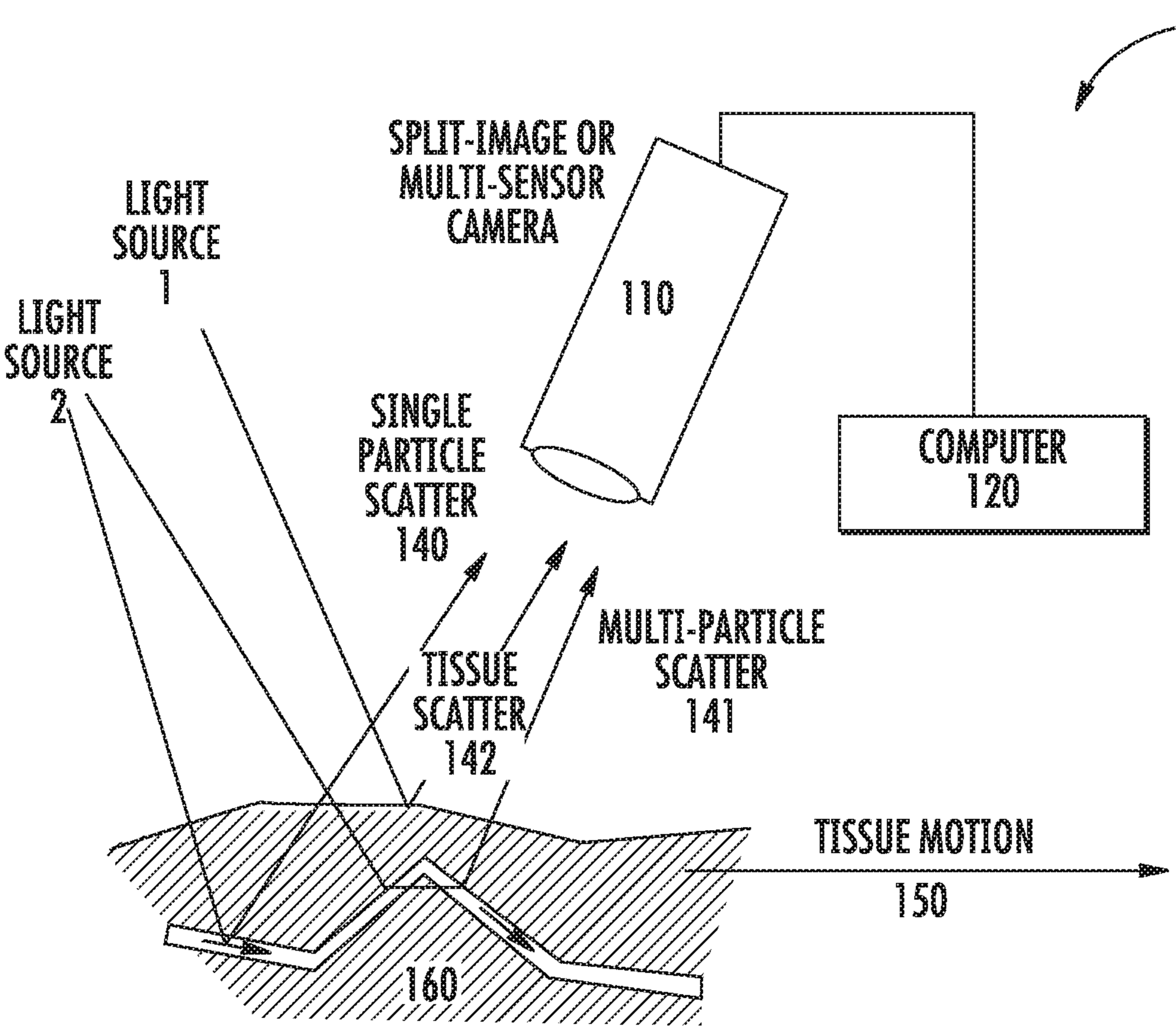

100
SPLIT-IMAGE OR MULTI-SENSOR CAMERA
110
LIGHT SOURCE 1
LIGHT SOURCE 2
SINGLE PARTICLE SCATTER 140
COMPUTER 120
MULTI-PARTICLE SCATTER 141
TISSUE SCATTER 142
TISSUE MOTION 150
160

FIG. 1