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(71) Applicant: **CALIFORNIA INSTITUTE OF TECHNOLOGY** [US/US]; 1200 E. California Boulevard, Pasadena, California 91125 (US).

(72) Inventors: **OU, Xiaoze**; 180 S. Catalina Avenue, #3, Pasadena, California 91106 (US). **CHUNG, Jaebum**; 1043 E. Del Mar Boulevard, Pasadena, California 91106 (US). **YANG, Changhuei**; 1918 La France Avenue, South Pasadena, California 91030 (US).

(74) Agents: **MARTINEZ-LEMKE, Sheila** et al.; WEAVER AUSTIN VILLENEUVE & SAMPSON LLP, P.O. Box 70250, Oakland, California 94614-0250 (US).

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(54) Title: LASER-BASED FOURIER PTYCHOGRAPHIC IMAGING SYSTEMS AND METHODS

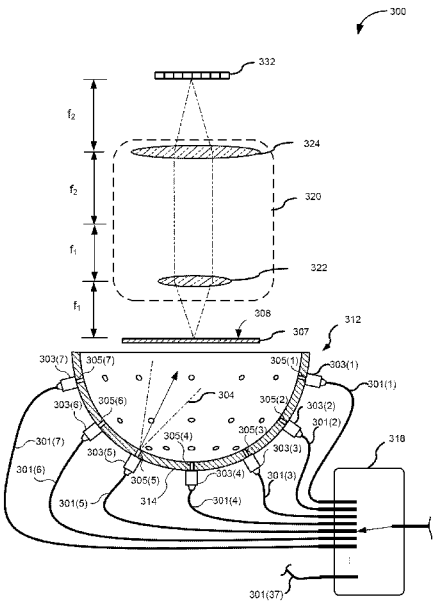

FIG. 3

(57) Abstract: Certain embodiments pertain to laser-based Fourier ptychographic (LFP) imaging systems, angle direction devices used in the LFP imaging systems, optical switches used in the LFP imaging systems, and LFP imaging methods. The LFP systems include an angle direction device for directing laser light to a sample plane at a plurality of illumination angles at different sample times. The LFP systems also include an optical system and a light detector. The optical system receives light issuing from the sample being imaged and propagates and focuses the light to the light detector acquiring raw intensity images.

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LASER-BASED FOURIER PTYCHOGRAPHIC IMAGING SYSTEMS AND METHODS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/165,084, titled "Laser Based Fourier Ptychographic Microscopy" and filed on May 21, 2015, which is hereby incorporated by reference in its entirety and for all purposes.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant No. OD007307 and AI096226 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD

[0003] Certain embodiments described herein are generally related to digital imaging techniques, more specifically, in high-resolution imaging for microscopy and photography in applications such as, for example, pathology, haematology, and semiconductor wafer inspection.

BACKGROUND

[0004] Imaging lenses ranging from microscope objectives to satellite-based cameras are physically limited in the total number of features they can resolve. These limitations are a function of the point-spread function size of the imaging system and the inherent aberrations across its image plane field of view. Referred to as the space-bandwidth product, the physical limitation scales with the dimensions of the lens but is usually on the order of 10 megapixels regardless of the magnification factor or numerical aperture (NA). While conventional imaging systems may be able to resolve up to 10 megapixels, there is typically a tradeoff between point-spread function and field of view. For example, certain conventional microscope objectives may offer a sharp point-spread function across a narrow field of view, while others imaging systems with wide-angle lenses can offer a wide field of view at the expense of a blurry point-spread function.

[0005] Traditionally, the resolution of an image sensor, such as in a digital camera, determines the fidelity of visual features in the resultant images captured by the image sensor. However, the resolution of any image sensor is fundamentally limited by geometric aberrations in the lens or lenses used to focus light onto the image sensor. This is because the number of resolvable points for a lens, referred to as the SBP, is fundamentally limited by geometrical aberrations. While CMOS and CCD technologies have been demonstrated having image sensors with pixels in the 1 micron (μm) range, it remains a challenge to design and manufacture lenses which have the resolving power to match the resolution of such image sensors.

[0006] Certain interferometric synthetic aperture techniques try to increase spatial-bandwidth product. Most of these interferometric synthetic aperture techniques include setups that record both intensity and phase information using interferometric holography such as off-line holography and phase-shifting holography. Interferometric holography has its limitations. For example, interferometric holography recordings typically use highly coherent light sources. As such, the constructed images typically suffer from coherent noise sources such as speckle noise, fixed pattern noise (induced by diffraction from dust particles and other optical imperfections in the beam path), and multiple interferences between different optical interfaces. Thus the image quality is typically worse than from a conventional microscope. On the other hand, using off-axis holography sacrifices spatial-bandwidth product (i.e., reduces total pixel number) of the image sensor. In addition, interferometric imaging techniques may be subject to uncontrollable phase fluctuations between different measurements. Hence, accurate a priori knowledge of the sample location may be needed to set a reference point in the image recovery process. Another limitation is that many of these interferometric imaging systems require mechanical scanning to rotate the sample and thus precise optical alignments, mechanical control at a sub-micron level, and associated maintenances are required by these systems. In terms of spatial-bandwidth product, these interferometric imaging systems may present little to no advantage as compared with a conventional microscope. Previous lensless microscopy such as in-line holography and contact-imaging microscopy also present drawbacks. For example, conventional in-line holography does not work well with contiguous samples and contact-imaging microscopy requires a sample to be in close proximity to the sensor.

[0007] A high spatial-bandwidth product is very desirable in imaging applications such as microscopy for biomedical imaging such as used in pathology, haematology, phytotomy, immunohistochemistry, and neuroanatomy. For example, there is a strong need in biomedicine and neuroscience to image large numbers of histology slides for evaluation.

SUMMARY

[0008] Certain embodiments pertain to laser-based Fourier ptychographic (LFP) imaging systems and their components. For example, some embodiments relate to LFP systems comprising an angle direction device, an optical system comprising a collection element and a focusing element, and a light detector(s). The angle direction device is configured or configurable to direct laser light from a laser light source(s) to a sample plane generally at a specimen surface. The laser light is directed at a sequence of illumination angles at different sample times. The collection element is configured to receive light issuing from a specimen when it is located on the specimen surface. The sequence of illumination angles and numerical aperture of the collection element correspond to overlapping regions in a Fourier domain. The light detector is configured or configurable to receive light focused by the focusing element of the optical system and to acquire a plurality of raw intensity images of the specimen when it is located on the specimen surface and illuminated. Each raw intensity image acquired by the light detector(s) corresponds to a different illumination angle of the sequence of illumination angles. The LFP systems may also include a processor configured or configurable to execute instructions for iteratively updating overlapping regions in the Fourier domain with the plurality of intensity images acquired by the light detector to generate a high resolution image of the specimen. In some cases, the processor is also configured or configurable to execute instructions for filtering out low spatial frequency artifacts associated laser light by using a differential phase contrast deconvolution procedure.

[0009] Certain embodiments pertain to angle direction devices configured or configurable to direct laser light from a laser light source(s) to a sample plane generally at a specimen surface of an LFP system.

[0010] In one embodiment, the angle direction device comprises a plurality of fixed mirrors and one or more rotatable mirrors. Each fixed mirror is oriented to reflect

laser light at one of the plurality of illumination angles to the specimen surface. The one or more rotatable mirrors are configured or configurable to reflect laser light from the laser light source sequentially to different fixed mirrors of the plurality of fixed mirrors at different sampling times. The fixed mirrors are oriented to reflect laser light received from the one or more rotatable mirrors to the specimen surface at the sequence of illumination angles.

[0011] In another embodiment, the angle direction device comprises a plurality of optical fibers and one or more optical switches. Each of the plurality of optical fibers has a first and second end portion. The one or more optical switches are in optical communication with a laser light source. The one or more optical switches are configured or configurable to switch at different sampling times to direct laser light from the laser light source to the first end portion of different optical fibers of the plurality of optical fibers when the laser light source is activated. The optical pathways are configured so that each second end portion directs laser light to one of the sequence of illumination angles when the one or more optical switches is switched to the corresponding optical fiber and the laser light source is activated.

[0012] In another embodiment, the angle direction device comprises a movable stage (e.g., X-Y stage) and an optical fiber coupled to the movable stage and optically coupled at one end to the laser light source. The movable stage is configured or configurable to translate and/or rotate the optical fiber to direct laser light from the other end of the optical fiber to illuminate the specimen surface at the plurality of illumination angles at the different sampling times.

[0013] In another embodiment, the angle direction device comprises one or more rotatable mirrors and a lens system. The one or more rotatable mirrors are configured or configurable to direct the laser light from the laser light source to the specimen surface through a lens system, the lens system configured such that rotation of the one or more rotatable mirrors causes the laser light to illuminate the specimen at the sequence of illumination angles.

[0014] In certain embodiments, the angle direction device comprises a surface and a plurality of fixed mirrors coupled to the surface, each fixed mirror oriented to receive laser light and reflect the laser light to one of the plurality of illumination angles.

[0015] Certain embodiments pertain to laser-based Fourier ptychographic imaging methods employing at least one laser light source. The methods comprise directing laser light from a laser light source to a specimen surface located at about a sample plane using an angle direction device at a sequence of illumination angles. The methods further comprise receiving light, at a light detector, issuing from a sample when the sample is located on the specimen surface, the light received from an optical system. The methods further comprise acquiring a plurality of intensity images based on light received at the light detector, wherein each intensity image corresponds to one of the illumination angles. The methods further compriseconstructing a higher resolution image by simultaneously updating a pupil function and a sample spectrum, wherein the sample spectrum is updated in overlapping regions with Fourier transformed intensity images, wherein each of the overlapping regions corresponds to one of the plurality of illumination angles.

[0016] These and other features are described in more detail below with reference to the associated drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIG. 1** shows a simplified block diagram of a laser-based Fourier ptychographic (LFP) imaging system, in accordance with some implementations.

[0018] **FIG. 2** shows a schematic drawing of a cut-away view of an example of an LFP imaging system, in accordance with some implementations.

[0019] **FIG. 3** shows a schematic drawing of a cut-away view of another example of an LFP imaging system, in accordance with some implementations.

[0020] **FIG. 4** shows a schematic drawing of a cut-away view of another example of an LFP imaging system, in accordance with some implementations.

[0021] **FIG. 5** shows a schematic drawing of a cut-away view of another example of an LFP imaging system, in accordance with some implementations.

[0022] **FIG. 6** shows a schematic drawing of an orthogonal view of another example of an LFP imaging system, in accordance with some implementations.

[0023] **FIG. 7A** shows a schematic layout of an example of a mirror array, in accordance with some implementations.

[0024] **FIG. 7B** shows a schematic layout of an example of a region in Fourier space that corresponds to the mirror array of **FIG. 7A**, in accordance with some implementations.

[0025] **FIG. 8** shows a schematic drawing of an orthogonal view of another example of an LFP imaging system, in accordance with some implementations.

[0026] **FIG. 9A** shows a schematic drawing of an orthogonal view of another example of a mirror array, in accordance with some implementations.

[0027] **FIG. 9B** shows a schematic drawing of an orthogonal view of another example of a mirror array, in accordance with some implementations.

[0028] **FIG. 10** shows a flowchart of an example of an LFP imaging method, in accordance with some implementations.

[0029] **FIG. 11** shows a flowchart depicting details of operations of an LFP imaging method that implements DPC deconvolution, in accordance with some implementations.

[0030] **FIG. 12** shows an example of a side-by-side comparison of LFP reconstructed images that have been enhanced with DPC deconvolution and LFP reconstructed images that have not been enhanced with DPC deconvolution, in accordance with some implementations.

[0031] **FIG. 13A** shows an example of a raw image of a sample acquired by an LFP imaging system, in accordance with some implementations.

[0032] **FIG. 13B** shows an example of an LFP reconstructed high resolution image of the sample of **FIG. 13A**, in accordance with some implementations.

[0033] **FIG. 14** shows a schematic representation of a phase and amplitude reconstruction process during the performance of an LFP imaging method, according to an implementation.

DETAILED DESCRIPTION

[0034] Embodiments of the present invention will be described below with reference to the accompanying drawings. The following description is directed to certain implementations for the purposes of describing various aspects of this disclosure. However, a person having ordinary skill in the art will readily recognize that the teachings herein can be applied in a multitude of different ways. Thus, the teachings are not intended to be limited to the implementations depicted solely in the Figures, but instead have wide applicability as will be readily apparent to one having ordinary skill in the art.

I. Introduction

[0035] Fourier ptychography imaging techniques generally involve synthesizing an image with a higher bandwidth using multiple low-bandwidth images captured at different spatial frequency regions. Fourier ptychography techniques provide resolution enhancement that can be implemented with an optical system to achieve resolution construction beyond the physical capabilities of the optical system alone. In certain cases, Fourier ptychography imaging involves the acquisition of multiple raw intensity images of a sample where each image is acquired using light at a different illumination angle than the others (variable illumination).

[0036] An imaging system that implements Fourier ptychography imaging techniques using a microscope optical system is collectively referred to as an FPM. Some examples of existing FPMs that use light-emitting diode (LED) illumination can be found in G. Zheng, R. Horstmeyer and C. Yang, "Wide-field, high-resolution Fourier ptychographic microscopy," Nature Photonics, 2013, X. Ou, R. Horstmeyer, C. Yang and G. Zheng, "Quantitative phase imaging via Fourier ptychographic microscopy," Optics Letters, 2013, X. Ou, R. Horstmeyer, G. Zheng and C. Yang, "High numerical aperture Fourier ptychography: principle, implementation and characterization," Optics Express, 2015, "A phase space model of Fourier ptychographic microscopy," Opt. Express 22(1), 338-358 (2014), X. Ou, G. Zheng, and C. Yang, "Embedded pupil function recovery for Fourier ptychographic microscopy," Opt. Express 22(5), 4960-4972 (2014), X. Ou, R. Horstmeyer, G. Zheng, and C. Yang, "Counting White Blood Cells from a Blood Smear Using Fourier Ptychographic Microscopy," PLoS One 10(7), e0133489 (2015), A. Williams,

J. Chung, X. Ou, G. Zheng, S. Rawal, Z. Ao, R. Datar, C. Yang, and R. Cote, "Fourier ptychographic microscopy for filtration-based circulating tumor cell enumeration and analysis," J. Biomed. Opt. 19(6), 066007 (2014), and R. Horstmeyer, X. Ou, G. Zheng, P. Willems, and C. Yang, "Digital pathology with Fourier ptychography," Comput. Med. Imaging Graphics 42, 38-43 (2015), all of which are hereby incorporated by reference for the discussion regarding FPMs.

[0037] As introduced above, existing implementations of FPMs typically used an array of average consumable light-emitting diodes (LEDs) to provide angularly varying illumination. The time need to acquire the raw intensity images and generate a high resolution image with the existing LED-based FPMs was typically relatively slow since LEDs provide low intensity illumination that required lengthy exposure times. Although high power LEDs could be used, they require cooling systems that made it difficult to design a compact system.

[0038] Various aspects described here relate generally to Laser-based Fourier ptychographic (LFP) imaging systems, devices, and methods that use a guided laser beam as illumination. In these aspects, the LFP imaging system includes an angle direction device to direct laser light illumination at different illumination angles. For example, certain LFP imaging systems discussed herein are configured to enable high resolution imaging of a sample (also referred to herein as a "specimen") on a specimen surface at a sample plane, using an angle direction device configured or configurable to direct laser light sequentially at different illumination angles to the sample plane. The use of a laser light sometimes present speckles during image acquisition due to reflections between glass surfaces in the system. If not mitigated, these speckles would appear as slowly varying background fluctuations in the final reconstructed image. The LFP imaging method is adapted to mitigate artifacts from any speckles.

[0039] Since LFP imaging systems use laser light, they have several advantages as compared to existing Fourier ptychography imaging systems that use average consumable LED array to provide variable illumination. First, laser light has brighter illumination as compared to conventional LEDs which enables shortening the exposure time of raw intensity image acquisition. For example, certain LFP systems have exposure times in the range of about 10 microseconds to about 100 milliseconds

per frame as compared to LED-based systems which have exposure times in the range of 10 millisecond to 10 seconds per frame that typically use average consumable LEDs with relatively low power. Shorter exposure time can provide an imaging scheme with higher temporal resolution suitable for resolving transient biological processes. Since the duration of an imaging cycle is mostly driven by the time intensive raw image acquisition process, using laser light significantly reduces the cycle time (e.g., range of 100 milliseconds to less than about 10 seconds) as compared to LED-based systems (e.g., range of more than 10 seconds to 1000 seconds). Additionally the broader range and finer spectrum of a laser light source potentially allows for spectral imaging capability. Conventional LED-based systems used LEDs with a broad spectrum and a limited number of choices for the central wavelength. Thus, the potential application of conventional LED-based Fourier ptychography systems was limited to the spectra offered by the LEDs.

[0040] As introduced above, various aspects described herein relate to LFP imaging systems that implement LFP methods to obtain high-resolution images of a sample. In certain implementations, LFP imaging systems include a specimen surface for receiving a sample being imaged, an angle direction device, an optical system, and a light detector such as an image sensor. The angle direction device is configured or configurable to direct laser light from a laser light source to the specimen surface at a sequence of illumination angles. The optical system comprises a collection element (e.g., lens) for receiving light issuing from the sample when it is illuminated from laser light directed by the angle direction device. The optical system also includes a focusing element (e.g., lens) for focusing light propagated from the collection element to the light detector. In some cases, the LFP imaging system further includes a controller in communication with the angle direction device and/or laser light source and configured to cause laser light from the laser light source to be directed by the angle direction device to the specimen surface at the sequence of illumination angles at particular sampling times. The controller may also be in communication with the light detector to synchronize exposure time of the light detector for measuring the intensity distribution data of each raw intensity image while laser light is illuminating from one of the sequence of illumination angles. Intensity distribution data is measured at the light detector when the sample is illuminated at each of the illumination angles. The intensity distribution data measured at the light detector

when the sample is illuminated at a particular illumination angle is referred to herein as an "intensity image" or "raw image" corresponding to the particular illumination angle. A sequence of intensity images of the sample is acquired over the course of an entire "image acquisition phase" during which the sample is sequentially illuminated at different illumination angles. The optical system focuses light scattered by the sample onto a light detector. Over the course of the image acquisition phase, the light detector takes a sequence of intensity distribution measurements (intensity images), one raw intensity image being measured for each illumination angle. A processor combines the raw intensity images in the spatial frequency domain using a reconstruction process to correct aberrations and to render a single high-resolution image of the sample.

[0041] Since the laser light is generally brighter than light from standard LEDs, holding the sensitivity of the light detector constant, use of a laser light source allows for a faster image acquisition phase than if standard LEDs were used. The LFP imaging systems and methods described herein allow for a compact imaging system that is able to acquire a sufficient number of intensity images to generate a high resolution image of a sample over a short image acquisition phase, e.g. potentially less than 1 second. In one embodiment, an LFP system can be configured to generate a high resolution image of a sample in less than 1 second. In another embodiment, an LFP system can be configured to generate a high resolution image of a sample in less than 10 seconds. In another embodiment, an LFP system can be configured to generate a high resolution image of a sample in less than 60 seconds. In one embodiment, an LFP system can be configured to generate a high resolution image of a sample in a range of 1 to 100 seconds. In one embodiment, an LFP system can be configured to acquire an intensity image at a rate of 100 per second. In one embodiment, an LFP system can be configured to acquire an intensity image at a rate of 1000 per second.

[0042] The LFP imaging systems disclosed herein have additional benefits compared to e Fourier ptychography imaging systems that use standard LEDs. By way of example, as discussed above, certain LFP imaging systems described herein are capable of capturing sufficient intensity images to generate a high resolution image of a specimen in a relatively short amount of time. Therefore, unlike slower FP

imaging systems that use standard LEDs, LFP imaging systems may more effectively image moving specimens.

[0043] Some examples of LFP imaging systems are described in further detail below with reference to the Figures.

I. Laser-based Fourier Ptychographic (LFP) Systems

[0044] A. LFP systems

[0045] **FIG. 1** is a block diagram of an LFP imaging system **100** capable of implementing an LFP imaging method, according to embodiments. At a high level, the LFP imaging system **100** is configured or configurable to sequentially illuminate a sample being imaged by laser light directed at different illumination angles, to capture a sequence of intensity images where each intensity image is captured when the sample is illuminated by laser light directed at one of the illumination angles, and process the raw image data to reconstruct a full resolution complex image of the sample while correcting for any aberrations from, for example, speckles generated through the use of laser light.

[0046] The LFP imaging system **100** includes a laser illumination system **110**, an optical system **120**, an imaging system **130**, a controller **140**, a communication interface **150**, and a display **152** in communication with the communication interface **150**, and internal memory **160**. The LFP imaging system **100** also optionally (denoted by dashed line) includes a communication interface **170** and an external computing device or system **172** in communication with the communication interface **170**. The LFP imaging system **100** also optionally includes a communication interface **180** and an external memory device or system **182** in communication with the communication interface **180** for optional storage of data to the external memory device or system **182**. The LFP imaging system **100** also optionally includes a network communication interface **190** and an external network **192** (for example, a wired or wireless network) communication with the communication interface **190**. Each of the communication interfaces **150**, **160**, **170**, **180**, **190** is in communication with the controller **140**. The laser illumination system **110** is configured or configurable to provide laser light illumination from a sequence of illumination angles. The optical system **120** generally has one or more lenses that propagate light issuing from the sample to one or more light detectors of the imaging system **130**. The controller **140** is in

communication with the internal memory **160** to retrieve and store data to the internal memory **160**. The controller **140** is configured or configurable to output raw image data, processed image data, and/or other data over a communication interface **150** for display on a display **152**. The controller **140** is in communication with the imaging system **130** which is in communication with the optical system **120**. The described communication between components of the LFP imaging system **100** may be in wired form and/or wireless form.

[0047] The controller **140** comprises one or more processors which are in communication with internal memory **160** and/or other computer readable memory (CRM). The controller **140** controls operations of the LFP imaging system **100** based on instructions stored in memory (e.g., internal memory **160**). The controller **140** is in electrical communication with the imaging system **130** to receive the raw image data from the imaging system **130**. Optionally (denoted by dotted lines), the controller **140** may also be in electrical communication with the laser illumination system **110** to control illumination, for example, in order to synchronize the illumination with the exposure times during acquisition of intensity images by the imaging system **130**. The controller **140** or another processor controls the illumination from laser light source(s). For example, the controller **140** or another processor may control the operation of an angle direction device that directs light from the laser light source(s) to at predefined illumination angles at particular times and for particular durations during various image acquisition exposures and/or control the activation of laser light source(s), for example, by powering on during the image acquisition phase. In some implementations, the controller **140** is further configured to execute instructions to perform processing operations on the raw image data such as operations performed as part of the LFP imaging method.

[0048] The imaging system **130** is in communication with the optical system **120** to receive light from the optical system **120** and capture raw images while the sample is on the specimen surface and illuminated, each raw image captured over an exposure time. The laser illumination system **110** is in communication with the optical system **120** to provide laser light to a sample being imaged on the specimen surface such that light scattered by or otherwise issuing from the sample is propagated through the optical system **120** to light detector(s) of the imaging system **130** which capture the raw images. When laser light illuminates the sample, light scattered or otherwise

issuing from the sample is propagated through the optical system **120** to the imaging system **130** which captures a sequence of intensity images.

[0049] The LFP imaging systems described herein include an angle direction device. An angle direction device generally refers to one or more components that are configured or configurable to guide a laser beam to the sample plane at N different illumination angles at N different image sampling times. During the image acquisition phase, the sample being imaged is located on the specimen surface at the sample plane and the laser light is directed to the sample being imaged. In certain embodiments, N has a value in a range of between 2 to 1000. In one case, N has a value in a range of between 90 and 200. In one case, $N = 95$. In one case, N is less than 100. In one case, N is less than 200. In one case, N has a value in a range of between 50 and 100. In one case, N has a value in a range of between 2 and 100. In one case, N has a value in a range of between 100 and 200.

[0050] Generally the angle direction device is designed to guide the laser beam to the sample plane to provide oblique illumination. The angle direction device is typically optically coupled with (or includes) one or more laser light sources. The angle direction device also includes one or more devices for directing the laser beam from the laser light source to the specimen surface at the N different illumination angles described in the preceding paragraph. For example, the angle direction device may include one or more mirrors and/or movement mechanisms to direct the laser beam sequentially to the different illumination angles at the appropriate sampling times. In one embodiment, the angle direction device includes an optical port for receiving an optical fiber coupled with the laser light source(s) and is able to translate and/or rotate the optical fiber to provide the oblique illumination described above. In one implementation, the angle direction device includes multiple optical ports for receiving multiple optical fibers. In this case, the angle direction device may also include one or more optical switches for switching laser light from the laser light source to a single optical fiber. In another implementation, the angle direction device may include one or more rotatable mirrors that direct light from the laser light source by way of an array of fixed mirrors, each of which may be positioned to illuminate the specimen surface at one of the illumination angles. In yet another implementation, the angle direction device may include one or more rotatable mirrors that direct light from the laser light source to the specimen surface by way of a system of lenses that

refract the light from the laser light source to illuminate the specimen surface at each of the illumination angles.

[0051] The angle direction device is generally configured or configurable to cause light from the laser light source to illuminate the sample plane at the specimen surface at different angles sequentially. The image data from the sequence of images captured based on illumination at different illumination angles corresponds to overlapping regions in Fourier space. The angle direction device is typically configured to direct laser light to illuminate at a sequence of illumination angles that provide for an overlapping area of neighboring regions of image data in Fourier space where the overlapping area is of at least a certain minimum amount (e.g. 65% overlap, 75% overlap, 70% overlap, 80% overlap, in a range of 10%-65% overlap, in a range of 65%-75% overlap, etc.). To provide this minimum amount of overlap of neighboring regions in Fourier space, the angle direction device may have elements that are configured so that the difference between adjacent illumination angles is less than a certain maximum angular difference.

[0052] The laser light source(s) may be a component of or may be separate from the LFP system **100**. The intensity of light provided by the laser light source may vary, for example, depending on the type of sample being imaged. By way of example, dead skin samples can tolerate a power per unit area of up to several W/cm^2. On the other hand, non-biological samples may be able to tolerate a much higher power per unit area without being degraded. Although examples described herein typically discuss a laser light source that provides visible light e.g. some of the LFP imaging systems disclosed herein generally operate with a laser light source with a wavelength in the visible range between 400 nm and 750 nm, the disclosure is not so limiting. In other embodiments, the laser wavelength may be in the ultra-violet region or in the infra-red region and the focusing element and glass surfaces of the LFP system would need to be appropriately substituted to work in these different wavelength ranges. In addition, other sources of laser radiation may be used, e.g. a maser, a free electron laser, an x-ray laser, an acoustic laser, etc. When different types of lasers are used, many of the optical elements, e.g. lenses or mirrors, disclosed herein may be substituted with suitable alternatives. By way of example, where an X-ray or microwave laser is used, mirrors and/or lenses that are configured to reflect or refract

x-rays or microwaves respectively may be included as substitutes for lenses and or mirrors described and/or depicted in figures herein.

[0053] The LFP systems described herein include an optical system that is generally configured to propagate illumination issuing from the sample to the one or more light detectors to be able to capture raw images of the sample. The optical system comprises at least a collection element and a focusing element. The collection element (e.g., lens) of an LFP system comprises one or more lenses and is designed to collect light scattered by or otherwise issuing from the specimen at the sample plane. Some examples of suitable collection elements include an $f = 50$ mm lens (e.g., f/1.8D AF Nikkor), 4x Olympus Plan Achromat objective 0.10 NA, CFI Plan Achromat 10x objective NA 0.25, an Valumax objective lens 5x 0.10 NA, and the like. The focusing element comprises one or more lenses and is located to receive incident light from the optical system and focus the light to the light detector. An example of suitable focusing elements include a tube lens (e.g., a $f = 200$ mm tube lens such as the Thorlabs® ITL200 tube lens). Although certain illustrated examples are shown with the collection and focusing elements arranged in a 4f arrangement, it would be understood that the disclosure is not so limiting and that other arrangements can be used. For example, the collection and focusing elements can be arranged in a 6f arrangement. In other examples, other optical elements such as bandpass filters, beam splitters, and/or mirrors can be included.

[0054] In some embodiments, the optical system of an LFP system has an iris located at the back focal plane of the collection element of the optical system. An iris generally refers to a circular aperture that can be adjusted in diameter to limit the amount of light collected by the optical system. Reducing the diameter and blocking out the outer region of light is equivalent to reducing the NA of the collection element, which means blocking out high spatial frequency information of the sample. Placing the iris is useful to accurately define the NA of the imaging system for the purpose of the LFP imaging method to prevent aliasing in the detected images, etc. There are different ways to control the size and shape of the iris. In one example, one can use a mechanical iris which has its diameter adjustable with a slider (e.g. Ring-actuated SM2 iris diaphragm from Thorlabs). As another example, an iris may be a transmissive liquid crystal display (LCD) that can adjust the contrast of its pixel elements to produce an aperture. As another example, light may be guided to an SLM

or DMD and then only a part of the beam reflected back into the system's optical path by turning on different elements on the SLM or DMD. With the use of an LCD, SLM, and DMD, the iris's shape is not limited to a circle because any discretized shape can be displayed on these displays and thus, the shape and size may be defined as the operator desires.

[0055] In some cases, the collection element, focusing element, illumination elements, and other components of the LFP system may be selected based on the particular imaging implementations. For example, when imaging a histological sample, the appropriate system NA (objective NA + illumination NA) provided by selecting a particular collection element and illumination elements may be in the range of 0.5-0.75. The objective NA may be 0.05 - 0.2 and the illumination NA 0.4 - 0.7 to provide this system NA, for example. On the other hand, if a blood smear is being analyzed for malaria, a system NA in the range of 1.2 - 1.5 may be desired.

[0056] Generally, the LFP systems described herein includes one or more light detectors. In certain examples, such as the one shown in FIG. 1, the LFP system includes an imaging system **130** comprising one or more light detectors. According to certain implementations, a light detector is configured to capture light and output a data signal including image data representative of the measured intensities of light received at particular locations of the active surface of the light detector (referred to herein as a "light intensity distribution," "intensity distribution," or simply as an "intensity image," "raw intensity image," "image," or "image frame"). The image data output by the light detector(s) is transmitted (or "sent" or "communicated") in a signal to a processor of, for example, a controller such as controller **140**. The light detector(s) of an LFP imaging system can acquire a sequence of N raw intensity images during the image acquisition process. Each light detector acquires an intensity image by measuring an intensity distribution of light incident on the sensing area of light detector during an exposure time. Some examples of suitable light detectors are CMOS sensors, a charge-coupled device (CCD), and other similar imaging devices. In one example, a light detector is a CCD having a pixel size of 5.5 μm such as the Prosilica GX6600 light detector. In certain implementations, the one or more light detectors in an LFP system are monochromatic light detectors.

[0057] The exposure time refers to the duration of time during which the light detector(s) measures a light intensity distribution of light received at its active surface to capture a single raw intensity image. The exposure time used to capture an intensity image by the light detector(s) of various implementations of the LFP systems depends on both the sensitivity of the light detector(s) and the intensity of the laser light from the one or more laser source(s). In other words, increasing the sensitivity of the light detector(s) and/or the intensity of the laser light source decreases the exposure time. As such, the LFP imaging systems described herein may be configured for particular exposure times based on the laser light source used. For example, a range of exposure times from 100 μs to 10 ms may be used for an LFP imaging system with a light detector in the form of a sCMOS detector and with a laser light source having a range of light intensities from 100μW to 10 mW.

[0058] Each of the LFP systems described herein is configured to implement an LFP imaging method. Each imaging cycle of the LFP imaging method generally includes a raw intensity image acquisition phase, a high-resolution reconstruction phase, and an optional display phase. During the raw image acquisition phase, light generated by the laser light source(s) and directed by the angular direction device illuminates the sample plane at different illumination angles sequentially. The sample is typically located on the specimen surface approximately located at the sample plane during the image acquisition phase. Laser light incident on the sample is scattered by the physical features of the sample as it passes through the sample. A portion of the scattered light then passes to the collection element of the optical system. The focusing element of the optical system focuses the scattered light from the sample to one or more light detectors. The one or more light detectors capture a sequence of raw intensity images during sequential illumination of the sample with laser light at the sequence of illumination angles. The processor(s) (e.g., processor of a controller) sends control signals to various system components to control their operations. The processor(s) controls the operations of the angular direction device and/or the light detectors by sending control signals.

[0059] During the high-resolution reconstruction phase, the processor(s) processes the raw image data from the image acquisition phase to generate processed image data. In some implementations, the processor(s) are configured or configurable to perform LFP processing operations on the image data of a sequence of intensity

images. In these cases, the processor(s) interpret raw image data from the sequence of acquired intensity images, transform the relatively low-resolution raw intensity image data into Fourier space, iteratively update the transformed raw image data in Fourier space to reconstruct amplitude and phase data for a single high-resolution image of the sample and the pupil function of the LFP imaging system. In some cases, the one or more processors use a differential phase contrast (DPC) deconvolution procedure to acquire the quantitative phase of the sample and use the phase data to correct for noise in the captured images such as those caused by speckles that may be caused by laser light illumination. During the optional display phase, display data is sent to a display such as display **152** to display data such as the high-resolution image and other data.

[0060] In certain implementations, the processor(s) of an LFP system may be configured to perform parallel image processing such as, for example, when processing multiple tile images (i.e. portions of the full field of view image) to reconstruct phase and amplitude data for multiple tile images in parallel. These tile images can then be combined to form the full field of view image. To perform parallel image processing, the controller would be configured to include at least one processor (or "processing unit"). Some examples of processors that can be used to perform parallel processing or non-parallel processing include, for example, a general purpose processor (CPU), an application-specific integrated circuit, an programmable logic device (PLD) such as a field-programmable gate array (FPGA), or a System-on-Chip (SoC) that includes one or more of a CPU, application-specific integrated circuit, PLD as well as a memory and various interfaces.

[0061] Returning to **FIG. 1**, the controller **140** is in communication with internal memory device **160**. The internal memory device **160** can include a non-volatile memory array for storing processor-executable code (or "instructions") that is retrieved by the processor to perform various functions or operations described herein for carrying out various operations on the image data. The internal memory device **160** also can store raw and/or processed image data. In some implementations, the internal memory device **160** or a separate memory device can additionally or alternatively include a volatile memory array for temporarily storing code to be executed as well as image data to be processed, stored, or displayed. In some

implementations, the controller **140** itself can include volatile and in some instances also non-volatile memory.

[0062] In **FIG. 1**, the controller **140** is in communication with the communication interface **150** which is in communication with the display **152**. In certain implementations, the controller **140** is configured or configurable by an operator to output raw image data or processed image data over the communication interface **150** for display on the display **152**. In some implementations, the controller **140** also can be configured or configurable by to output raw image data as well as processed image data (for example, after image processing) over a communication interface **170** to an external computing device or system **172**. Indeed, in some implementations, one or more of the LFP operations can be performed by such an external computing device **172**. In some implementations, the controller **140** also can be configured or configurable by a user to output raw image data as well as processed image data over a communication interface **180** for storage in an external memory device or system **182**. In some implementations, the controller **140** also can be configured or configurable by a user to output raw image data as well as processed image data (for example, after image processing) over a network communication interface **190** for communication over an external network **192** (for example, a wired or wireless network). The network communication interface **190** also can be used to receive information such as software or firmware updates or other data for download by the controller **140**. In some implementations, the LFP imaging system **100** further includes one or more additional interfaces such as, for example, various Universal Serial Bus (USB) interfaces or other communication interfaces. Such additional interfaces can be used, for example, to connect various peripherals and input/output (I/O) devices such as a wired keyboard or mouse or to connect a dongle for use in wirelessly connecting various wireless-enabled peripherals. Such additional interfaces also can include serial interfaces such as, for example, an interface to connect to a ribbon cable. It should also be appreciated that the laser illumination system **110** can be electrically coupled to communicate with the controller **140** over one or more of a variety of suitable interfaces and cables such as, for example, USB interfaces and cables, ribbon cables, Ethernet cables, among other suitable interfaces and cables.

[0063] In some embodiments, the data signals output by the light detectors of an LFP system may be multiplexed, serialized or otherwise combined by a multiplexer, serializer or other electrical component of the imaging system before being communicated to the controller. In certain implementations, the controller can further include a demultiplexer, deserializer or other device or component for separating the image data from each of the light detectors so that the image frames can be processed in parallel by the controller or for separating the image data from each tile image so that the sequence of image frames of each tile image can be processed in parallel by the controller.

[0064] Some examples of LFP systems are shown in **FIGS. 2 - 6**. Some of these LFP systems have certain components that are similar to those of the LFP system **100** in **FIG. 1** above.

B. LFP systems with a scanning fiber port

[0065] Certain implementations of an LFP imaging system include an angle direction device with a scanning fiber port. In these implementations, laser light is coupled to one end of an optical fiber and the other end of the optical fiber is attached with a fiber connector or directly to an optical port (also called output port) in a movable stage. The movable stage is configured to translate the end of the optical fiber in a plane and/or rotate the end of the optical fiber. An example of a movable stage is a two-axial motorized stage such as an X-Y stage. The movable stage is generally configured to move (also referred to as scan) the optical fiber to provide the sample plane with angularly varying illumination from a sequence of illumination angles.

[0066] **FIG. 2** is schematic drawing of an example of an LFP imaging system **200** with a scanning fiber port, in accordance with some implementations. An optical fiber **201** is shown with an end **202** within a fiber connector **203** coupled to an optical port **205** in a movable stage **212**. Although shown with a fiber connector **203**, it would be understood that the optical fiber **201** could be placed directed in the optical port **205** in another embodiment. The other end (not shown) of the optical fiber **201** is optically coupled with a laser light source(s) and receives laser light from the laser light source(s) when activated. In the illustrated example, the laser light source(s) is activated and laser light **204** spreads conically from the end **202** proximal to the fiber

connector **203**. A transparent layer **207** with a specimen surface **208** is also shown. The specimen surface **208** can receive a sample (not shown) being imaged. In some cases, one or both of the fiber connector **203** and the optical fiber **201** are components of the LFP imaging system **200**. In other cases, the fiber connector **203** and optical fiber **201** are separate components from the LFP imaging system **200**.

[0067] The LFP imaging system **200** comprises an angular direction device **212** having a movable stage **214** with an optical port **205** configured to receive the optical fiber **201**. With the optical fiber **201** coupled to the optical port **205**, the movable stage **214** is configured or configurable to move (translate/rotate) the optical fiber **201** to direct illumination at a plurality of illumination angles to the sample plane. The LFP imaging system **200** further comprises an optical system **220** having a collection element **222** and a focusing element **224**, and a light detector **232** for receiving light propagated by the optical system **220**. Although the illustrated example shows the movable stage **214** as having an optical port **205** in the form of a circular aperture, it would be understood that other receiving configurations for the optical port **205** could be used. A series of arrows are depicted in **FIG. 2** to show an example of the direction of translational movement of the optical fiber **201** during an image acquisition phase of one implementation. Other implementations may include other translation patterns or may include rotational movement as well. In yet other implementations, the movable stage **214** may be configured for two axis rotational movement.

[0068] In one embodiment, the LFP imaging system **200** also includes one or more processors and computer readable medium (CRM) (e.g., memory) in communication with the processor(s). The one or more processors and computer readable medium may be part of a controller such as the controller **140** described with respect to **FIG. 1**. The processor-executable code (or "instructions") for performing the LFP imaging method can be stored on the CRM or other memory. The processor(s) and can retrieve the processor-executable code from the CRM or other memory to perform various functions or operations of the LFP imaging method. The CRM or other memory can store raw and/or processed image data. In some implementations, the CRM or other memory can additionally or alternatively include a volatile memory array for temporarily storing code to be executed as well as image data to be processed, stored, or displayed.

[0069] The LFP Imaging system **200** also includes an optical system **220** having a collection element **222** (e.g., lens) having a focal length, f_1, and a focusing element **224** (e.g., lens) having a focal length, f_2. The collection element has an objective NA and is configured to receive light issuing from a specimen when it is located on the specimen surface **207**. The focusing element **224** is configured to focus light propagated from the collection element **222** to the light detector **232**. The sequence of illumination angles and the objective NA correspond to overlapping regions in the Fourier domain, as described in further detail below. In the illustration, the optical system **220** is in a 4f arrangement where the collection element **222** is located so that the sample plane is f_1 apart from it. The sample's Fourier plane is located f_1 away on the other side of 222. Fourier plane of the sample at the sample plane at specimen surface **208** is a focal length, f_2, away from the focusing element **224** and the focusing element **224** is located a distance of a focal length, f_2, away from the light detector. Other arrangements can be used. For example, a 6f arrangement can be used. As another example, other optical elements (e.g., mirrors, bandpass filters, beam splitters, etc.) can be introduced into the optical path for other arrangements.

[0070] The LFP imaging system **200** further comprises a light detector **232** with eight discrete light elements. Although shown with eight discrete light elements, it would be understood that other numbers of light elements could be used. The collection element **222** is configured to receive light issuing from the sample (not shown) being illuminated on the specimen surface **208** and the focusing element **224** focuses light propagated from the collection element **222** to the light detector **232**.

[0071] While laser light **204** issuing from the optical fiber **201** is depicted as spreading conically outwards with a particular width, the width of this cone depends on the NA of the optical fiber **201**. Different types of optical fibers may be used to achieve the desired illumination. By way of example, a single mode fiber optic with an NA in the range of 0.1-0.4 may be used to achieve a narrower cone. In another example, a multi-mode fiber with an NA in the range of 0.1 to 0.8 may be used to achieve a wider cone.

[0072] The movable stage **214** may be any kind of stage to which the optical fiber **201** with or without the fiber connector **203** can be attached and that can be configured to translate and/or rotate the optical fiber **201** to direct the optical fiber **201**

to provide a laser beam to a sample plane at a specimen surface **208** at a sequence of illumination angles as described in the section above in the context of the **FIG. 1**. For instance, at a particular sampling time, the movable stage **214** may cause the optical fiber **201** to translate/rotate to a particular position/direction at which laser light from the optical fiber **201** illuminates a sample plane at the specimen surface **207** at a particular one of the illumination angles in the sequence of illumination angles. Once the fiber connector **203** has illuminated the sample plane at the particular illumination angle for at least the exposure time such that a light detector **232** may acquire an intensity image, the movable stage **214** may cause the optical fiber **201** to translate/rotate to another position/direction at which the optical fiber **201** provides the laser beam to the sample plane at another one of the illumination angles. The optical fiber **201** may remain at each position/direction for at least the exposure time of the light detector **232** for generating an intensity image. This raw image acquisition phase may continue until the sample has been illuminated at all of the illumination angles at the different sampling times.

[0073] The movable stage **214** may cause optical fiber **201** to translate and/or rotate in the positions described in the preceding paragraph in a variety of manners. In the illustrated example, the movable stage **214** is configured to cause the optical fiber **201** to translate linearly in the x-direction and then shift to different locations in the y-direction in order to scan back and forth in the X-Y plane. In this manner, the optical fiber **201** provides a laser beam to illuminate the sample from N different positions in the X-Y plane along the arrows in **FIG. 2**. Alternatively, the movable stage **214** may be configured to cause the optical fiber **201** to translate spirally outwards such that the optical fiber **201** may illuminate the sample from N positions along a spiral moving to successive points in an r-θ plane. In one implementation, the movable stage **214** is an X-Y stage.

[0074] The movable stage **214** may be controlled in a variety of manners. By way of example, a processor (e.g. a processor of the controller **140** of **FIG. 1**) or processors may be in communication with movable stage **214**. The processor may retrieve instructions stored in a memory (e.g. internal memory **160** of **FIG. 1**) and the instructions may be executable to cause the movable stage **214** to translate and/or rotate such that the optical fiber **201** is positioned/directed to provide the laser light to the sample sequentially at different illumination angles at different sampling times. In

some case, the movable stage **214** holds the optical fiber **201** at the position for at least the duration of the exposure time used acquire an intensity image by the light detector **232**. Alternatively, the movable stage **214** may have an internal memory and processor and may be programed to cause the fiber connector **203** to follow a pre-determined path.

[0075] It would be understood to those skilled in the art that the fiber connector **203** may have shapes other than the one illustrated in **FIG. 2**. In addition, it would be understood that the optical fiber **201** may be directly attached to the movable stage **214** or may be attached through the fiber connector **203** to the movable stage **214**.

[0076] At the instant in time illustrated in **FIG. 2**, the angle direction device **212** is illuminating a sample (not shown) on the specimen surface **208**. Generally, light incident on the specimen is scattered by the physical features of the specimen as it passes through the specimen. The collection element **222**, which have a particular objective NA, receives light passing through the specimen. If an iris is included in optical system **220**, it would be placed at the back focal plane of **222**. The optional iris receives light propagated by the collection element **222** and passes light incident on its pupil area. Light incident on the area of the iris around the pupil area is substantially blocked. The focusing element **224** receives the light passed by the iris and focuses the light to the light detector **232**. The light detector **232** is configured to receive light focused by the focusing element **224** and acquire an intensity image of the sample over each exposure time when the sample is on the specimen surface and illuminated by laser light from the angle direction device **212**. The light detector **232** is configured to capture a sequence of raw intensity images while the angle direction device **212** provides laser light sequentially from different illumination angles at the different sampling times.

[0077] During the high-resolution reconstruction phase, one or more processors of the LFP system **200** interpret and process the raw image data from the sequence of images acquired during the image acquisition phase to generate processed image data. The one or more processors interpret raw image data from the sequence of acquired intensity images, transform the relatively low-resolution raw image data frames into Fourier space, iteratively update the transformed raw image data in Fourier space to reconstruct amplitude and phase data for a single high-resolution image of the sample

and the associated pupil function of the imaging system. In some cases, the one or more processors uses a differential phase contrast (DPC) deconvolution procedure to acquire the quantitative phase of the sample and use the phase data to correct for noise in the captured images such as those caused by speckles that may be caused by laser light illumination. During the optional display phase, display data is sent to a display such as display **152** in **FIG. 1** to display data such as the high-resolution image and other data.

C. LFP Systems with a Fiber Array Structure

[0078] Certain implementations of an LFP imaging system include an angular direction device with a fiber array dome or other shaped structure for receiving an array of optical fibers at fixed locations. In these implementations, the surface wall of the fiber array dome or other structure has an array of optical ports for receiving and holding in place an array of optical fibers in directions that will provide laser illumination from a plurality of illumination angles. For example, the optical ports may be apertures or other holder, each designed to receive a fiber connector at one end of an optical fiber or the one end of the fiber directly without the fiber connector. The other end of each of the optical fibers is optically coupled to an optical switch. The optical switch is connected to another optical switch or directly to the laser source(s). The optical switch(es) may be controlled by a controller or other computing device. In certain implementations, an optical switch may couple one fiber channel to the laser light source at a time, providing illumination on the specimen surface plane at a certain angle. Although the surface wall is a dome-shape in the illustrated example below, it would be understood that other shapes can be used. In one embodiment, the surface of the fiber array structure may be a flat plate with slanted apertures. In another embodiment, the fiber array structure may be a truss structure.

[0079] As mentioned above, the LFP imaging systems with a fiber array dome, one or more optical switches can be used. In implementations where a single optical switch is used, the optical switch is configured to switch to each of the optical fibers. In other cases, multiple optical switches are used. For example, a cascade arrangement of multiple optical switches in series can be used. Each output end of an optical switch may be connected to the input end of a separate optical switch to

increase the number of optical fiber outputs from the laser source(s) available for the fiber array dome design. An example of an optical switch that can be used in series is a mol 1x16 19" 2 HU from Leoni Fiber Optics® that can switch the light output to 16 optical fibers given one input light source.

[0080] **FIG. 3** is a schematic drawing of a cutaway view of an LFP imaging system **300** comprising an angle direction device **312** having a fiber array dome **314**, in accordance with some implementations. The fiber array dome **314** is configured to receive and direct optical fibers **301(1)** through **301(37)** at a plurality of illumination angles to the sample plane. The fiber array dome **314** comprises thirty-seven (37) optical ports **305(1)** through **305(37)** for receiving and holding optical fibers **301(1)** through **301 (37)** with respective fiber connectors **303(1)** through **303 (37)**. In this cutaway view, seven (7) optical ports **305(1)** through **305(7)** are shown with fiber connectors **303(1)** through **303 (37)** and their respective optical fibers **301(1)** through **301 (37)**. It would be understood that the fiber array dome **314** may be configured with more or fewer optical ports **305**. For example, the fiber array dome **314** could be configured with ninety-seven (97) optical ports **305**.

[0081] The LFP imaging system **300** further includes an optical switch **318**. Each optical fiber **301** is shown with a first end located within a respective fiber connector **303** held within a respective fiber port **305**. The second end of each optical fiber **301** is optically coupled to an optical switch **318**. Each optical port **305** holds the respective optical fiber **301** in a position/direction such that when the laser light source(s) (not shown) is activated and the optical switch **318** is switched to provide laser light to the respective optical fiber **301** at its second end, laser light is propagated through the optical fiber **301** and directed out the first end at a particular illumination angle. In the illustrated example, the respective fiber port **305(5)** holds the respective optical fiber **301(5)** in a position/direction to direct the laser light **304** spreading conically from the first end of optical fiber **301(5)** proximal of the optical port **305(5)**. A bisecting arrow through the cone is shown depicting the illumination angle of the laser light from the first end of the optical fiber **301(5)**. A transparent layer **307** (e.g., slide) with a specimen surface **308** is also shown. The specimen surface **308** can receive a sample (not shown) to be imaged. The transparent layer **307**, the optical fibers **301**, the optical ports **305**, fiber connectors **303**, and/or the optical switch **318**,

may be components of or may be separate components from the LFP imaging system **300**.

[0082] In the illustrated example, the LFP imaging system **300** further comprises an optical system **320** having a collection element **322** and a focusing element **324**, and a light detector **332** for receiving light propagated by the optical system **320**. The collection element **322** (e.g., lens) has a focal length, f_1, and the focusing element **324** (e.g., lens) has a focal length, f_2. The collection element has an objective NA and is configured to receive light issuing from a specimen when it is located on the specimen surface **308**. The focusing element **324** is configured to focus light propagated from the collection element **322** to the light detector **332**. The sequence of illumination angles and the objective NA correspond to overlapping regions in the Fourier domain, as described in further detail below. In the illustration, the optical system **320** is in a 4f arrangement where the collection element **322** is located so that the sample plane is f_1 apart from it. The sample's Fourier plane is located f_1 away on the other side of **322**. Fourier plane of the sample at the sample plane at specimen surface **308** is a focal length, f_2, away from the focusing element **324** and the focusing element **324** is located a distance of a focal length, f_2, away from the light detector. Other arrangements can be used. For example, a 6f arrangement can be used. As another example, other optical elements (e.g., mirrors) can be introduced into the optical path for other arrangements.

[0083] The LFP imaging system **300** further comprises a light detector **332** with eight discrete light elements. Although shown with eight discrete light elements, it would be understood that other numbers of light elements could be used. The collection element **322** receives light issuing from the sample (not shown) on the specimen surface **308** and the focusing element **324** focuses light propagated from the collection element **322** to the light detector **332**.

[0084] In one embodiment, the LFP imaging system **300** also includes one or more processors and computer readable medium (CRM) (e.g., memory) in communication with the processor(s). The one or more processors and computer readable medium may be part of a controller such as the controller **140** described with respect to **FIG. 1**. The processor-executable code (or "instructions") for performing the LFP imaging method can be stored on the CRM or other memory. The processor(s) and can

retrieve the processor-executable code from the CRM or other memory to perform various functions or operations of the LFP imaging method. The CRM or other memory can store raw and/or processed image data. In some implementations, the CRM or other memory can additionally or alternatively include a volatile memory array for temporarily storing code to be executed as well as image data to be processed, stored, or displayed.

[0085] While laser light **304** issuing from the optical fiber **301(5)** is depicted as spreading conically outwards with a particular width, the width of this cone depends on the NA of the optical fiber **301(5)**. Different types of optical fibers may be used to achieve desired illumination. By way of example, a single mode fiber optic with an NA in the range of 0.1-0.4 may be used to achieve a narrower cone or a multi-mode fiber with an NA in the range of 0.1 to 0.8 may be used to achieve a wider cone.

[0086] The LFP system **300** also includes an optical switch **318** in optical communication with the laser light source (not shown). The optical switch **318** is also in optically communication with optical fibers **301(1)** through **301(37)**. The optical fibers **301(1)** through **301(37)** are coupled to respective fiber connectors **305(1)** through **305(37)**. As introduced above, the optical ports **305(1)** through **305(37)** are designed to direct laser light from the optical fibers **301(1)** through **301(37)** within the fiber connectors **305(1)** through **305(37)** to the specimen surface **308** such that a specimen on the specimen surface **308** may be sequentially illuminated with the laser light from thirty-seven (37) different illumination angles during the image acquisition phase. During this image acquisition phase, the optical switch **318** switches to the different optical fibers **301(1)** through **301(37)**. By way of illustration, at the sampling time shown in **FIG. 3**, the optical switch **318** is switched to provide laser light to optical fiber **301(5)** within fiber connector **303(5)** located within optical port **303(5)** to illuminate at the illumination angle depicted by the arrow. Once the sample has been illuminated at a particular illumination angle, e.g., for at least the exposure time, the optical switch **318** switches to a different optical fiber **301** to illuminate at a different illumination angle. This process of illumination and switching may continue until the sample has been illuminated at each of illumination angles at each of the different sampling times. The laser light source may be activated during the entire process or may be switched on at each exposure time during the process.

[0087] In **FIG. 3**, the optical ports **305(1)** through **305(37)** are shown as circular apertures. It would be understood that the optical ports have other shapes in other embodiments.

[0088] As shown in **FIG. 3**, each optical port **305** is configured along a fiber array dome **314** to position the respective optical fiber **301** in a particular direction. In order to approximate uniform illumination at each illumination angle, the fiber array dome **314** can be designed so that there is a uniform distance between each optical port **305** and the sample plane. In one embodiment, the fiber array dome **314** is hemisphere shaped with a constant radius so that there is a constant distance between each optical port **305** and the center of the circular surface at the surface of the hemisphere. The circular surface at the surface of the hemisphere is kept near the outer surface of the transparent layer **307** to approximate uniform illumination of the sample plane.

[0089] In certain implementations, the spacing between adjacent optical ports **305** and the direction of the optical ports **305** are configured so that the laser light directed by the optical ports **305** generates adjacent illumination angles with overlapping regions in Fourier space of at least a minimum amount (e.g. 60%, 65%, 70%, 80%, or 90%). The spacing between adjacent optical ports **305** and the size the fiber array dome **314** may vary across implementations. In one embodiment, the spacing between adjacent optical ports **305** may be uniform. In one particular implementation, the radius of the dome surface of the fiber array dome **314** along which each optical port **305** is positioned is approximately 8mm. In this implementation, each optical port **303(i)** may be spaced 1-5 cm apart. A closer spacing, e.g. 1 cm, and a wider radius may be used if the collection element **322** of the optical system **320**, as discussed further below, has a relative low NA for wide field-of-view imaging. On the other hand, a larger spacing, e.g. 5 cm, may be used if the collection element **322** of the optical system **320** has a higher NA.

[0090] The optical switch **318** and/or other components of LFP system **300** may be controlled by control signals from a processor(s) (e.g. process of the controller **140** of **FIG. 1**). The processor(s) retrieves instructions stored in the CRM (e.g. internal memory **160** of **FIG. 1**) for performing various functions of the LFP system **300**. For example, the processor(s) may be coupled with the optical switch **318** to send control

signals to switch the laser light from the laser source(s) to a particular optical fiber **301** at a particular sampling time to direct the laser beam at one of the illumination angles. In some cases, the controller may also activate the laser light source(s). Alternatively, the optical switch **318** may have an internal memory and processor and may be programed to cause the optical switch **318** to switch between optical fibers **301** at pre-determined times.

[0091] While the optical switch **318** is described as a single optical switch, an array of optical switches may be used in another implementation. In this case, an array of optical switches is connected in a cascading series to achieve *N* outputs. For example, each of the 16 outputs of a 1x16 19" 2 HU from Leoni Fiber Optics® may be connected with an input of another 1x16 19" 2 HU from Leoni Fiber Optics®. In such an arrangement, 256 outputs could be achieved. While such a series may be extended indefinitely (e.g. the output of one switch may be connected with the input of another switch, which may be connected with the input of yet another switch, etc.), each interface between switches introduces a certain amount of insertion loss/reflection. Accordingly, such a series of switches will cause some decrease in intensity of the laser light from the laser light source. Thus, in this scenario, the intensity of the laser light source should be adjusted accordingly. This insertion loss may be avoided by using a single optical switch with the appropriate number of outputs.

[0092] At the instant in time illustrated in **FIG. 3** angle direction device **312** illuminates the specimen on specimen surface **308**. The light incident on the specimen is scattered by the physical features of the specimen as it passes through the specimen. The collection element **322,** which has a particular objective NA, receives light passing through the specimen. If an iris is included in optical system **320**, it would be placed at the back focal plane of collection element **322**. The iris receives light propagated by the collection element **322** and passes light incident on its pupil area. Light incident on the area of the iris around the pupil area is substantially blocked. The focusing element **324** receives the light passed by the iris and focuses the light to the light detector **332**. The light detector **332** is configured to receive light focused by the focusing element **324** and acquire a sequence of intensity images of the sample when the sample is on the specimen surface **308** and while the optical switch

318 switches laser light between the different optical fibers **301** at the different sampling times.

[0093] During the high-resolution reconstruction phase, one or more processors of the LFP system **300** interpret and process the raw image data from the sequence of images acquired during the image acquisition phase to generate processed image data. The one or more processors interpret raw image data from the sequence of acquired intensity images, transform the relatively low-resolution raw image data frames into Fourier space, iteratively update the transformed raw image data in Fourier space to reconstruct amplitude and phase data for a single high-resolution image of the sample and the associated pupil function of the imaging system. In some cases, the one or more processors uses a differential phase contrast (DPC) deconvolution procedure to acquire the quantitative phase of the sample and use the phase data to correct for noise in the captured images such as those caused by speckles that may be caused by laser light illumination. During the optional display phase, display data is sent to a display such as display **152** in **FIG. 1** to display data such as the high-resolution image and other data.

D. LFP Systems with a rotatable mirror and a mirror array

[0094] Certain implementations of an LFP imaging system include an angular direction device with a two-axis rotatable mirror and a mirror array (e.g., faceted bowl shaped mirror array). In these implementations, a collimated laser beam is directed to the reflective surface of the two-axis rotatable mirror system such as Galvo mirror. The two-axis rotatable mirror system rotates its one or more lenses to receive and reflect the laser beam to each of a plurality of mirror elements in the mirror array. The laser beam is reflected by each of the mirror elements to direct the laser beam at the illumination angles to the sample plane. Changing the tilt angle of the two-axis rotatable mirror system allows the laser beam to be incident on different mirror elements and provides for angularly varying illumination of the sample plane during the raw image acquisition process.

[0095] **FIG. 4** shows a schematic drawing of a cutaway view of an LFP imaging system imaging system **400** comprising an angle direction device **412** with a two-axis rotatable mirror **413** and a mirror array **414**, in accordance with some implementations. In **FIG. 4**, a laser light source (not depicted) is activated and

providing collimated laser light **404** to a two-axis rotatable mirror **413** of the angle direction device **412**. A transparent layer **407** (e.g., slide) with a specimen surface **408** generally at the sample plane is also shown. The specimen surface **408** can receive a sample (not shown) to be imaged. One or more components of the angle direction device **412** may be separate from the LFP imaging system **400**. The LFP imaging system **400** further comprises an optical system **420** and a light detector **432** for receiving light propagated by the optical system **420**.

[0096] The LFP Imaging system **400** also includes an optical system **420** having a collection element **422** (e.g., lens) having a focal length, f_1, and a focusing element **424** (e.g., lens) having a focal length, f_2. The collection element has an objective NA and is configured to receive light issuing from a specimen when it is located on the specimen surface **407**. The focusing element **424** is configured to focus light propagated from the collection element **422** to the light detector **432**. The sequence of illumination angles and the objective NA correspond to overlapping regions in the Fourier domain, as described in further detail below. In the illustration, the optical system **420** is in a 4f arrangement where the collection element **422** is located so that the sample plane is f1 apart from it. The sample's Fourier plane is located f1 away on the other side of 422. Fourier plane of the sample at the sample plane at specimen surface **408** is a focal length, f_2, away from the focusing element **424** and the focusing element **424** is located a distance of a focal length, f_2, away from the light detector. **408**Other arrangements can be used. For example, a 6f arrangement can be used. As another example, other optical elements (e.g., mirrors) can be introduced into the optical path for other arrangements.

[0097] Angle direction device **412** includes a rotatable mirror **413** and a mirror array **414** having N mirrors **415**. Rotatable mirror **413** may be configured or configurable to rotate along two axes to orient its reflective surface at different positions during the image acquisition phase in order to reflect laser light from the laser light source to each of the N mirrors **415** of the mirror array **414** at different sampling times. Each mirror **415** of the mirror array **414** is oriented such that it reflects laser light received from the rotatable mirror **413** to a sample plane at the specimen surface **408**. During the image acquisition phase, the rotatable mirror **413** rotates to the different positions, in some cases holding at each position for at least an exposure time, to reflect laser light from the laser light source to different mirrors **415**

of the mirror array **414** to reflect the laser light at the sequence of illumination angles described above in the context of **FIG. 1**. As such, the rotatable mirror **413** rotates such that collimated laser light **404** is reflected by the rotatable mirror **413** to the mirrors of the mirror array **414** to illuminate the specimen at different illumination angles at different sampling times. At the instant in time shown in the illustrated in **FIG. 4**, the rotatable mirror **413** is rotated to a particular position to cause the collimated laser light **404** to be directed to one mirror **415** of the mirror array **414** from which the collimated laser light **404** is reflected, illuminating the specimen at one of the illumination angles. Once the collimated laser light **404** has illuminated the specimen at the particular illumination angle, typically for at least an exposure time, the light detector **432** measures an intensity image and the rotatable mirror **413** is rotated to another position to cause the collimated laser light **404** to be directed to another mirror of the mirror array **414**, illuminating the specimen from another one of the illumination angles and the light detector measures another intensity image. This process may continue until the specimen has been illuminated at each of the illumination angles at each of the different sampling times.

[0098] While a single two-axis rotatable mirror **413** is depicted in **FIG. 4**, two mirrors, each of which is rotatable about a single axis such as in a Galvo mirror system, may be used in another embodiment. An example of two mirrors that can be used in such an embodiments are the mirrors **613** and **614** discussed in greater detail below in the context of **FIGs. 6** and **8**.

[0099] The rotatable mirror **413** may be controlled in a variety of manners. By way of example, a controller, e.g. controller **140** of **FIG. 1**, may be coupled with the rotatable mirror **413** of **FIG. 4**. The controller may access instructions stored in a memory, e.g. internal memory **160** of **FIG. 1**. The instructions may be executable by the controller to cause the rotatable mirror **413** of **FIG. 4** to or rotate to any given position such that the specimen is illuminated at each of the illumination angles at each of the different sampling times, as described in the preceding paragraph. Alternatively, the rotatable mirror **413** may have an internal memory and processor and may be programed to cause the rotatable mirror **413** to follow a pre-determined rotation path.

[0100] The rotatable mirror **413** and/or other components of LFP system **400** may be controlled by a controller (e.g. controller **140** of **FIG. 1**) with a processor and a computer readable medium (CRM) in communication with the processor. The controller retrieves instructions stored in the CRM (e.g. internal memory **160** of **FIG. 1**) for performing various functions of the LFP system **400**. For example, the controller may be coupled with the rotatable mirror **413** to send control signals to rotate the rotatable mirror **413** at a particular sampling time to direct the laser beam at one of the illumination angles. In some cases, the controller may also activate the laser light source(s). In one case, the laser light source(s) are turned on during the entire image acquisition phase. In another case, the laser light source(s) are activated to be turned on during at least the exposure time of each intensity image acquisition. Alternatively, the rotatable mirror **413** or other system components may have an internal memory and processor and may be programed to cause rotatable mirror **413** to rotate at pre-determined times.

[0101] Optical system **420** may have some of the same or similar components and operate in the same or similar manner as optical systems **220** and **320** of **FIGs. 3 and 4**. Like in **FIGs. 3** and **4**, when the specimen on specimen surface **408** of **FIG. 4** is illuminated at each of illumination angles at each of the different sampling times, some of the light illuminating the specimen is scattered by the specimen and passes through an optical system **420**. Optical system **420** includes a collection element **422** and a focusing element **424**.

[0102] In one embodiment, the LFP imaging system **300** also includes one or more processors and computer readable medium (CRM) (e.g., memory) in communication with the processor(s). The one or more processors and computer readable medium may be part of a controller such as the controller **140** described with respect to **FIG. 1**. The processor-executable code (or "instructions") for performing the LFP imaging method can be stored on the CRM or other memory. The processor(s) and can retrieve the processor-executable code from the CRM or other memory to perform various functions or operations of the LFP imaging method. The CRM or other memory can store raw and/or processed image data. In some implementations, the CRM or other memory can additionally or alternatively include a volatile memory array for temporarily storing code to be executed as well as image data to be processed, stored, or displayed.

[0103] At the instant in time illustrated in **FIG. 4**, angle direction device **412** illuminates the specimen on specimen surface **408**. The light incident on the specimen is scattered by the physical features of the specimen as it passes through the specimen. The collection element **422**, which has a particular objective NA, receives light issuing from the specimen being illuminated. If an iris is included in optical system **420**, it would be placed at the backfocal plane of **422**. The iris receives light propagated by the collection element **422** and passes light incident on its pupil area. Light incident on the area of the iris around the pupil area is substantially blocked. The focusing element **424** receive the light passed by the iris and focuses the light to the light detector **432**.

[0104] Like in **FIGs. 2-3**, a light detector **432** of **FIG. 4** receives light focused by the focusing element **424** and acquires a sequence of intensity images of the specimen when the specimen is illuminated sequentially by the plurality of illumination angles at the different sampling times.

[0105] During the high-resolution reconstruction phase, one or more processors of the LFP system **400** interpret and process the raw image data from the sequence of images acquired during the image acquisition phase to generate processed image data. The one or more processors interpret raw image data from the sequence of acquired intensity images, transform the relatively low-resolution raw image data frames into Fourier space, iteratively update the transformed raw image data in Fourier space to reconstruct amplitude and phase data for a single high-resolution image of the sample and the associated pupil function of the imaging system. In some cases, the one or more processors uses a differential phase contrast (DPC) deconvolution procedure to acquire the quantitative phase of the sample and use the phase data to correct for noise in the captured images such as those caused by speckles that may be caused by laser light illumination. During the optional display phase, display data is sent to a display such as display **152** in **FIG. 1** to display data such as the high-resolution image and other data.

E. LFP systems with a rotatable mirror and lenses

[0106] Certain implementations of an LFP imaging system include an angular direction device with a rotatable mirror and lenses. In these implementations, a collimated laser beam is directed to the reflective surface of the two-axis rotatable

mirror such as Galvo mirror. The two-axis rotatable mirror is placed at the focal plane of a first lens. The two-axis rotatable mirror is rotated to reflect the laser beam at different angles to the first lens. The laser beam reflected from the two-axis rotatable mirror is focused by the first lens on the first lens' back-focal plane, which coincides with the focal plane of a second lens. The laser beam exits the second lens as a collimated beam and shines on the sample plane at an illumination angle, which is proportional to the angle at which the laser beam is reflected off the rotatable mirror. In this way, the rotatable mirror can rotate (tilt) to direct the laser light at different illumination angles to the sample plane to illumination a sample to be imaged.

[0107] **FIG. 5** shows a schematic drawing of a side view of an example of an LFP imaging system **500** comprising an angular direction device **512** with a two-axis rotatable mirror **513** and a first lens **514** and a second lens **515**, in accordance with some implementations. In **FIG. 5**, a laser light source (not depicted) is activated and providing collimated laser light **504** to a two-axis rotatable mirror **513** of the angle direction device **512**. A transparent layer **507** (e.g., slide) with a specimen surface **508** is also shown. The specimen surface **508** can receive a sample (not shown) to be imaged. One or more components of the angle direction device **512** may be separate from the LFP imaging system **500**.

[0108] The LFP Imaging system **500** also includes an optical system **520** having a collection element **522** (e.g., lens) having a focal length, f_1, and a focusing element **524** (e.g., lens) having a focal length, f_2. The collection element has an objective NA and is configured to receive light issuing from a specimen when it is located on the specimen surface **507**. The focusing element **524** is configured to focus light propagated from the collection element **522** to the light detector **532**. The sequence of illumination angles and the objective NA correspond to overlapping regions in the Fourier domain, as described in further detail below. In the illustration, the optical system **520** is in a 4f arrangement where the collection element **522** is located so that the sample plane is f_1 apart from it. The sample's Fourier plane is located f_1 away on the other side of collection element **522**. Fourier plane of the sample at the sample plane at specimen surface **508** is a focal length, f_2, away from the focusing element **524** and the focusing element **524** is located a distance of a focal length, f_2, away from the light detector. Other arrangements can be used. For example, a 6f

arrangement can be used. As another example, other optical elements (e.g., mirrors) can be introduced into the optical path for other arrangements.

[0109] Angle direction device **512** includes a rotatable mirror **513** and a lens system including a first lens **514** and a second lens **515**. The rotatable mirror **513** propagates collimated laser light **504** to the specimen surface **508** via the lens system including first and second lenses **514** and **515**. During an image acquisition phase, the rotatable mirror **513** is configured to rotate along two axes to direct collimated laser light **504** to the first lens **514** at a sequence of incidence angles. The first lens **514** focuses light **504**. The first and second lenses **514** and **515** are positioned such that the focal plane **516** of the first lens **514** coincides with the back focal plane of the second lens **515**. As such, the light **504** focused by the first lens **514** is unfocused by the second lens **515** back into a collimated beam. The rotatable mirror **513** is configured to rotate such that collimated laser light **504** is refracted by the first and second lenses **514** and **515** to illuminate the specimen with a collimated beam at each illumination angle of a sequence of illumination angles, as described above in the context of **FIG. 1**. By way of illustration, at the instant in time shown in **FIG. 5**, the rotatable mirror **513** is rotated to a particular position to cause the collimated laser light **504** to be refracted by the lenses **514** and **515**, illuminating the specimen at one of the illumination angles. While the collimated laser light **504** illuminates the specimen at the particular illumination angle, e.g., for at least the exposure time, the light detector **532** measures an intensity image. Once the intensity image is acquired, the rotatable mirror **513** may be rotated to another position to acquire another intensity image. When the rotatable mirror **513** is in the other position it causes the collimated laser light **504** to be refracted at a different angle by the lenses **514** and **515**, illuminating the specimen at a different one of the illumination angles and the light detector **532** acquires another intensity image. This process may continue until the specimen has been illuminated at each of illumination angles at each of the different sampling times and the light detector **532** acquires a sequence of intensity images.

[0110] In one embodiment, the LFP imaging system **500** also includes one or more processors and computer readable medium (CRM) (e.g., memory) in communication with the processor(s). The one or more processors and computer readable medium may be part of a controller such as the controller **140** described with respect to **FIG. 1**. The processor-executable code (or "instructions") for performing the LFP imaging

method can be stored on the CRM or other memory. The processor(s) and can retrieve the processor-executable code from the CRM or other memory to perform various functions or operations of the LFP imaging method. The CRM or other memory can store raw and/or processed image data. In some implementations, the CRM or other memory can additionally or alternatively include a volatile memory array for temporarily storing code to be executed as well as image data to be processed, stored, or displayed.

[0111] In addition to mitigating aberrations from speckles in image due to the use of laser light, other mitigation measures may be taken to account for other aberrations in the LFP systems according to certain embodiments. For example, mitigation measures may be taken into account for aberration introduced when collimated laser light **504** enters the lenses **514** and **515** in different places. For example, in some implementations, the lenses **514** and **515** are both F-theta lenses rather than conventional spherical lenses to reduce spherical aberration. Examples of F-theta lenses are commercially available such as an F-theta lens produced by Thor Labs®.

[0112] The rotatable mirror **513** may be controlled in a variety of manners. By way of example, a processor (e.g., processor of controller **140** of **FIG. 1**) may be in communication with the rotatable mirror **513** of **FIG. 5**. The processor may access instructions stored in a memory, e.g. internal memory **160** of **FIG. 1**. The instructions may be executable by the processor to cause the rotatable mirror **513** of **FIG. 5** to rotate to any given position such that the specimen is illuminated at each of the illumination angles at each of the different sampling times. Alternatively, the rotatable mirror **513** may have an internal memory and processor and may be programed to cause the rotatable mirror **513** to follow a pre-determined rotation path.

[0113] Generally, the rotatable mirror **513** and/or other components of LFP system **500** may be controlled with control signals sent by a processor(s) (e.g. processor of controller **140** of **FIG. 1**). The processor retrieves instructions stored in a computer readable medium (CRM) (e.g. internal memory **160** of **FIG. 1**) for performing various functions of the LFP system **500**. For example, the controller may be coupled with the rotatable mirror **513** to send control signals to rotate the rotatable mirror **513** at a particular sampling time to direct the laser beam at one of the illumination angles. In some cases, the controller may also activate the laser light source(s). In one case, the

laser light source(s) are turned on during the entire image acquisition phase. In another case, the laser light source(s) are activated to be turned on during at least the exposure time of each intensity image acquisition. Alternatively, the rotatable mirror **513** may have internal memory and processor and may be programed to cause rotatable mirror **513** to rotate at pre-determined times and/or other system components may have internal memory and processor and may be programed in its operations.

[0114] While a single two-axis rotatable mirror **513** is depicted in **FIG. 5**, two mirrors, each of which is rotatable about a single axis, may be used in another embodiment. An example of two mirrors that can be used in such an embodiments are the mirrors **613** and **614** and **813** and **814** discussed in greater detail below in the context of **FIGs. 6** and **8**.

[0115] Optical system **520** may have the same or similar elements and operate in the same or similar manner as optical systems **220, 320** and **420** of **FIGs. 3, 4**, and **5**. Like in **FIGs. 3, 4,** and **5,** when the specimen on specimen surface **508** of **FIG. 5** is illuminated at each of illumination angles at each of the different sampling times, some of the light illuminating the specimen is scattered by the specimen and passes through an optical system **520**. Optical system **520** includes a collection element **522** and a focusing element **524**.

[0116] At the instant in time illustrated in **FIG. 5** angle direction device **512** illuminates the specimen on specimen surface **508**. The light incident on the specimen is scattered by the physical features of the specimen as it passes through the specimen. The collection element **522**, which has a particular objective NA, receive light passing through the specimen. If an iris is included in optical system **520**, it would be placed at the back focal plane of collection element **522**. The iris receives light propagated by the collection element **522** and passes light incident on its pupil area. Light incident on the area of the iris around the pupil area is substantially blocked. The focusing element **524** receives the light passed by the iris and focuses the light to the light detector **532**.

[0117] Like in **FIGs. 2-4**, a light detector **532** of **FIG. 5** receives light focused by the focusing element **524** and acquires a sequence of intensity images of the specimen when the specimen is illuminated sequentially by the plurality of illumination angles at the different sampling times.

[0118] During the high-resolution reconstruction phase, one or more processors of the LFP system **500** interpret and process the raw image data from the sequence of images acquired during the image acquisition phase to generate processed image data. The one or more processors interpret raw image data from the sequence of acquired intensity images, transform the relatively low-resolution raw image data frames into Fourier space, iteratively update the transformed raw image data in Fourier space to reconstruct amplitude and phase data for a single high-resolution image of the sample and the associated pupil function of the imaging system. In some cases, the one or more processors uses a differential phase contrast (DPC) deconvolution procedure to acquire the quantitative phase of the sample and use the phase data to correct for noise in the captured images such as those caused by speckles that may be caused by laser light illumination. During the optional display phase, display data is sent to a display such as display **152** in **FIG. 1** to display data such as the high-resolution image and other data.

F. LFP Systems with a Circular Mirror Array on flat surface

[0119] Certain implementations of an LFP imaging system include an angular direction device with a two-axis rotatable mirror system such as a Galvo mirror system and a circular array of mirrors (mirror array) arranged on a flat surface. For example, the mirror array may be an arrangement of mirrors in concentric circles along a flat surface. In these implementations, a collimated laser beam is directed to the reflective surface of the two-axis rotatable mirror. The two-axis rotatable mirror system rotates its one or more mirrors to receive and reflect the laser beam to each of a plurality of mirror elements in the mirror array. The laser beam is reflected by each of the mirror elements to direct the laser beam at the illumination angles to the sample plane. Changing the tilt angle of the two-axis rotatable mirror system allows the laser beam to be incident on different mirror elements and provides for angularly varying illumination of the sample plane during the raw image acquisition process.

[0120] **FIG. 6** shows a schematic drawing of an orthogonal view of an LFP imaging system imaging system **600** with a circular mirror array **615** on a flat surface, in accordance with some implementations. A transparent layer **607** (e.g., slide) with a specimen surface **608** generally at the sample plane is also shown. The specimen surface **608** can receive a sample (not shown) to be imaged.

[0121] The LFP imaging system imaging system **600** comprises an angle direction device **612** with a first rotatable mirror **613** and second rotatable mirror **614**, and a circular mirror array **615** having ninety five (95) mirrors **616(1)-616(95)**. More or fewer mirrors **616** may be used. One or more components of the angle direction device **612** may be separate from the LFP imaging system **600**. The LFP imaging system **600** further comprises an optical system **620** and an image sensor system **630** with one or more light detectors for receiving light propagated by the optical system **620**.

[0122] The optical system **620** comprises a collection element **622** (e.g., lens) having a focal length, f_1, and a focusing element **624** (e.g., lens) having a focal length, f_2. The collection element has an objective NA and is configured to receive light issuing from a specimen when it is located on the specimen surface. The focusing element **624** is configured to focus light propagated from the collection element **622** to the light detector of the image sensor system **630**. The sequence of illumination angles and the objective NA correspond to overlapping regions in the Fourier domain, as described in further detail below. In the illustration, the optical system **620** is in a 4f arrangement where the collection element **622** is located so that the sample plane is f1 apart from it. The sample's Fourier plane is located f1 away on the other side of **622**. Fourier plane of the sample at the sample plane at specimen surface **608** is a focal length, f_2, away from the focusing element **624** and the focusing element **624** is located a distance of a focal length, f_2, away from the light detector. **608**Other arrangements can be used. For example, a 6f arrangement can be used. As another example, other optical elements (e.g., mirrors) can be introduced into the optical path for other arrangements.

[0123] Angle direction device **612** includes first and second rotatable mirrors **613** and **614** and a circular mirror array **615** having ninety five (95) mirrors **616(1)-616(95)**. First and second rotatable mirrors **613** and **614** may rotate each rotate about a different axis such that they are configured to be rotated to reflect laser light from the laser light source to each of the ninety five (95) mirrors **616(1)-616(95)**. The first rotatable mirror **613** is configured to reflect collimated laser light **604** from the laser light source (not shown) to the second rotatable mirror **614**. The second rotatable mirror **614** is configured to reflect to reflect the collimated laser light **604** from the first rotatable mirror **613** to one of the mirrors **616(1)-616(95)**. Each of the mirror

616(1)-616(95) in the mirror array **615** may be oriented such that it reflects laser light received from the second rotatable mirrors **614** to the sample plane at one of the illumination angles in a sequence of illumination angles, as described above in the context of **FIG. 1**. As such, the first and second rotatable mirrors **613** and **614** are configured to rotate such that collimated laser light **604** may be reflected by the first and second rotatable mirrors **613** and **614** and directed to the mirrors **616(1)-616(95)** to illuminate the specimen at each illumination angles in the sequence of illumination angles. By way of illustration, at a particular sampling time, the first and second rotatable mirrors **613** and **614** may be rotated to particular orientations to cause the collimated laser light **604** to be directed to a particular mirror **616(i)** of the circular mirror array **615** from which the collimated laser light **604** is reflected, illuminating the specimen at a particular one of the illumination angles. While the collimated laser light **604** illuminates the sample plane at a particular illumination angle, e.g., for at least an exposure time, a light detector(s) of the image sensor system **630** acquires an intensity image and then the rotatable mirrors **613** and **614** may be rotated to cause the collimated laser light **604** to be directed to another mirror **616(i+1)**, illuminating the specimen from another one of the illumination angles so that the light detector may generate another intensity image. This process may continue until the specimen has been illuminated at each of illumination angles at each of the different sampling times.

[0124] At the instant in time depicted in **FIG. 6**, a laser light source (not depicted) is activated and providing collimated laser light **604** to the first rotatable mirror **613** of the angle direction device **612**. The first rotatable mirror **613** is oriented to reflect collimated laser light **604** to the second rotatable mirror **614** which reflects the collimated laser light **604** to a particular mirror **616(i)** of the circular mirror array **615** from which the collimated laser light **604** is reflected to the specimen plane which is approximately at the specimen surface **608**.

[0125] In one embodiment, the LFP imaging system **600** also includes one or more processors and computer readable medium (CRM) (e.g., memory) in communication with the processor(s). The one or more processors and computer readable medium may be part of a controller such as the controller **140** described with respect to **FIG. 1**. The processor-executable code (or "instructions") for performing the LFP imaging method can be stored on the CRM or other memory. The processor(s) and can

retrieve the processor-executable code from the CRM or other memory to perform various functions or operations of the LFP imaging method. The CRM or other memory can store raw and/or processed image data. In some implementations, the CRM or other memory can additionally or alternatively include a volatile memory array for temporarily storing code to be executed as well as image data to be processed, stored, or displayed.

[0126] Although the mirror array **615** is illustrated with ninety five (95) mirrors **616**, other numbers of mirrors can be used in the mirror array **615**. Each mirror **616(i)** of the mirror array **615** is coupled at one end of a rectangular tower coupled to a plate. In one embodiment, the rectangular towers are 3D-printed structures. Each tower's top surface is sloped at a certain angle such that collimated laser light **604** may be reflected towards the sample plane at one of the illumination angles in the sequence of illumination angles described above.

[0127] **FIG. 7A** is a plan view of a mirror array **715** with a similar configuration the mirror array **615** shown in **FIG. 6**, according to an embodiment. The mirror array **715** has a diameter, *d*. **FIG. 7B** is an illustration of the Fourier spectrum region **750** covered by the angular varying illumination of the mirror array **715** shown in **FIG. 7A**. As shown by **FIG. 7B**, the mirrors **616(1)-616(95)** are arranged to provide illumination to the sample plane such that contiguous regions produce 60% overlap of the specimen's spectrum in the Fourier domain, as shown in **FIG. 7B**. As discussed above, other embodiments may have a greater overlap, for example, at least 70%, at least 80%, etc.

[0128] In some implementations, a neutral density filter is placed on each of the mirrors **616(1)-616(95)** in the mirror array **615**. Use of such neutral density filters allows for increasing the input laser intensity to obtain higher SNR in dark field images while preventing the bright field images from over-exposure.

[0129] The first and second rotatable mirrors **613** and **614** may be controlled in a variety of manners. By way of example, a controller, e.g. controller **140** of **FIG. 1**, may be coupled with the first and second rotatable mirrors **613** and **614** of **FIG. 6**. The controller may access instructions stored in a memory, e.g. internal memory **160** of **FIG. 1**. The instructions may be executable by the controller to cause the first and second rotatable mirrors **613** and **614** of **FIG. 6** to or rotate to any given position such

that the specimen is illuminated at each of the illumination angles at each of the different sampling times, as described in the preceding paragraph. Alternatively, the first and second rotatable mirrors **613** and **614** may have an internal memory and processor and may be programed to cause each of the first and second rotatable mirrors **613** and **614** to follow a pre-determined rotation path.

[0130] The first and second rotatable mirrors **613** and **614** and/or other components of LFP system **600** may be controlled by control signals from a processor(s) (e.g., a processor of the controller **140** of **FIG. 1**). The processor(s) retrieves instructions stored in a computer readable medium (CRM) (e.g. internal memory **160** of **FIG. 1**) for performing various functions of the LFP system **400**. For example, the processor(s) may be coupled with the first and second rotatable mirrors **613** and **614** to send control signals to rotate the first and second rotatable mirrors **613** and **614** at particular sampling times to direct the laser beam to the different illumination angles. In some cases, the processor(s) may also send control signals to activate the laser light source(s). In one case, the laser light source(s) are turned on during the entire image acquisition phase. In another case, the laser light source(s) are activated to be turned on during at least the exposure time of each intensity image acquisition. Alternatively, the first and second rotatable mirrors **613** and **614** may have an internal memory and processor and may be programed to cause first and second rotatable mirrors **613** and **614** to rotate at pre-determined times. Also, other system components may have an internal memory and a processor and may be programed in its operations.

[0131] While two rotatable mirrors **613** and **614** are depicted in **FIG. 6**, a single mirror rotatable about two different axes can be used in another embodiment. Such a configuration of a single mirror is discussed in greater detail above in the context of **FIGs. 4** and **5**.

[0132] Optical system **620** may have the same or similar elements and operate in the same or similar manner as optical systems **220, 320, 420** and **520** of **FIGs. 3, 4, 5** and **6**. Like in **FIGs. 3, 4, 5** and **6**, when the specimen on specimen surface **605** of **FIG. 6** is illuminated at each of illumination angles at each of the different sampling times, some of the light illuminating the specimen is scattered by the specimen and

passes through an optical system **620**. Optical system **620** includes a collection element **622** and a focusing element **624**.

[0133] At the instant in time illustrated in **FIG. 6**, angle direction device **602** illuminates the specimen on specimen surface **605**. The light incident on the specimen is scattered by the physical features of the specimen as it passes through the specimen. The collection element **622**, which has a particular objective NA, receives light passing through the specimen. If an iris is included in optical system **620**, it would be placed at the back focal plane of collection element **622**. The iris receives light propagated by the collection element **622** and passes light incident on its pupil area. Light incident on the area of the iris around the pupil area is substantially blocked. The focusing element **624** receives the light passed by the iris and focuses the light to the light detector **630**.

[0134] A light detector of the image sensor system **630** of **FIG. 6** receives light focused by the focusing element **624** and acquires an intensity image of the specimen when the specimen is illuminated at each of the illumination angles at each of the different sampling times. During the high-resolution reconstruction phase, one or more processors of the LFP system **600** interpret and process the raw image data from the sequence of images acquired during the image acquisition phase to generate processed image data. The one or more processors interpret raw image data from the sequence of acquired intensity images, transform the relatively low-resolution raw image data frames into Fourier space, iteratively update the transformed raw image data in Fourier space to reconstruct amplitude and phase data for a single high-resolution image of the sample and the associated pupil function of the imaging system. In some cases, the one or more processors uses a differential phase contrast (DPC) deconvolution procedure to acquire the quantitative phase of the sample and use the phase data to correct for noise in the captured images such as those caused by speckles that may be caused by laser light illumination. During the optional display phase, display data is sent to a display such as display **152** in **FIG. 1** to display data such as the high-resolution image and other data.

1) Example of an LFP imaging system with circular mirror array on a flat surface

[0135] The dimensions and/or types of components of LFP Imaging systems disclosed herein may vary across implementations depending on the desired resolution of reconstructed images generated by the system, the field of view of view and/or NA of optical systems used in the LFP Imaging system, types of specimens being imaged by the LFP Imaging system, and other system parameters.

[0136] One particular implementation of an LFP Imaging system with a circular array on a flat surface has components similar to those described with respect to **FIG. 6** and **FIG. 7A**. In this particular implementation, the optical system is a 4f system with collection element being a 0.1 NA Olympus® 4x objective lens and focusing element being a Thorlabs® 200-mm-focal-length tube lens. The light detector is a PCO.edge® 5.5 16bit sCMOS sensor. The sensor has a pixel size of 6.5 mm and a maximum frame-rate of 100 Hz at 1920x1080 pixel resolution for a global shutter mode. In this implementation, the sensor size limits the available field of view of a specimen to be 2.7 mm by 1.5 mm. In this implementation, the laser light source is a 457 nm 1 W laser beam, which is pinhole-filtered and collimated, using standard collimation techniques. After collimation, the collimated laser light is 1 cm in diameter and has a 150 mW in power. Since the beam diameter the collimated laser light 1 cm, the collimated laser light covers the entire field of view captured by the light detector (2.7 mm by 1.5 mm after magnification).

[0137] In this particular implementation, the circular mirror array of mirrors has a height h of 30 cm. The mirrors are placed 40 cm away from the plane of the specimen surface. Rotatable mirror, which is a 2D Galvo mirror device (GVS 212), guides collimated laser light such that the central part of its Gaussian profile (approximately 40% of total output area) is incident on the input of the rotatable mirror, which is also 2D Galvo mirror device (GVS 212). Rotatable mirror then guides collimated laser light to individual mirrors. Each mirror is a 19mm x 19mm first surface mirror attached to a 3D-printed rectangular tower. Each tower's top surface is sloped at a certain angle such that collimated laser light may be reflected towards the specimen surface at one of the illumination angles in the sequence of illumination angles described above.

[0138] The mirrors of the mirror array are arranged to provide illumination to a sample plane such that contiguous elements produce 60% overlap of the specimen's spectrum in the Fourier domain. In this implementation, the total illumination (NA_{ti}) is 0.325 with the resulting system NA (NA_{sys}) being $NA_{obj}+NA_{ti}$ =0.1+0.325=0.425. In this particular implementation, the image reconstruction effectively increases the NA of the optical system by a factor of 4.25.

[0139] In this particular implementation, to achieve the maximum frame rate of the sCMOS sensor in light detector, the exposure time is set to its minimum, at 500 microseconds. The light detector and rotatable mirrors and are externally triggered every 10 milliseconds, resulting in 0.96 seconds of total capturing time for 95 intensity images (1 for each illumination angle) and 1 dark noise image.

G. LFP Systems with a Rectangular Mirror Array on flat surface

[0140] Certain implementations of an LFP imaging system include an angular direction device with a two-axis rotatable mirror system such as a Galvo mirror system and a rectangular array of mirrors (mirror array) arranged on a flat surface. For example, the mirror array may be an arrangement of mirrors in a rectangular array along a flat surface. In these implementations, a collimated laser beam is directed to the reflective surface of the two-axis rotatable mirror. The two-axis rotatable mirror system rotates its one or more lenses to receive and reflect the laser beam to each of a plurality of mirror elements in the mirror array. The laser beam is reflected by each of the mirror elements to direct the laser beam at the illumination angles to the sample plane. Changing the tilt angle of the two-axis rotatable mirror system allows the laser beam to be incident on different mirror elements and provides for angularly varying illumination of the sample plane during the raw image acquisition process.

[0141] **FIG. 8** shows a schematic drawing of an orthogonal view of an LFP imaging system imaging system **800** with a rectangular mirror array **815** on a flat surface, in accordance with some implementations. The LFP imaging system imaging system **800** comprises an angle direction device **812** with a first rotatable mirror **813** and second rotatable mirror **814**, and a rectangular mirror array **815** having a hundred (100) mirrors **804(1)-804(100)**. In **FIG. 8**, a laser light source (not depicted) is activated and providing collimated laser light **804** to an angle direction device **812**. A transparent layer **807** (e.g., slide) with a specimen surface generally at the sample

plane is also shown. The specimen surface can receive a sample (not shown) to be imaged. One or more components of the angle direction device **812** may be separate from the LFP imaging system **800**. The LFP imaging system **800** further comprises an optical system **820** and an image sensor system **830** with one or more light detectors for receiving light propagated by the optical system **820**.

[0142] The LFP Imaging system **800** also includes an optical system **820** having a collection element **822** (e.g., lens) having a focal length, f_1, and a focusing element **824** (e.g., lens) having a focal length, f_2. The collection element has an objective NA and is configured to receive light issuing from a specimen when it is located on the specimen surface. The focusing element **824** is configured to focus light propagated from the collection element **822** to the light detector of the image sensor system **830**. The sequence of illumination angles and the objective NA correspond to overlapping regions in the Fourier domain, as described in further detail below. In the illustration, the optical system **820** is in a 4f arrangement where the collection element **822** is located so that the sample plane is f_1 apart from it. The sample's Fourier plane is located f_1 away on the other side of the collection element **822**. Fourier plane of the sample at the sample plane at specimen surface **808** is a focal length, f_2, away from the focusing element **824** and the focusing element **824** is located a distance of a focal length, f_2, away from the light detector. Other arrangements can be used. For example, a 6f arrangement can be used. As another example, other optical elements (e.g., mirrors) can be introduced into the optical path for other arrangements.

[0143] The angle direction device **812** includes first and second rotatable mirrors **613** and **814** and a rectangular mirror array **815** having a hundred (100) mirrors **804(1)-804(100)**. First and second rotatable mirrors **813** and **814** may rotate each rotate about a different axis such that they are configured to be rotated to reflect laser light from the laser light source to each of the hundred (100) mirrors **804(1)-804(100)**. By way of illustration, the first rotatable mirror **813** may reflect collimated laser light **804** from the laser light source (not shown) to the second rotatable mirror **814**. The second rotatable mirror **814** may then reflect the collimated laser light **804** from the first rotatable mirror **813** to any of the mirrors **816(1)-816(100)**. Each of the mirror **816(1)-816(100)** in the mirror array **815** may be oriented such that it reflects laser light received from the first and second rotatable mirrors **813** and **814** to the sample plane at one of the illumination angles in a sequence of illumination angles, as

described above in the context of **FIG. 1**. As such, the first and second rotatable mirrors **813** and **814** may be configured to rotate such that collimated laser light **804** may be reflected by the first and second rotatable mirrors **813** and **814** and directed to the mirrors **816(1)-816(100)** to illuminate the specimen at each illumination angles in the sequence of illumination angles. By way of illustration, at a particular sampling time, the first and second rotatable mirrors **813** and **814** may be rotated to a particular position to cause the collimated laser light **804** to be directed to a particular mirror **816(i)** of the rectangular mirror array **815** from which the collimated laser light **804** is reflected, illuminating the specimen at a particular one of the illumination angles. Once the collimated laser light **804** has illuminated the specimen at the particular illumination angle, e.g., for at least an exposure time, a light detector of the image sensor system **630** acquires an intensity image and the rotatable mirrors **813** and **814** may be rotated to another position to cause the collimated laser light **804** to be directed to another mirror **816(i+1),** illuminating the specimen from another one of the illumination angles so that the light detector may generate another intensity image. This process may continue until the specimen has been illuminated at each of illumination angles at each of the different sampling times.

[0144] In one embodiment, the LFP imaging system **800** also includes one or more processors and computer readable medium (CRM) (e.g., memory) in communication with the processor(s). The one or more processors and computer readable medium may be part of a controller such as the controller **140** described with respect to **FIG. 1**. The processor-executable code (or "instructions") for performing the LFP imaging method can be stored on the CRM or other memory. The processor(s) and can retrieve the processor-executable code from the CRM or other memory to perform various functions or operations of the LFP imaging method. The CRM or other memory can store raw and/or processed image data. In some implementations, the CRM or other memory can additionally or alternatively include a volatile memory array for temporarily storing code to be executed as well as image data to be processed, stored, or displayed.

[0145] Although the mirror array **815** is illustrated with one hundred (100) mirrors **816**, other numbers of mirrors can be used in the mirror array **815**. Each mirror 816(i) of the mirror array **815** comprises a surface mirror affixed at one end of a rectangular tower. In one embodiment, the rectangular towers are 3D-printed structures. Each

tower's top surface is sloped at a certain angle such that collimated laser light **804** may be reflected towards the sample plane at one of the illumination angles in the sequence of illumination angles described above.

[0146] In some implementations, a neutral density filter is placed on each of the mirrors **816(1)-816(100)** in the mirror array **815**. Use of such neutral density filters allows for increasing the input laser intensity to obtain higher SNR in dark field images while preventing the bright field images from over-exposure.

[0147] The first and second rotatable mirrors **813** and **814** may be controlled in a variety of manners. By way of example, a controller, e.g. controller **140** of **FIG. 1**, may be coupled with the first and second rotatable mirrors **813** and **814** of **FIG. 6**. The controller may access instructions stored in a memory, e.g. internal memory **160** of **FIG. 1**. The instructions may be executable by the controller to cause the first and second rotatable mirrors **813** and **814** of **FIG. 6** to or rotate to any given position such that the specimen is illuminated at each of the illumination angles at each of the different sampling times, as described in the preceding paragraph. Alternatively, the first and second rotatable mirrors **813** and **814** may have an internal memory and processor and may be programed to cause each of the first and second rotatable mirrors **813** and **814** to follow a pre-determined rotation path.

[0148] The first and second rotatable mirrors **813** and **814** and/or other components of LFP system **800** may be controlled by a controller (e.g. controller **140** of **FIG. 1**) with a processor and a computer readable medium (CRM) in communication with the processor. The controller retrieves instructions stored in the CRM (e.g. internal memory **160** of **FIG. 1**) for performing various functions of the LFP system **400**. For example, the controller may be coupled with the first and second rotatable mirrors **813** and **814** to send control signals to rotate the first and second rotatable mirrors **813** and **814** at a particular sampling time to direct the laser beam at one of the illumination angles. In some cases, the controller may also activate the laser light source(s). In one case, the laser light source(s) are turned on during the entire image acquisition phase. In another case, the laser light source(s) are activated to be turned on during at least the exposure time of each intensity image acquisition. Alternatively, the first and second rotatable mirrors **813** and **814** or other system components may have an

internal memory and processor and may be programed to cause first and second rotatable mirrors **813** and **814** to rotate at pre-determined times.

[0149] While two rotatable mirrors **813** and **814** are depicted in **FIG. 6**, a single mirror rotatable about two different axes can be used in another embodiment. Such a configuration of a single mirror is discussed in greater detail above in the context of **FIGs. 4** and **5**.

[0150] Optical system **820** may have the same or similar elements and operate in the same or similar manner as optical systems **220, 320, 420** and **520** of **FIGs. 3, 4, 5** and **6**. Like in **FIGs. 3, 4, 5** and **6**, when the specimen on specimen surface **605** of **FIG. 6** is illuminated at each of illumination angles at each of the different sampling times, some of the light illuminating the specimen is scattered by the specimen and passes through an optical system **820**. Optical system **820** includes a collection element **822** and a focusing element **824**.

[0151] At the instant in time illustrated in **FIG. 6** angle direction device **802** illuminates the specimen on specimen surface **805**. The light incident on the specimen is scattered by the physical features of the specimen as it passes through the specimen. The collection element **822**, which has a particular objective NA, receives light passing through the specimen. If an iris is included in optical system **820**, it would be placed at the back focal plane of **822**. The iris receives light propagated by the collection element **822** and passes light incident on its pupil area. Light incident on the area of the iris around the pupil area is substantially blocked. The focusing element **824** receives the light passed by the iris and focuses the light to the light detector of imaging system **830**.

[0152] A light detector of the image sensor system **830** of **FIG. 8** receives light focused by the focusing element **824** and acquires an intensity image of the specimen when the specimen is illuminated at each of the illumination angles at each of the different sampling times. During the high-resolution reconstruction phase, one or more processors of the LFP system **800** interpret and process the raw image data from the sequence of images acquired during the image acquisition phase to generate processed image data. The one or more processors interpret raw image data from the sequence of acquired intensity images, transform the relatively low-resolution raw image data frames into Fourier space, iteratively update the transformed raw image

data in Fourier space to reconstruct amplitude and phase data for a single high-resolution image of the sample and the associated pupil function of the imaging system. In some cases, the one or more processors uses a differential phase contrast (DPC) deconvolution procedure to acquire the quantitative phase of the sample and use the phase data to correct for noise in the captured images such as those caused by speckles that may be caused by laser light illumination. During the optional display phase, display data is sent to a display such as display **152** in **FIG. 1** to display data such as the high-resolution image and other data.

[0153] As described above, mirror arrays in angular direction devices of LFP Imaging systems can take a wide variety of shapes and sizes. By way of example, **FIGs. 9A** and **B** show examples of an 8x8 mirror array **900** and a 6x6 mirror array **904**, respectively, according to embodiments.

[0154] While **FIGs. 5-9B** show various examples of shapes and sizes of mirror arrays in angular direction devices of LFP Imaging systems, such mirror arrays are not limited to the bowl-shaped surface, flat concentric circular ring surface, and/or flat rectangular surface arrangements shown in **FIGs. 5-9B**, respectively. Rather, such mirror arrays may take any symmetric or asymmetric round or polygonal shape, e.g. a rectangle, an octagon, an oval, an ellipse, etc.

II. LFP Imaging Methods

[0155] According to certain implementations, the LFP imaging method discussed in the context of **FIG. 11** operates most effectively for a thin sample. The thinness of the sample depends on the bigger value between the illumination NA and the objective NA. In one case, the thinness can be related to these value by pi/(kzmax) where kzmax is defined by max(k0^2 – sqrt(k0^2 – (k0*NAobj)^2), k0^2 – sqrt(k0^2 – (k0*NAillu)^2)) which is described in Ou, Xiaoze etal., "High numerical aperture Fourier ptychography: principle, implementation and characterization," Optics Express , p 3472-3492 (February 9, 2015). For example, for NAillu = 0.7 and NAobj = 0.5, hmax will be 1.75*lambda, which corresponds to 822 nm for lambda = 470 nm. The sample may be thicker than this in many cases.

[0156] For thin samples, the sample may be approximated as two dimensional, similar to a thin transparent film with a particular absorption and phase profile. In a general application of LFP methods to a specific implementation using an optical

system such as optical system **120** of **FIG. 1** (e.g. a 4f microscope), when a sample is on a specimen surface (e.g. on the stage of the 4f microscope) it may be perpendicularly illuminated by a light source that is coherent both temporally (i.e. monochromatic) and spatially (i.e. plane wave). The light field scattered by features of the sample the sample is Fourier transformed when it passes through the collection optical element of the optical system (e.g. objective lens microscope) and arrives at the back-focal plane of the collection optical element. The field is then Fourier transformed again as it propagates through the focusing optical element (e.g. a microscope's tube lens) to be imaged onto a light detector (e.g. a camera sensor or in a microscopist's eyes). The amount of the sample's detail that the optical system can capture is defined by the NA (NA_{obj}) of the collection element, which physically limits the extent of the sample's Fourier spectrum being transmitted to the light detector. Thus, the NA_{obj} acts as a low-pass filter in the optical system with a coherent illumination source.

[0157] For illustrative purposes, the following discussion is limited to a one dimensional case. The one dimensional case described below may be directly extended to two dimensions for a thin sample. Under the illumination of a same light source at an angle θ with respect to the sample's normal, the field at the sample plane, $\psi_{oblique}(x)$, can be described as:

$$\psi_{oblique}(x) = \psi_{sample}(x)exp(jk_0 sin\theta)$$

(Eqn. 1)

where $\psi_{sample}(x)$ is the sample's complex spatial distribution, x is a one-dimensional spatial coordinate, and k_0 is given by $2\pi/\lambda$ where λ is the illumination wavelength. This field is Fourier transformed by the collection element, becoming:

$$\Psi_{oblique}(k) = \int_{-\infty}^{\infty} \psi_{sample}(x)exp(jk_0 sin\theta)exp(-jkx)dx = \Psi_{sample}(k - k_0 sin\theta)$$

(Eqn. 2)

at the back-focal plane of the collection element, where $\Psi_{oblique}$ and Ψ_{sample} are the Fourier transforms of $\psi_{oblique}$ and ψ_{sample}, respectively, and k is a one dimensional coordinate in k-space. $\Psi_{sample}(k)$ is shown to be laterally shifted at the collection element's back-focal plane by $k_0 sin\theta$. Because NA_{obj} is physically fixed, a different sub-region of $\Psi_{sample}(k)$ is relayed down the imaging system. Thus, more regions of

$\Psi_{sample}(k)$ are acquirable by capturing many intensity images under varying illumination angles than would be acquirable by only capturing a single image under particular illumination. Each sub-sampled Fourier spectrum from the collection element's back-focal plane is Fourier transformed again by the focusing optical element, and the field's intensity value may captured by a light detector of the imaging system.

[0158] Due to the loss of phase information in the intensity measurement, the sub-sampled images cannot be directly combined in the Fourier domain. As such, an LFP procedure, e.g. the process described below in the context of **FIG. 11**, may be used to reconstruct the phase and amplitude of the expanded Fourier spectrum. As discussed above, an angle direction mechanism may illuminate a sample with laser light at a sequence of illumination angles such that each the intensity images overlap in Fourier space at least a certain minimum amount (e.g. 65% overlap, 75% overlap, 70% overlap, 80% overlap, in a range of 10%-65% overlap, in a range of 65%-75% overlap, etc.). This redundancy allows the LFP procedure to be used to infer the missing phase information through an iterative method which is described below in the context of **FIG. 11**.

A. An Exemplary LFP Imaging Method

[0159] **FIG. 10** is a flowchart of operations of an LFP imaging method **1000** that can be implemented by various LFP imaging systems described herein, in accordance with certain embodiments. At **1010**, a sample plane is successively illuminated at a sequence of N illumination angles using an angle direction device to direct laser light from a laser light source. During an image acquisition phase, the sample being imaged is located at the sample plane typically on a specimen surface and the sample is illuminated at a sequence of N illumination angles using a laser light source. By way of example, as described above in the context of **FIG. 6**, the rotatable mirrors **613** and **614** are rotated to direct collimated laser light **604** to different mirrors **816(1)-816(95)** of the mirror array **615** at different sampling times during the image acquisition phase. The different mirrors **816(1)-816(95)** of the mirror array **615** reflect the collimated laser light **604** at a sequence of N illumination angles at different sample times. At each sampling time, the rotatable mirrors **613** and **614** are

positioned such that they direct the collimated laser light **604** to one of mirrors **816(1)-816(95)**, illuminating the specimen at a sequence of 95 illumination angles.

[0160] At **1020**, N raw intensity images of the sample are acquired by the light detector(s). By way of example, the light detector(s) of the imaging system **630** in **FIG. 6** may capture a sequence of ninety five (95) intensity images of the sample when it is on the specimen surface **608** and while collimated laser light **604** is reflected by the ninety five (95) different mirrors **616(1)-616(95)** at ninety five (95) illumination angles to the sample plane at the specimen surface **608**.

[0161] In one embodiment, the light detector may also capture an additional "dark field" intensity image of the sample when the sample is not illuminated.

[0162] At **1030**, a high resolution image of the sample and/or pupil function is constructed using an Embedded Pupil Function Recovery (EPRY) process with one or more processors of the FLP imaging system. An example of an EPRY process is described below in detail with reference to **FIG. 11**. At optional operation **1040**, a display may receive image data such as the higher-resolution image data and/or other data, and display the data on a display (e.g., display **152** of **FIG. 1**). Then, the LFP method ends (**1050**) an imaging cycle.

[0163] **FIG. 11** is a flowchart depicting operations of an exemplary EPRY process **1100**, according to embodiments. At operation **1110**, the sample spectrum and pupil function are initialized as $S_0(\boldsymbol{u})$ and $P_0(\boldsymbol{u})$ respectively. In addition, the outer loop index variable, b, is set to 1 (first iteration) and the inner loop index variable, a, is set to 0. Outer loop index variable, b is the index incrementing the reconstruction process iterations and inner loop index variable, a, is the index incrementing the incidence angle. In the cycles of the inner loop, N acquired raw intensity images are addressed in the sequence: $I_{U_a}(\boldsymbol{r})$, $a = 0$ to N-1, where N is the number of acquired images, and each is considered in turn, with both the pupil function and sample spectrum updated at each loop.

[0164] In one embodiment, the initial sample spectrum $S_0(\boldsymbol{u})$ may be determined by first initializing a sample image in the spatial domain, and then applying a Fourier transform to obtain an initialized sample spectrum in the Fourier domain. In some cases, the initial guess may be determined as a random complex matrix (for both intensity and phase). In other cases, the initial guess may be determined as an

interpolation of the low-resolution intensity measurement with a random phase. An example of an initial guess for $S_0(\boldsymbol{u})$ may be interpolated from one of the captured intensity images. Another example of an initial guess is a constant value. The Fourier transform of the initial guess can be a broad spectrum in the Fourier domain.

[0165] In one embodiment, the initial pupil function guess $P_0(\boldsymbol{u})$ may be a circular shaped low-pass filter, with all ones inside the pass band, zeros out of the pass band and uniform zero phase. In one example, the radius of the pass band is $NA \times 2\pi/\lambda$, where NA is the numerical aperture of the filtering optical element (e.g., objective lens) and λ is the illumination wavelength. An example of an initial pupil function guess would be based on assuming the system is aberration free, phase = 0.

[0166] At operation **1112**, it is determined whether $b = 1$ i.e. it is the first iteration of the outer loop. If it is determined that it is not the first iteration, then the initial pupil function and the sample spectrum in the Fourier domain are set to the data determined in the last cycle of the inner loop: $S_0(\boldsymbol{u}) = S_{M-1}(\boldsymbol{u})$ and $P_0(\boldsymbol{u}) = P_{M-1}(\boldsymbol{u})$ at operation **1114**.

[0167] If it is determined that it is the first iteration at operation **1112**, then the EPRY process proceeds to operation **1113**. At operation **1113**, using a processor, a differential phase contrast (DPC) deconvolution procedure is applied. DPC deconvolution is a partially coherent method to acquire the quantitative phase of a sample. DPC deconvolution is based on the assumption that the absorption and phase of a specimen being imaged are small such that the specimen's complex transmission function, $\psi(x) = exp(-\mu(x) + j\theta(x))$ can be approximated as $\psi(x) \approx 1 - \mu(x) + j\theta(x)$. Under this condition, performing arithmetic operations on the intensity images captured at different illumination angles generates multiple-axis DPC images and a transfer function associated with the specimen's phase and DPC images. De-convolving the transfer function from the DPC images results in the quantitative phase image of the sample with the spatial frequency information extending to $2k_0\mathrm{NA}_{\mathrm{obj}}$ in k-space.

[0168] The phase of the initial guess is updated with the DPC-deconvolved quantitative phase as: $\psi_{2NA}(x) = |\mathcal{F}\{\Psi(k)P_{2NA}(x)\}| exp(j\theta_{DPC})$ where $\Psi(k)$ is the high-resolution Fourier spectrum of a sample, P_{2NA} is the low-pass filter with the spatial frequency extent of $2k_0NA_{obj}$ in k-space, $\mathcal{F}$ is Fourier transform operator, θ_{DPC}

is the quantitative phase obtained from DPC deconvolution, and ψ_{2NA} is the simulated image with its phase updated with θ_{DPC}. Unlike intensity image updates in techniques without using a DPC-updated phase, an update with the phase from DPC deconvolution requires use of a pupil function extending to $2NA_{obj}$ instead of just NA_{obj} because the deconvolved phase contains information up to $2NA_{obj}$ resolution.

[0169] Intensity images captured at different angles are used to reconstruct the high resolution Fourier spectrum and the pupil function of the microscope as done in earlier versions of the FP techniques. DPC phase needs to be recalculated at the beginning of each iteration of process **1100** because the pupil function of the microscope changes during pupil function update procedure.

[0170] Further details of an example of an DPC deconvolution process can be found in L. Tian and L. Waller, "Quantitative differential phase contrast imaging in an LED array microscope," Opt. Express 23(9), 11394–11403 (2015), which is hereby incorporated by reference for the discussion herein.

[0171] In certain embodiments, DPC deconvolution is included in an LFP imaging method to address low spatial frequency artifacts or "speckle noise" that may be introduced when a laser light source is used. Such artifacts may be optionally removed through use of DPC deconvolution because DPC deconvolution is a partially coherent method and is, therefore, robust to such speckle noise.

[0172] By way of illustration, **FIG. 12** shows an example of a side-by-side comparison of LFP reconstructed images (both phase and amplitude) of cells that have been enhanced using a DPC procedure and LFP reconstructed images (both phase and amplitude) of the same cells that have not been enhanced using a DPC procedure. As shown, the images for which optional DPC-deconvolution was not used in the reconstruction process, the resulting phase image of the sample shows an uneven background signal, producing speckles in the cells' phase amplitude. On the other hand, in the images for which optional DPC-deconvolution was used in the reconstruction process, the background is uniform and the cells show similar phase values. Notably, the modification has little to no effect on the amplitude image.

[0173] Returning to **FIG. 11**, in the a^{th} cycle of the inner loop, with the knowledge of the reconstructed $S_a(\boldsymbol{u})$ and $P_a(\boldsymbol{u})$ from the previous cycle of the inner loop, the exit wave at the pupil plane while the sample is illuminated by a wavevector U_n can be

simulated using: $\phi_a(u) = P_a(u)S_a(u - U_n)$ with the $S_a(\boldsymbol{u})$ and $P_a(\boldsymbol{u})$ from the previous cycle. At operation **1116**, the processor shifts the sample spectrum according to the illumination angle and multiplies by the pupil function according to: $\phi_a(u) = P_a(u)S_a(u - U_n)$. The pupil function comprises both an amplitude and a phase factor. The phase factor of the pupil function is generally associated with defocus or other aberration associated with the optical system. The amplitude of the pupil function is usually associated with the objective lens aperture shape of the optical system. By multiplying the sample spectrum by the pupil function in the Fourier domain, the processor(s) both filters the higher-resolution solution by multiplying by the modulus (computed amplitude component) of the pupil function and also multiplies by the phase factor of the pupil function. Multiplying the sample spectrum by the modulus filters the higher-resolution image in the Fourier domain for a particular plane wave incidence angle (θ_x^a, θ_y^a) with a wave vector $U_a = (k_x, k_y)$. An image captured with illumination U_a based on the ath illumination incidence angle is referred to in this section as $I_{Ua}(r)$. By multiplying the sample spectrum by the modulus, the processor(s) filters a region from the sample spectrum $S(u)$ in the Fourier domain. In cases with a filtering optical element in the form of an objective lens, this region takes the form of a circular pupil aperture with a radius of $NA*k_0$, where k_0 equals $2\pi/\lambda$ (the wave number in vacuum), given by the coherent transfer function of an objective lens. The center of the circular region in Fourier space corresponds to the associated illuminating incidence angle of this ath cycle of the inner loop. For an oblique plane wave incidence with a wave vector $U_a = (k_x, k_y)$, the region is centered about a position (k_x, k_y) in the Fourier domain.

[0174] At operation **1118**, the processor takes the inverse Fourier transform as follows: $\phi_a(r) = F^{-1}\{\phi_a(u)\}$. At operation **1120**, the processor imposes an intensity constraint. In this operation **1120**, the modulus (computed amplitude component) of the simulated region in Fourier space is replaced with the low resolution intensity measurement $I_{U_a}(r)$ captured by the radiation detector associated with an illumination wavevector U_a. The computed amplitude component is replaced by the square-root of the real intensity measurement $I_{Ua}(r)$ according to: $\phi'_a(r) = \sqrt{I_{U_a}(r)}\frac{\phi_a(r)}{|\phi_a(r)|}$. This forms an updated lower resolution image.

[0175] At operation **1122**, a Fourier transform is applied to the updated lower resolution image. In this operation, an updated exit wave is calculated via a Fourier transform according to: $\phi'_a(\boldsymbol{u}) = \mathcal{F}\{\Phi'_a(\boldsymbol{r})\}$.

[0176] At operation **1124**, the processor refreshes the Fourier spectrum guess of the higher resolution solution by updating the exit wave data and replacing data in a corresponding region of the Fourier domain as the updated exit wave data associated with incidence wave vector $U_n = (k_x, k_y)$. The processor updates the exit wave data using a sample spectrum update function. An example of a sample spectrum update function is given by:

$$S_{a+1}(\boldsymbol{u}) = S_a(\boldsymbol{u}) + \alpha \frac{P_a^*(u+U_a)}{|P_a\,(u+U_a)|^2_{max}} \left[\phi'_a(\boldsymbol{u}+U_a) - \phi_a(\boldsymbol{u}+U_a)\right]$$

(Eqn. 3)

By using such a spectrum update function, the updated value of the sample spectrum may be extracted from the difference of the two exit waves by dividing out the current pupil function. By multiplying with the conjugates using **Eqn. 3** and **Eqn. 4**, the sample spectrum can be separated from the pupil function so that the sample spectrum can be refreshed separately from the pupil function. In some cases, a correction is added to the sample spectrum guess with weight proportional to the intensity of the current pupil function estimate. The constant α adjusts the step size of the update. In one example, α =1. During the cycles of the inner loop, the data is updated as overlapping regions in the Fourier domain.

[0177] Concurrently with operation **1124**, at operation **1126** the processor refreshes the guess of the pupil function in the Fourier domain as: $P_{a+1}(\boldsymbol{u})$. An example of a pupil update function that can be used here is given by:

$$P_{a+1}(u) = SP_a(u) + \beta \frac{S_a^*(u+U_a)}{|S_a\,(u+U_a)|^2_{max}} \left[\phi'_a(\boldsymbol{u}) - \phi_a(\boldsymbol{u})\right]$$

(Eqn. 4)

The constant β adjusts the step size of the pupil function update and β = 1 is used in this paper. Using this pupil update function, the correction of the pupil function is extracted from the difference of the two exit waves by dividing out the current sample spectrum estimate, and added to the current pupil function guess with weight proportional to the intensity of the current sample spectrum estimate. By multiplying

by the conjugate using **Eqn. 4**, the pupil function can be separated from the sample spectrum and refreshed separately.

[0178] At operation **1128**, the processor imposes a pupil function constraint on the updated pupil function. Imposing the pupil function constraint may suppress noise. In the example of a microscope system, a physical circular aperture stop may be set to define the NA, thus the area in the pupil function that corresponds to the stop should always be zero. The non-zero points in the updated pupil function in the region corresponding to the stop are caused by the noise in image acquisition, and are set to zero to eliminate the noise.

[0179] The inner loop of the method continues to cycle until all N captured images in the sequence $I_{U_a}(r)$ are used to update the pupil and sample spectrum, at which point an iteration of the outer loop is complete. The cycles run from a = 0 to N -1. At operation **1130**, the processor determines whether $a = N$ -1. If the processor determines that a does not equal N -1, then not all the N captured images have been used. In this case, the loop index a will be incremented at operation **1132**, and the method will return to operation **1116** based on the next captured image associated with another incidence angle.

[0180] If the processor determines that a does equal N -1, the method continues to operation **1134**. If the processor determines that a does not equal N -1, the method continues to operation **1132**. At operation **1132**, the outer loop index is incremented a = a+1 to the next incidence angle. The method will then return to start a new cycle at operation **1116**.

[0181] At operation **1134**, the processor determines whether $b = B$. If the processor determines that b does not equal B, the loop index b will be incremented at operation **1136** to $b = b + 1$ and the loop index a will be reset to 0. The method will then return to start a new iteration at operation **1112**.

[0182] If the processor determines that b does equal B, then the iterations stop and the LFP method continues to operation **1138**. At operation **1138**, the sample spectrum is inverse Fourier transformed back to the spatial domain to generate image data for the improved resolution image of the specimen. Both the image data for the improved resolution image of the specimen and the pupil function are output of the

EPRY process. The pupil function that we are left with at the end of the operation (i.e. b = B) is the output for the reconstructed pupil function.

[0183] III. Experimental Results

[0184] An LFP imaging system similar to the LFP imaging system **600** shown in **FIG. 6** was used to perform the LFP imaging method **1000** described with respect to **FIGS. 10** and **11** to generate high resolution of a sample a sample. **FIG. 13A** shows a raw image of the sample, and **FIG. 13B** shows an re-constructed image of the sample generated using the LFP imaging method **1000** of **FIG. 10**, performed using the particular implementation of LFP imaging system **600** of **FIG. 6**, described above. The re-constructed image shown in **FIG. 12B** includes resolvable features corresponding to 1.1 μm periodicity. Since periodicity is calculated by dividing λ (the wavelength of the laser illumination) by NA, a 1.1 μm periodicity is consistent with the calculated NA of 0.425 of the particular implementation of LFP imaging system **600** of **FIG. 6**, which uses 457 nm laser illumination, as described above. As such, in this particular implementation, LFP imaging system **600** has been shown to have an NA that is approximately 4.25 times the NA of collection element **622.**

[0185] **FIG. 14** shows a schematic representation of a phase and amplitude reconstruction process **1400** during the performance of an LFP imaging method, according to an implementation. As depicted in **FIG. 14**, the reconstruction begins at **1410** with a raw image of a sample captured with the illumination from the center mirror element **816(1)** of **FIG. 6** as an initial guess of the sample field. The iteration process starts by forming the sample's quantitative phase image with 2NA resolution by DPC deconvolution with the initial guess of the pupil function, as described above in the context of **FIG. 11**. At **1420** of **FIG. 14**, the phase of the sample field up to the 2NA resolution extent is updated, as described above in the context of **FIG. 11**. In the end of process **1400** of **FIG. 14**, the complex sample field **1430** and the pupil function **1440** are reconstructed.

[0186] Modifications, additions, or omissions may be made to any of the above-described embodiments without departing from the scope of the disclosure. Any of the embodiments described above may include more, fewer, or other features without departing from the scope of the disclosure. Additionally, the steps of the described

features may be performed in any suitable order without departing from the scope of the disclosure.

[0187] It should be understood that the present invention as described above can be implemented in the form of control logic using computer software in a modular or integrated manner. Based on the disclosure and teachings provided herein, a person of ordinary skill in the art will know and appreciate other ways and/or methods to implement the present invention using hardware and a combination of hardware and software.

[0188] Any of the software components or functions described in this application, may be implemented as software code to be executed by a processor using any suitable computer language such as, for example, Java, C++ or Perl using, for example, conventional or object-oriented techniques. The software code may be stored as a series of instructions, or commands on a CRM, such as a random access memory (RAM), a read only memory (ROM), a magnetic medium such as a hard-drive or a floppy disk, or an optical medium such as a CD-ROM. Any such CRM may reside on or within a single computational apparatus, and may be present on or within different computational apparatuses within a system or network.

[0189] Although the foregoing disclosed embodiments have been described in some detail to facilitate understanding, the described embodiments are to be considered illustrative and not limiting. It will be apparent to one of ordinary skill in the art that certain changes and modifications can be practiced within the scope of the claims.

[0190] One or more features from any embodiment may be combined with one or more features of any other embodiment without departing from the scope of the disclosure. Further, modifications, additions, or omissions may be made to any embodiment without departing from the scope of the disclosure. The components of any embodiment may be integrated or separated according to particular needs without departing from the scope of the disclosure.

[0191] As used herein, the conjunction "or" is intended herein in the inclusive sense where appropriate unless otherwise indicated; that is, the phrase "A, B or C" is intended to include the possibilities of A, B, C, A and B, B and C, A and C and A, B

and C. Additionally, a phrase referring to "at least one of" a list of items refers to any combination of those items, including single members. As an example, "at least one of: A, B, or C" is intended to cover: A, B, C, A-B, A-C, B-C, and A-B-C.

WHAT IS CLAIMED IS:

1. A laser-based Fourier ptychographic imaging system, comprising:

an angle direction device configured to direct laser light from a laser light source to a specimen surface at a sequence of illumination angles;

an optical system comprising a collection element and a focusing element, the collection element configured to receive light issuing from a specimen when it is located on the specimen surface, wherein the sequence of illumination angles and a numerical aperture of the collection element correspond to overlapping regions in a Fourier domain; and

a light detector configured to receive light focused by the focusing element from the optical system and acquire a plurality of intensity images of the specimen when it is located on the specimen surface and illuminated, each intensity image corresponding to a different illumination angle of the sequence of illumination angles.

2. The laser-based Fourier ptychographic imaging system of claim 1, further comprising a processor configured to execute instructions for iteratively updating overlapping regions in the Fourier domain with the plurality of intensity images acquired by the light detector.

3. The laser-based Fourier ptychographic imaging system of claim 1, wherein the plurality of intensity images are acquired in less than 1 second.

4. The laser-based Fourier ptychographic imaging system of claim 2, wherein the processor is further configured to execute instructions for filtering out low spatial frequency artifacts associated laser light by using a differential phase contrast deconvolution procedure.

5. The laser-based Fourier ptychographic imaging system of claim 1, wherein the angle direction device comprises:

a plurality of fixed mirrors, each fixed mirror oriented to reflect laser light at one of the plurality of illumination angles to the specimen surface; and

one or more rotatable mirrors configured to reflect laser light from the laser light source sequentially to different fixed mirrors of the plurality of fixed mirrors at different sampling times,

wherein the plurality of fixed mirrors are oriented to reflect laser light received from the one or more rotatable mirrors to the specimen surface at the sequence of illumination angles.

6. The laser-based Fourier ptychographic imaging system of claim 5, wherein the one or more rotatable mirrors comprise:

a first rotatable mirror configured to rotate along a first axis; and

a second rotatable mirror configured to rotate along a second axis orthogonal to the first axis;

wherein the first rotatable mirror is configured to reflect laser light from the laser light source to the first rotatable mirror and the second rotatable mirror is configured to reflect light from the first rotatable mirror to the specimen surface,

wherein the first rotatable mirror and second rotatable mirror are configured to rotate to direct laser light to different fixed mirrors of the plurality of mirrors to direct laser light at the sequence of illumination angles.

7. The laser-based Fourier ptychographic imaging system of claim 5, wherein the one or more rotatable mirrors comprise a dual axis rotatable mirror configured to rotate along two orthogonal axes to reflect laser light from the laser light source to different fixed mirrors of the plurality of mirrors to direct laser light at the sequence of illumination angles.

8. The laser-based Fourier ptychographic imaging system of claim 5, wherein the plurality of mirrors are arranged in concentric rings along a bowl-shaped surface.

9. The laser-based Fourier ptychographic imaging system of claim 5, wherein the plurality of fixed mirrors are arranged in a flat structure.

10. The laser-based Fourier ptychographic imaging system of claim 5, wherein each fixed mirror of the plurality of mirrors comprises a neutral density filter.

11. The laser-based Fourier ptychographic imaging system of claim 1, wherein the angle direction device comprises:

a plurality of optical fibers, each optical fibers having first and second end portions; and

one or more optical switches in optical communication with the laser light source, the one or more optical switches configured to switch at different sampling times to direct laser light from the laser light source to the first end portion of different optical fibers of the plurality of optical fibers when the laser light source is activated;

wherein the plurality of optical pathways are configured so that each second end portion directs laser light to one of the sequence of illumination angles when the one or more optical switches is switched to the corresponding optical fiber and the laser light source is activated.

12. The laser-based Fourier ptychographic imaging system of claim 1, wherein in the angle direction device comprises:

a movable stage; and

an optical fiber coupled to the movable stage and optically coupled at one end to the laser light source,

wherein the movable stage is configured or configurable to translate and/or rotate the optical fiber to direct laser light from the other end of the optical fiber to illuminate the specimen surface at the plurality of illumination angles at the different sampling times.

13. The laser-based Fourier ptychographic imaging system of claim 12, wherein the movable stage is an X-Y stage, and wherein the optical fiber is coupled to the X-Y stage with a connector.

14. The laser-based Fourier ptychographic imaging system of claim 1, wherein in the angle direction device comprises:

one or more rotatable mirrors ; and

a lens system,

wherein the one or more rotatable mirrors are configurable to direct the laser light from the laser light source to the specimen surface through a lens system, the lens system configured such that rotation of the one or more rotatable mirrors causes the laser light to illuminate the specimen at the sequence of illumination angles.

15. The laser-based Fourier ptychographic imaging system of claim 18, wherein the lens system comprises a first lens and a second lens, wherein a back-focal plane of the first lens coinciding with a focal plane of the second lens, wherein at least one of the first and second lenses is a f-theta lens.

16. A laser angle direction device for directing laser light at a sequence of illumination angles, the laser angle direction device comprising:

a surface; and

a plurality of fixed mirrors coupled to the surface, each fixed mirror oriented to receive laser light and reflect the laser light to one of the plurality of illumination angles.

17. The laser angle direction device of claim 16, further comprising one or more rotatable mirrors configured to reflect laser light to the plurality of fixed mirrors at different sampling times.

18. The laser angle direction device of claim 16, wherein the surface is bowl-shaped.

19. The angle direction device of claim 16,

wherein the surface is flat;

further comprising a plurality of structures, each one of the fixed mirrors attached to one end of the structures, wherein the plurality of structures are attached to the surface.

20. The angle direction device of claim I, wherein each fixed mirror comprises a neutral density filter.

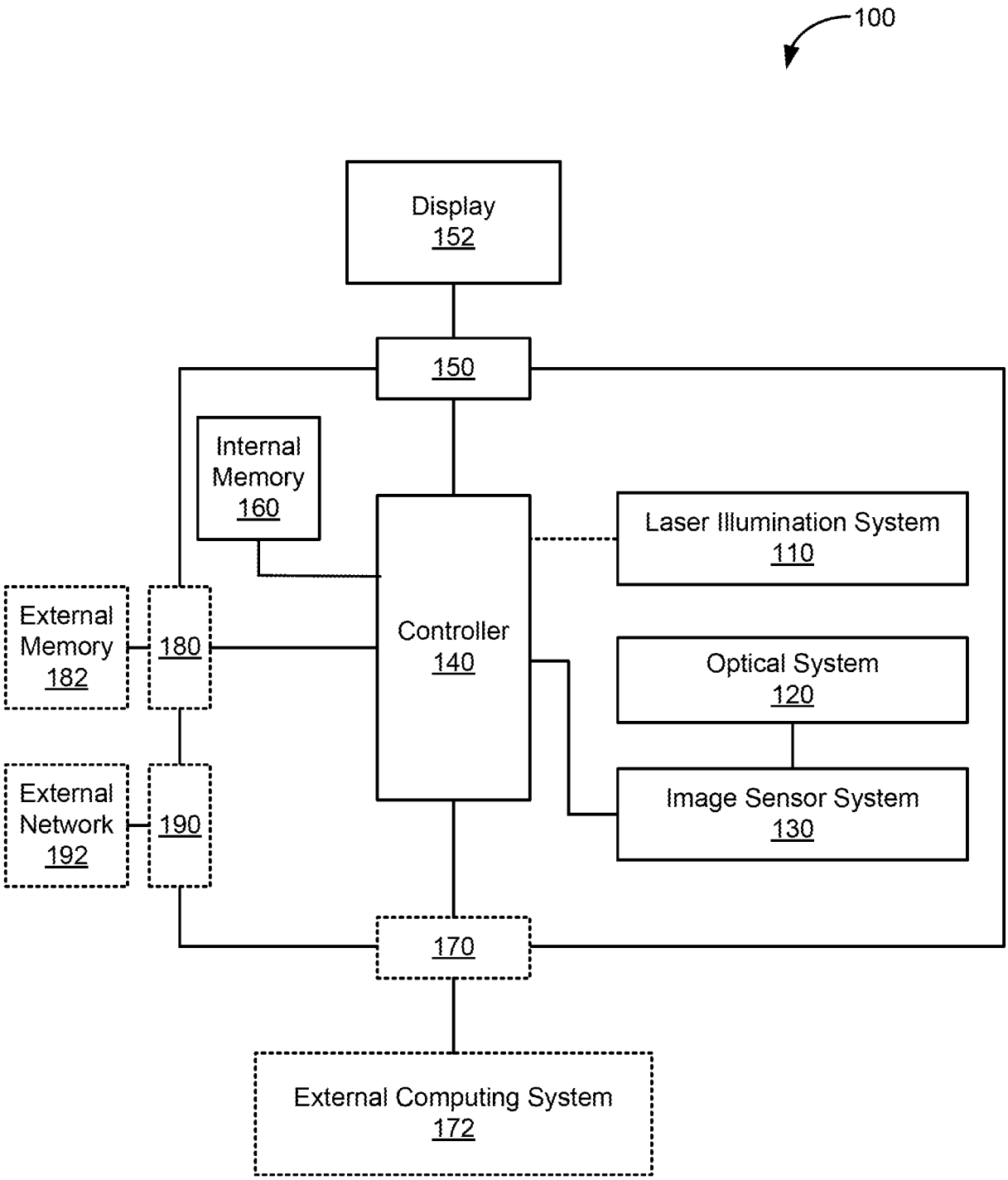
21. A laser-based Fourier ptychographic imaging method employing a laser light source, the method comprising:

directing laser light from a laser light source to a specimen surface located at about a sample plane using an angle direction device at a sequence of illumination angles;

receiving light, at a light detector, issuing from a sample when the sample is located on the specimen surface, the light received from an optical system;

acquiring a plurality of intensity images based on light received at the light detector, wherein each intensity image corresponds to one of the illumination angles; and

constructing a higher resolution image by simultaneously updating a pupil function and a sample spectrum, wherein the sample spectrum is updated in overlapping regions with Fourier transformed intensity images, wherein each of the overlapping regions corresponds to one of the plurality of illumination angles.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External Computing System
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FIG. 1

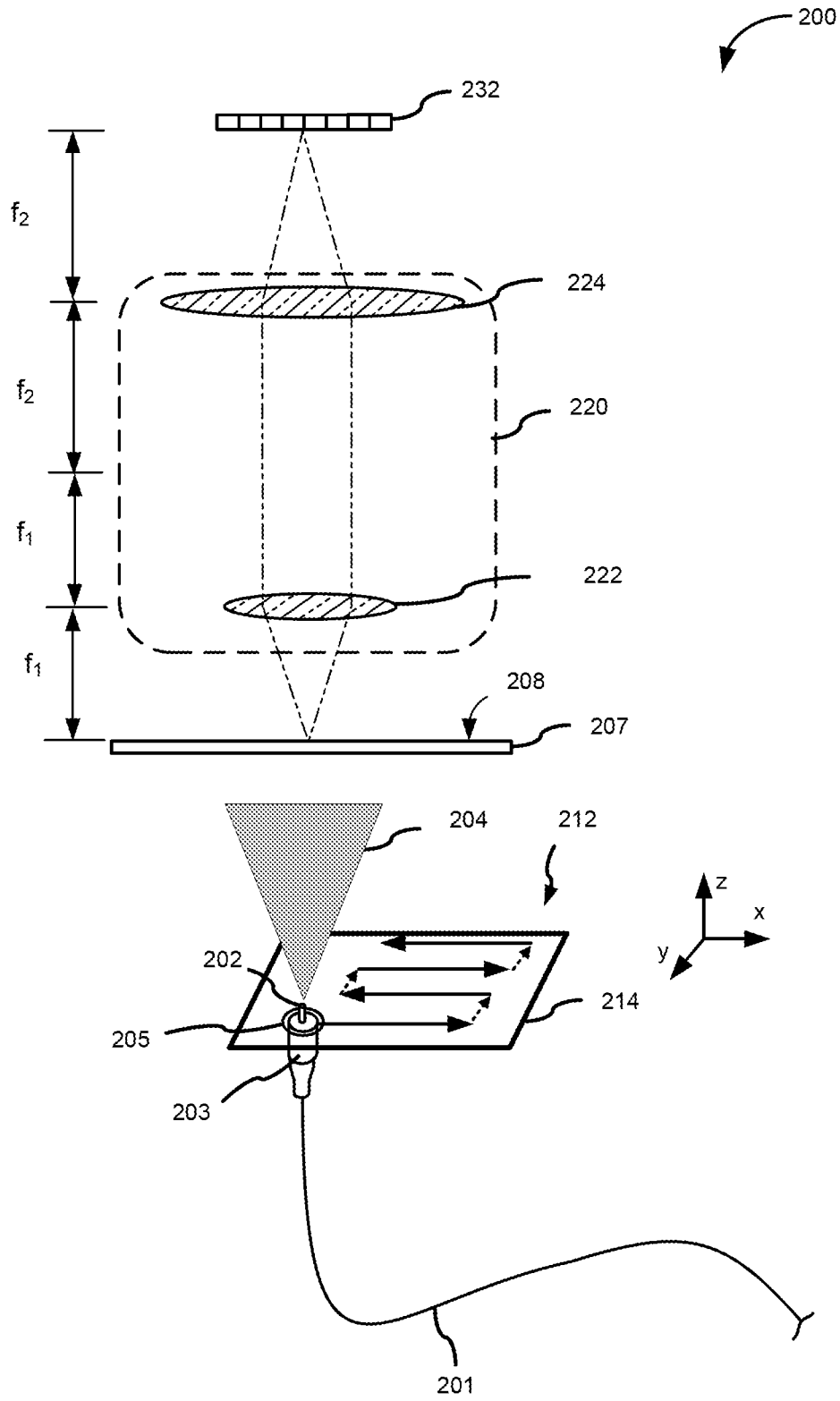
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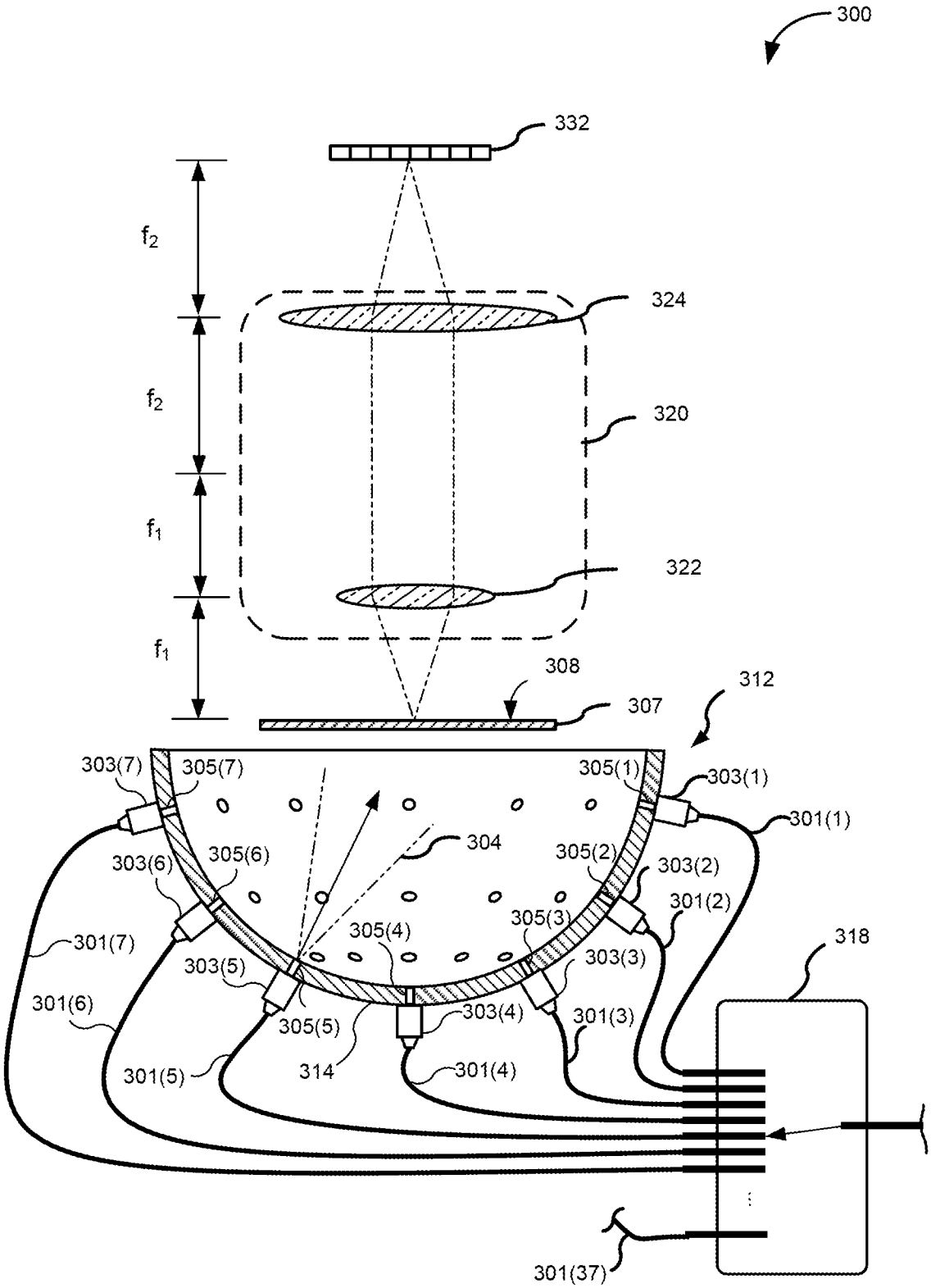
FIG. 2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303(5)
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301(5)
301(4)
301(37)

FIG. 3

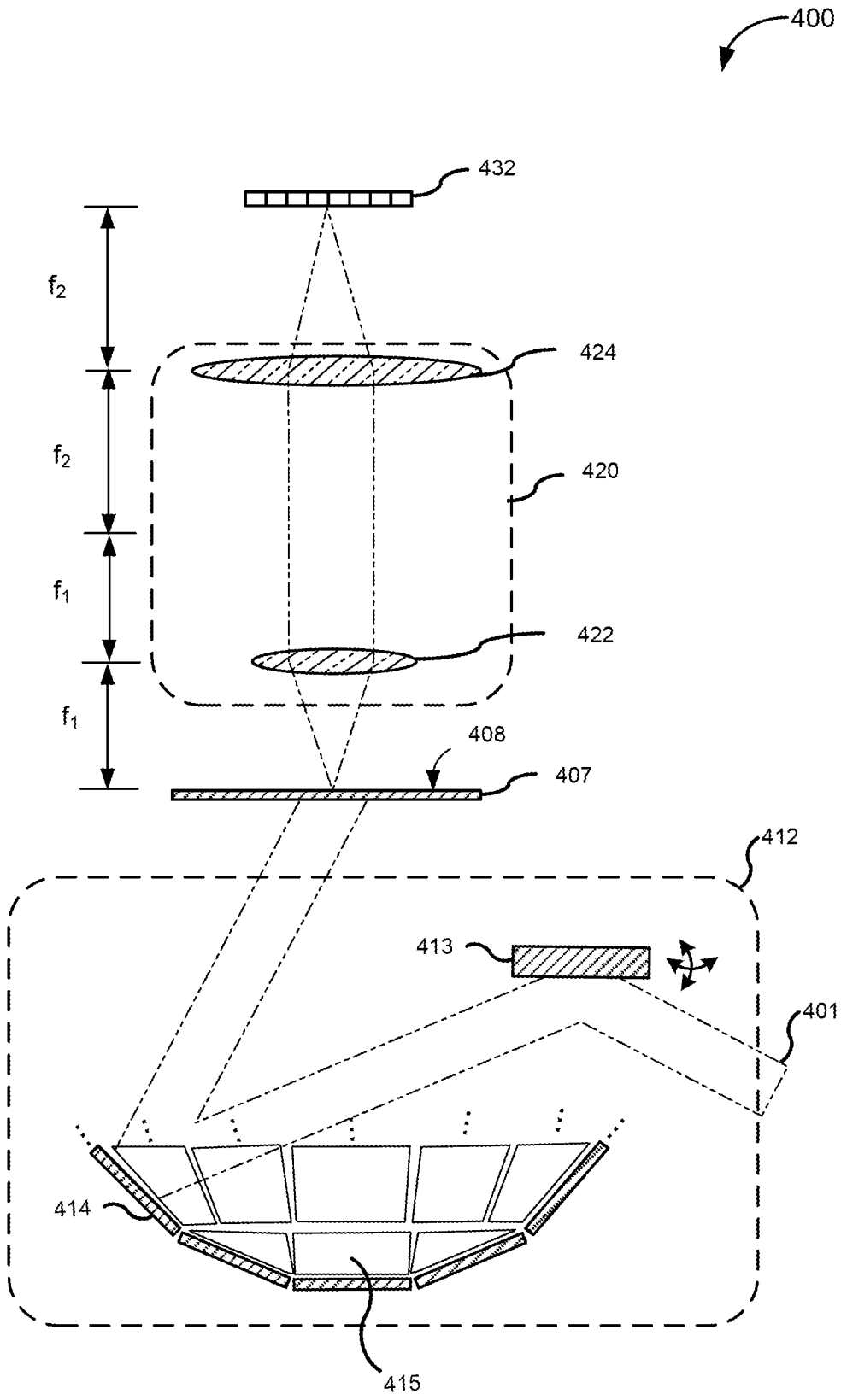

FIG. 4

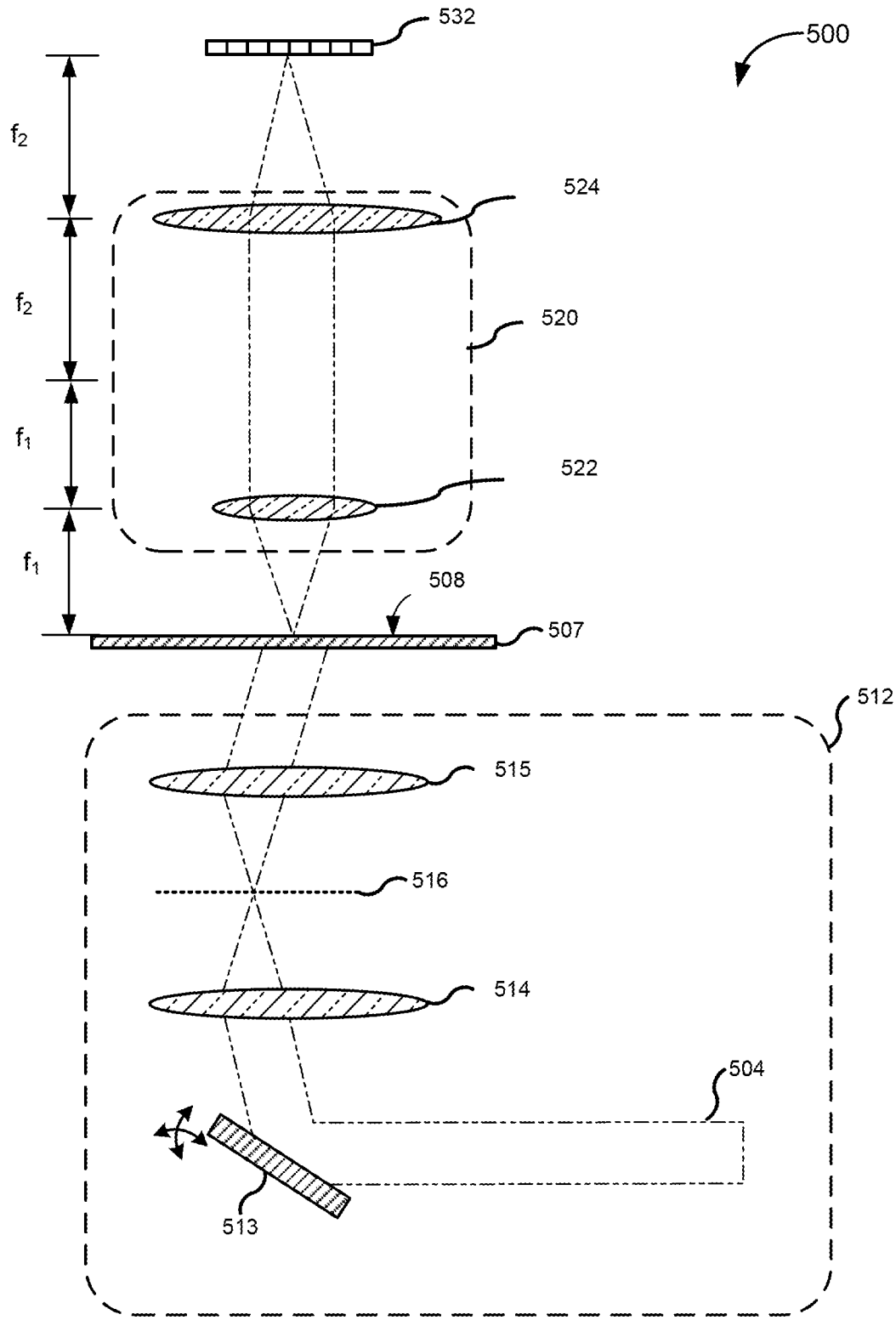

FIG. 5

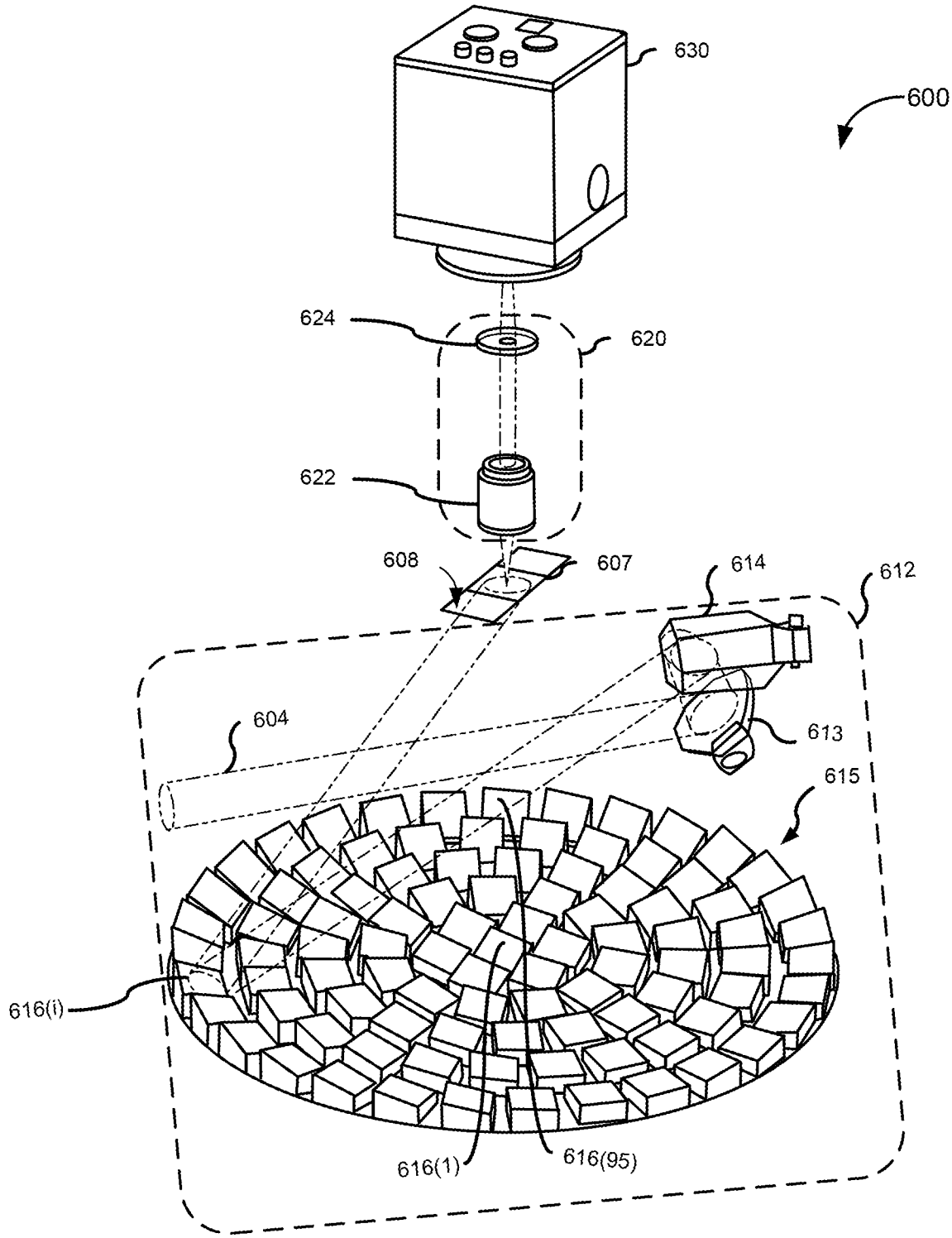

FIG. 6

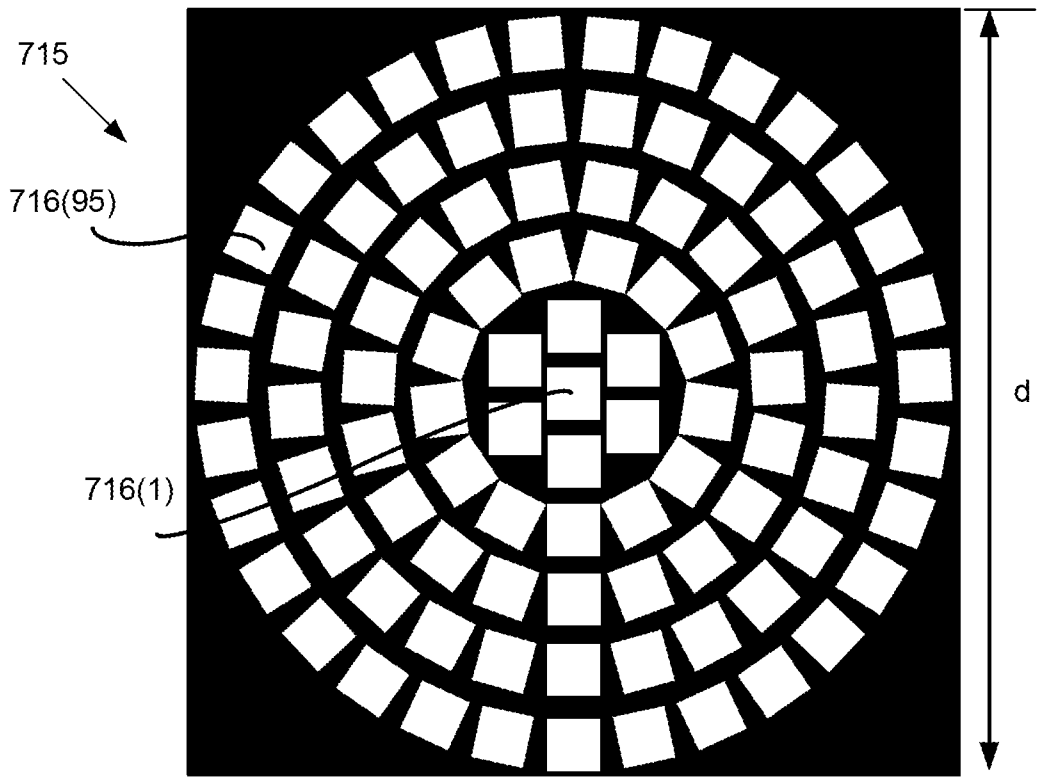

FIG. 7A

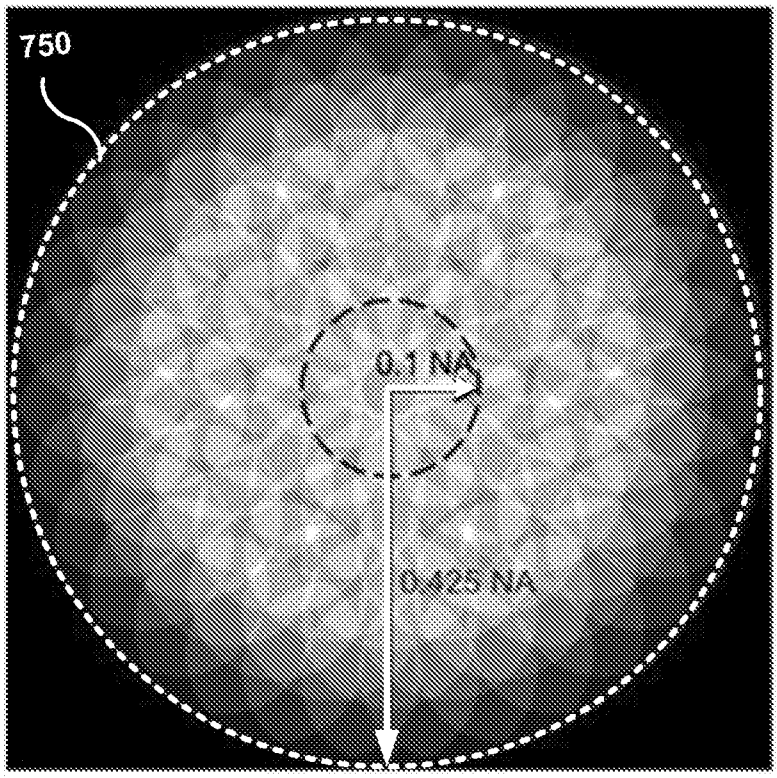

FIG. 7B

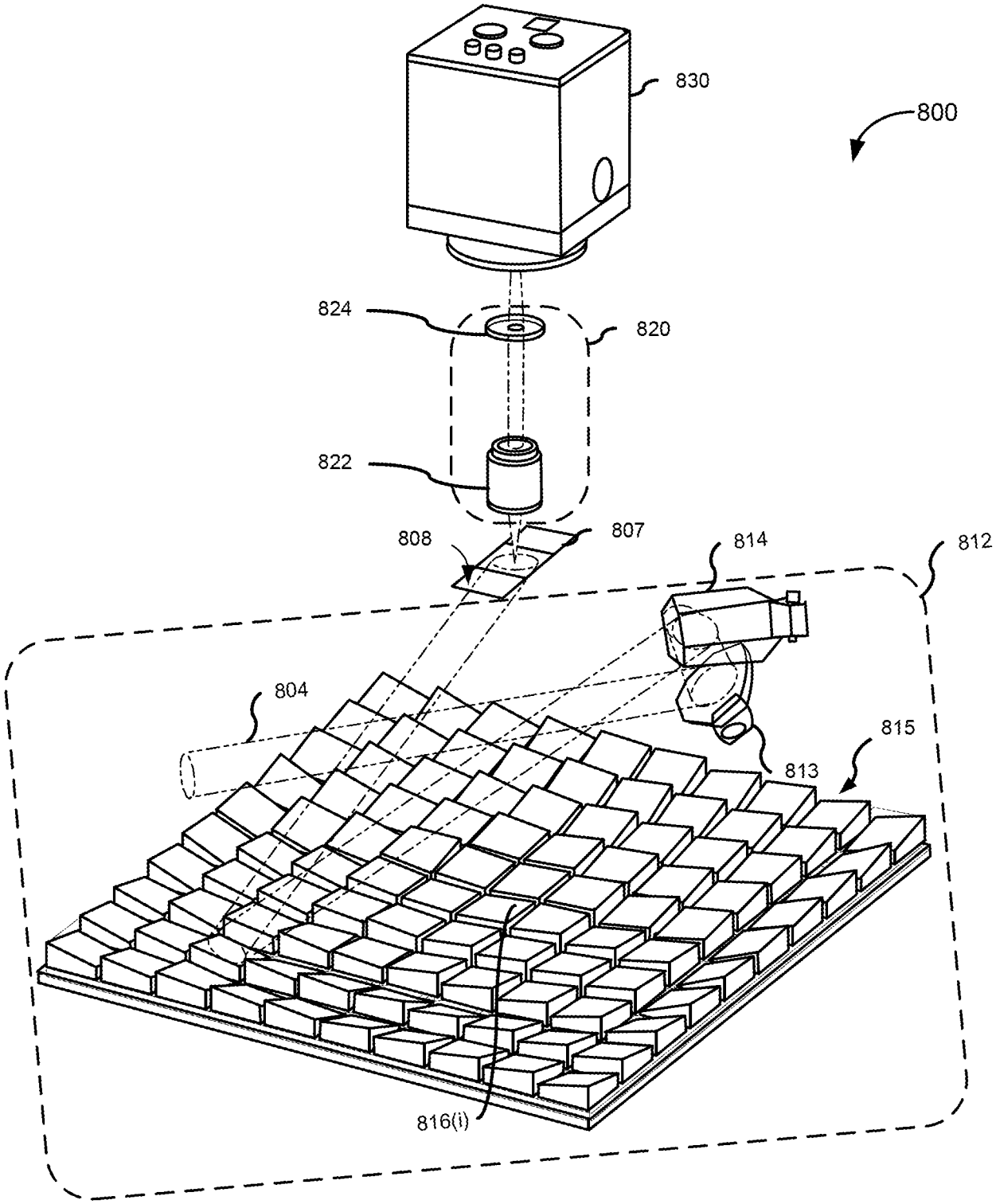

FIG. 8

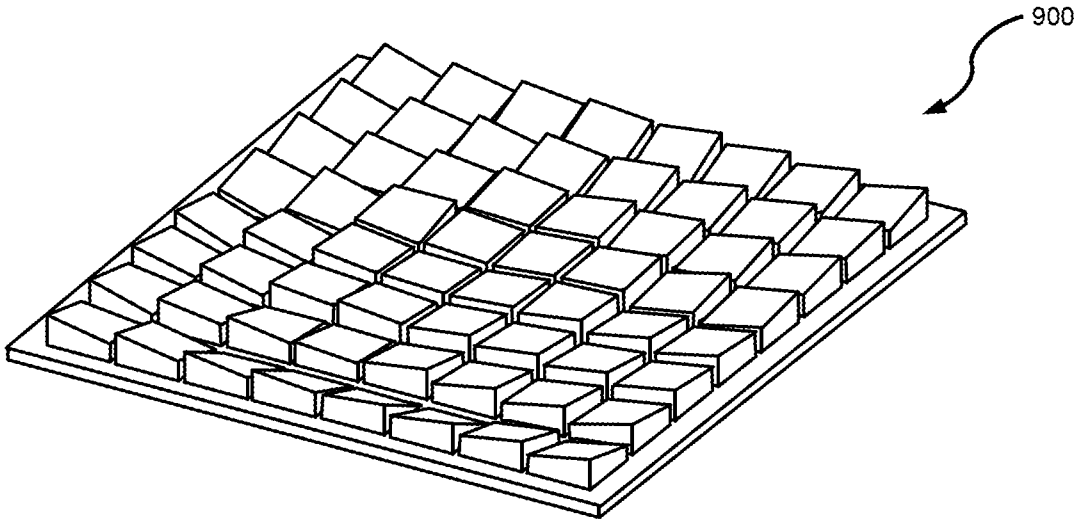

FIG. 9A

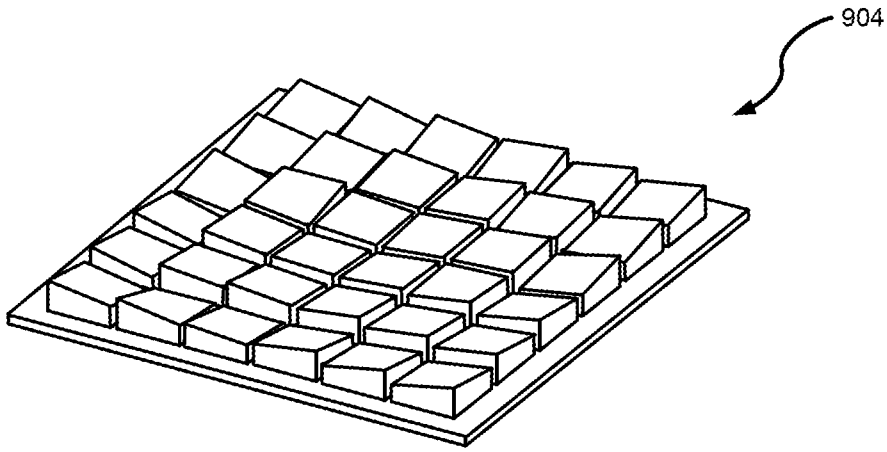

FIG. 9B

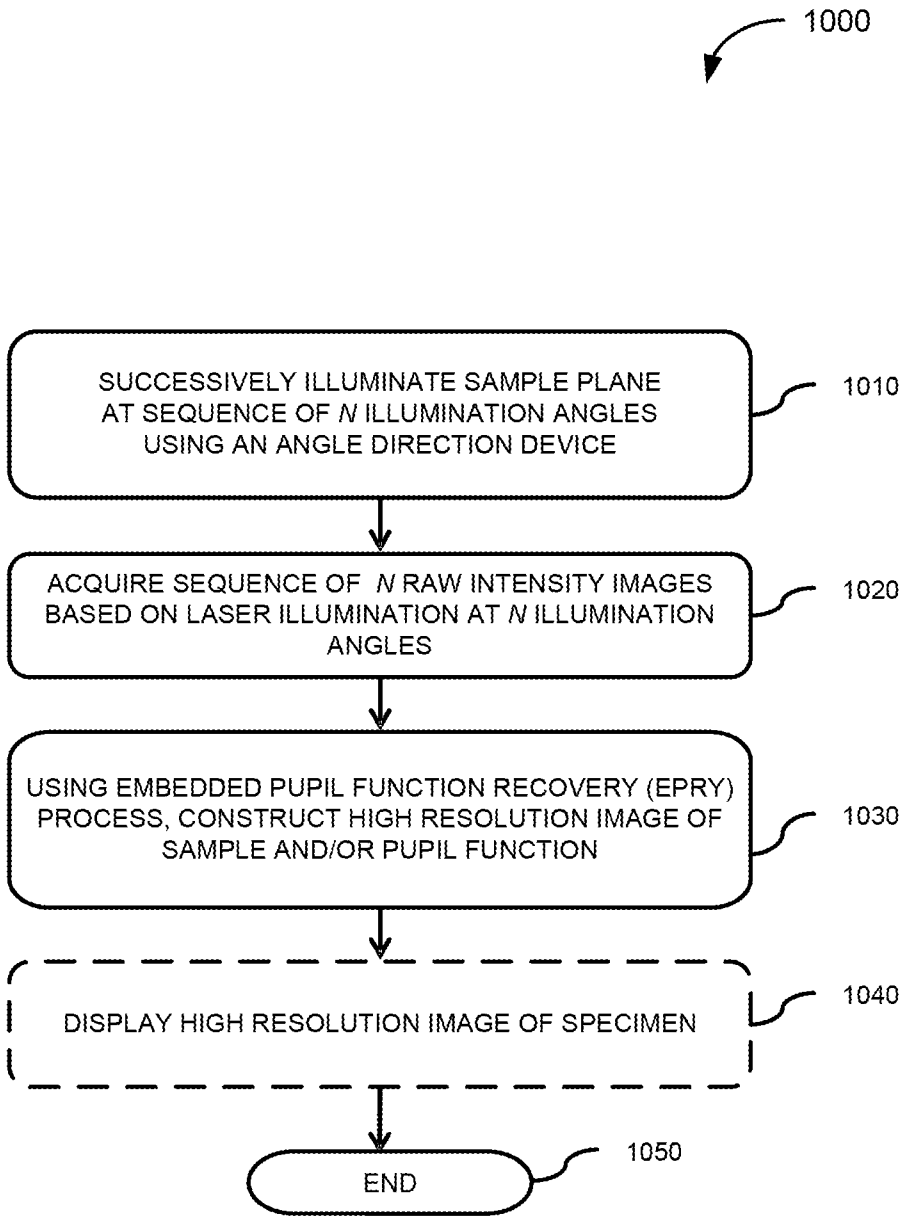
1000
SUCCESSIVELY ILLUMINATE SAMPLE PLANE AT SEQUENCE OF N ILLUMINATION ANGLES USING AN ANGLE DIRECTION DEVICE
1010
ACQUIRE SEQUENCE OF N RAW INTENSITY IMAGES BASED ON LASER ILLUMINATION AT N ILLUMINATION ANGLES
1020
USING EMBEDDED PUPIL FUNCTION RECOVERY (EPRY) PROCESS, CONSTRUCT HIGH RESOLUTION IMAGE OF SAMPLE AND/OR PUPIL FUNCTION
1030
DISPLAY HIGH RESOLUTION IMAGE OF SPECIMEN
1040
END
1050

Fig. 10

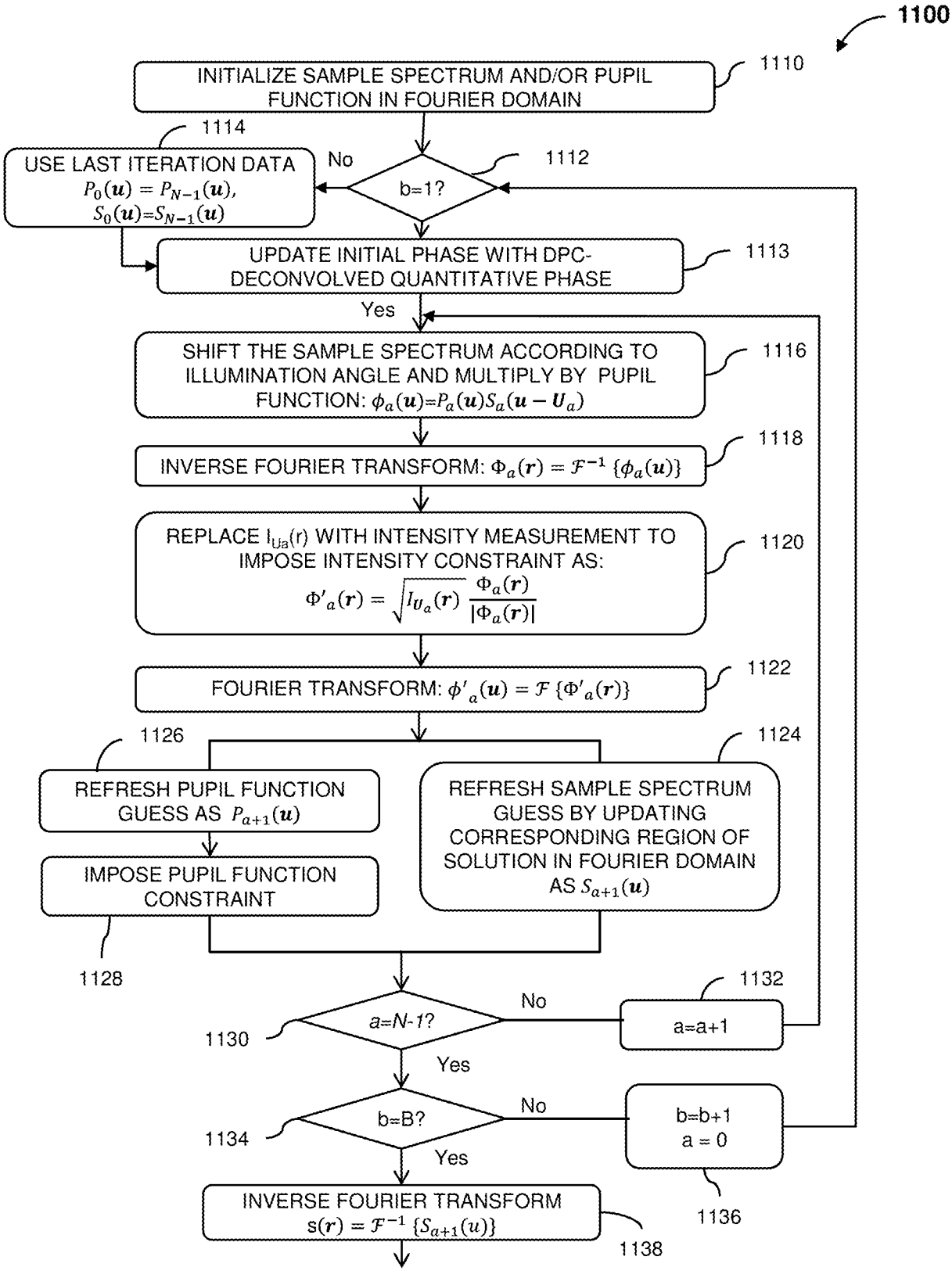
1100
INITIALIZE SAMPLE SPECTRUM AND/OR PUPIL FUNCTION IN FOURIER DOMAIN
1110
1114
USE LAST ITERATION DATA
$P_0(u) = P_{N-1}(u)$,
$S_0(u)=S_{N-1}(u)$
No
b=1?
1112
UPDATE INITIAL PHASE WITH DPC-DECONVOLVED QUANTITATIVE PHASE
1113
Yes
SHIFT THE SAMPLE SPECTRUM ACCORDING TO ILLUMINATION ANGLE AND MULTIPLY BY PUPIL FUNCTION: $\phi_a(u)=P_a(u)S_a(u-U_a)$
1116
INVERSE FOURIER TRANSFORM: $\Phi_a(r) = \mathcal{F}^{-1}\{\phi_a(u)\}$
1118
REPLACE $I_{Ua}(r)$ WITH INTENSITY MEASUREMENT TO IMPOSE INTENSITY CONSTRAINT AS:
$\Phi'_a(r) = \sqrt{I_{U_a}(r)}\frac{\Phi_a(r)}{|\Phi_a(r)|}$
1120
FOURIER TRANSFORM: $\phi'_a(u) = \mathcal{F}\{\Phi'_a(r)\}$
1122
1126
REFRESH PUPIL FUNCTION GUESS AS $P_{a+1}(u)$
IMPOSE PUPIL FUNCTION CONSTRAINT
1128
1124
REFRESH SAMPLE SPECTRUM GUESS BY UPDATING CORRESPONDING REGION OF SOLUTION IN FOURIER DOMAIN AS $S_{a+1}(u)$
a=N-1?
1130
No
1132
a=a+1
Yes
b=B?
1134
No
b=b+1
a = 0
1136
Yes
INVERSE FOURIER TRANSFORM
$s(r) = \mathcal{F}^{-1}\{S_{a+1}(u)\}$
1138

Fig. 11

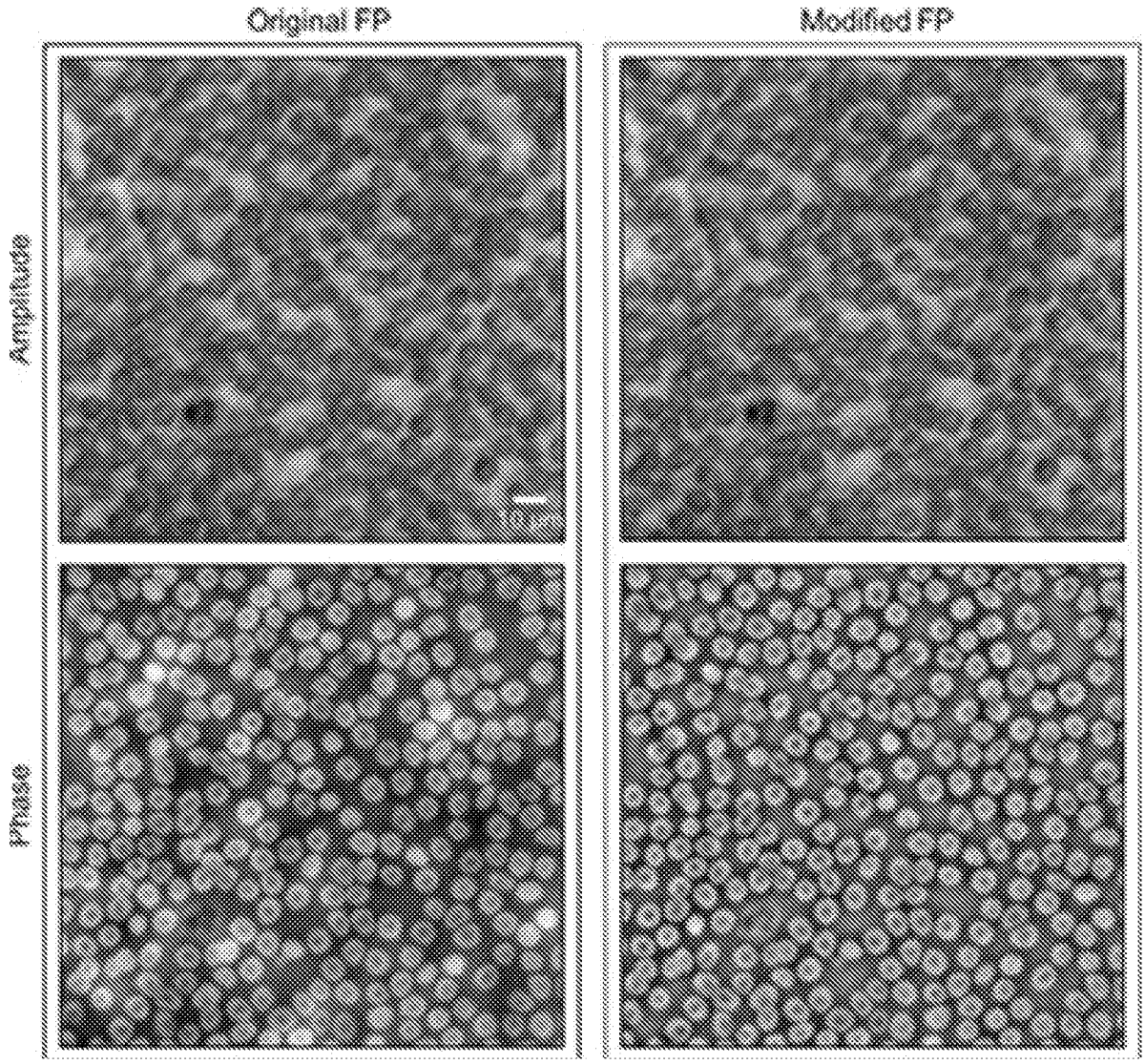

Fig. 12

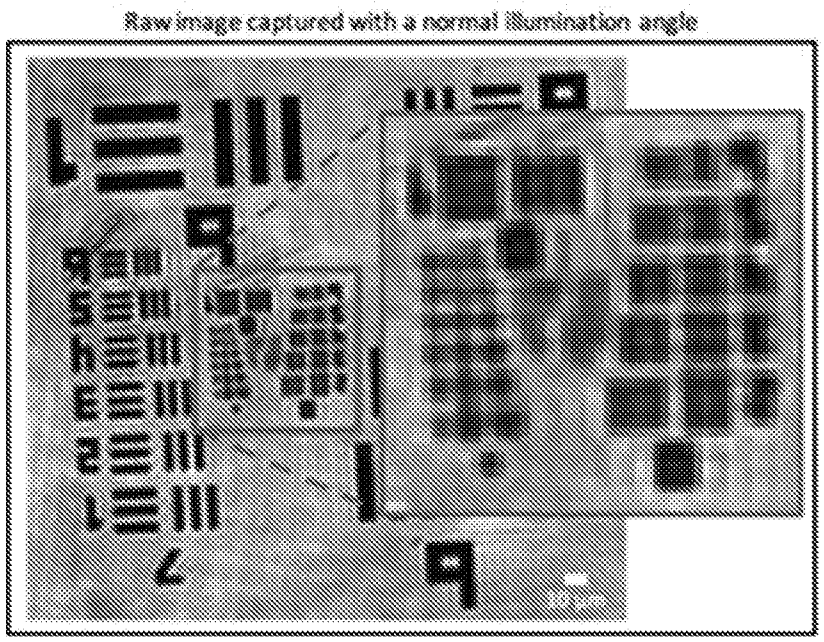

Fig. 13A

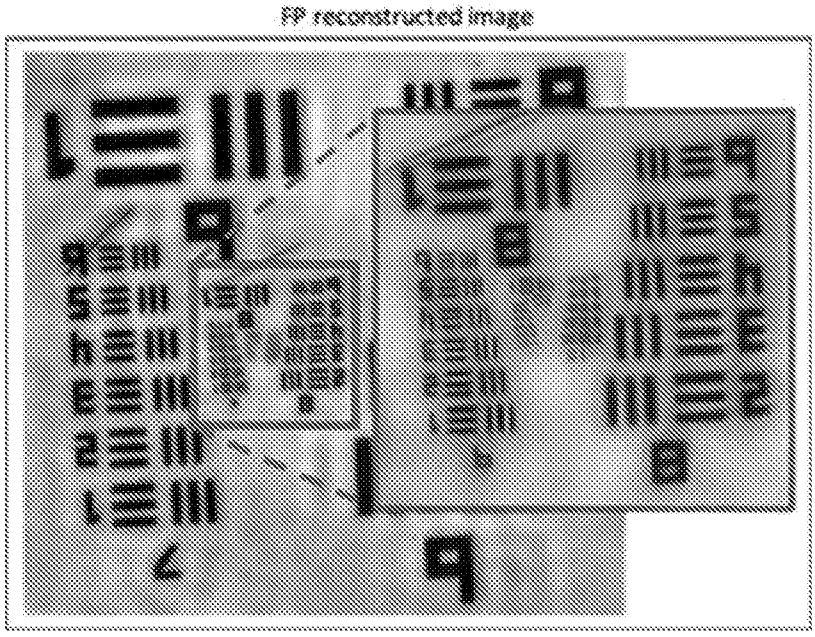

Fig. 13B

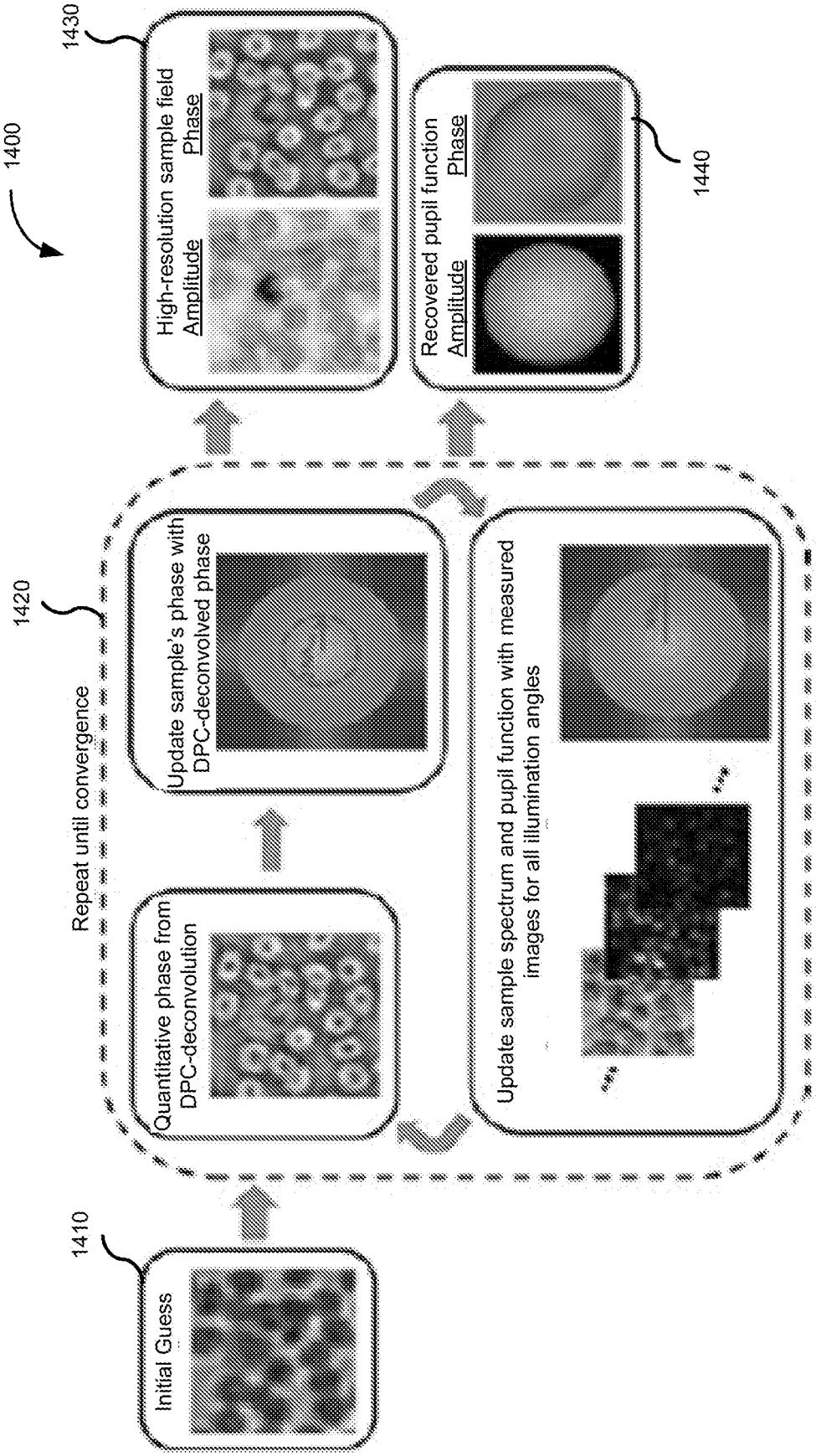
1400
1410
Initial Guess
1420
Repeat until convergence
Quantitative phase from DPC-deconvolution
Update sample's phase with DPC-deconvolved phase
Update sample spectrum and pupil function with measured images for all illumination angles
1430
High-resolution sample field
Amplitude
Phase
1440
Recovered pupil function
Amplitude
Phase

Fig. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/033638

A. CLASSIFICATION OF SUBJECT MATTER

G02B 21/36(2006.01)I, G02B 27/58(2006.01)I, G06K 9/00(2006.01)I, G02B 21/00(2006.01)I, G02B 21/24(2006.01)I

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)
G02B 21/36; G01B 11/03; G02B 3/04; H04N 5/243; G21K 7/00; G01S 17/42; G06E 3/00; H04N 9/47; G02B 27/46; G02F 1/01; G02B 27/58; G06K 9/00; G02B 21/00; G02B 21/24

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords:LFP, SBP, DPC, artifact, phase, contrast, deconvolution, optical switch, filter, speckle, rotatable, mirror, laser, light, imaging system, specimen, angle, illumination, intensity and overlapping region

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014-0126691 A1 (CALIFORNIA INSTITUTE OF TECHNOLOGY) 08 May 2014 See paragraphs [0011], [0027], [0053]-[0085], [0105], [0179]-[0206] and figures 1A-4B.	1-10,12-13,16-21
Y		11,14-15
Y	WO 03-062744 A1 (FARO TECHNOLOGIES, INC.) 31 July 2003 See page 3, lines 24-30, page 4, lines 3-13, page 13, lines 30-32 and figures 1-3.	11,14-15
A	US 2015-0036038 A1 (CALIFORNIA INSTITUTE OF TECHNOLOGY) 05 February 2015 See paragraphs [0007]-[0011], [0067]-[0080] and figure 3A.	1-21
A	US 8624968 B1 (HERSEE et al.) 07 January 2014 See column 1, line 56 - column 2, line 60 and figures 1A-3.	1-21
A	US 6747781 B2 (TRISNADI, JAHJA I.) 08 June 2004 See column 3, line 20 - column 4, line 13 and figures 1-3.	1-21

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"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	Date of mailing of the international search report
05 September 2016 (05.09.2016)	**05 September 2016 (05.09.2016)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-481-8578

Authorized officer
LEE, Hun Gil
Telephone No. +82-42-481-8525

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2016/033638

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2014-0126691 A1	08/05/2014	AU 2013-338193 A1	21/05/2015
		CA 2889495 A1	08/05/2014
		CN 104885187 A	02/09/2015
		EP 2915180 A1	09/09/2015
		EP 2915180 A4	27/04/2016
		JP 2015-535348 A	10/12/2015
		US 2014-0118529 A1	01/05/2014
		WO 2014-070656 A1	08/05/2014
WO 03-062744 A1	31/07/2003	EP 1466136 A1	13/10/2004
		EP 1466136 B1	03/08/2011
		EP 2275775 A2	19/01/2011
		EP 2275775 B1	23/09/2015
US 2015-0036038 A1	05/02/2015	AU 2014-296034 A1	18/02/2016
		CA 2919985 A1	05/02/2015
		EP 3028088 A1	08/06/2016
		WO 2015-017730 A1	05/02/2015
US 8624968 B1	07/01/2014	None	
US 6747781 B2	08/06/2004	CN 1543585 A	03/11/2004
		EP 1425625 A1	09/06/2004
		JP 2004-534265 A	11/11/2004
		KR 10-2004-0012971 A	11/02/2004
		US 2004-0008399 A1	15/01/2004
		WO 03-001281 A1	03/01/2003