SYSTEMS FOR *IN VIVO* IMAGING BIOLOGICAL PHENOMENA

Technical Field

[0001] Various embodiments relate to systems for *in vivo* imaging biological phenomena, in particular, fluorescence microscopy systems for *in vivo* imaging biological phenomena.

Background

[0002] For the mechanistic dissection of operational principles of biological phenomena, visualization of dynamic cellular activities *in vivo* is of great importance. Compared to other approaches, such as functional magnetic resonance imaging and electroencephalogram, only optical approaches have enabled real-time imaging of biological structure and function. Two-photon laser-scanning microscopy (2p-LSM), combined with genetically encoded calcium (Ca^{2+}) indicators or genetically encoded voltage indicators, has emerged as the standard modality for recording *in vivo* neuronal activity in the mammalian brain, which scatters and distorts lights. However, fundamental limits and tradeoffs between resolution, speed, and size of the acquisition volume prevent scalability of the sequential point-by-point scanning approach of a diffraction-limited spot as used in conventional 2p-LSM. For example, commercially available 80 MHz ultrashort pulse lasers that are commonly used in 2p-LSM can theoretically generate ~300 images per second with a 512 by 512 pixels (full frame) resolution in the XY plane; assuming one pulse per pixel condition, while this configuration will not generate sufficient fluorescence. As a result, practically 30-60 full-frame images per second can be acquired with a high-speed 8 kHz resonant scanner. In addition, typical piezo-driven z-axial scanning is significantly slower than

other scanners due to the inertia of the objective lens. Thus, the speed of volumetric imaging is highly limited.

[0003] To address this limitation, a pulse-splitting technique that can increase imaging speed has been developed. However, it is still challenging to record the activity of a large population of neurons in scattering tissue, at sufficient depth and single-cell resolution, and in physiological timescales. Until recently, the most advanced technology has been the Light beads microscopy, which can image approximately $3 \times 5 \times 0.5$ mm^3 volume of specimen at the rate of 5 Hz (5 volumes per second), although the number of pulses in this technique cannot be increased to cover more than 500 μm due to physical and theoretical constraints.

[0004] It has been challenging to achieve volumetric 3D imaging larger than $1mm^3$ with a video rate (24 Hz), due to slow Z-axis scanning speed compared with X- or Y-axis scanning.

[0005] Wide-field imaging can image larger areas, but volumetric imaging cannot be achieved in a scattering medium like brain tissues or brain organoids.

[0006] 3D-AOD (3-dimensional acousto-optic deflector)-based 2p microscope can achieve fast volumetric imaging in relatively small volume ($0.5 \times 0.5 \times 0.6$ mm^3) with limited pixel numbers (510 x 510 x 40 pixels/second). Due to poor transmittance of the AOD scanner, 3D-AOD-based 2p microscopes need higher laser power to achieve deep imaging in a scattering medium like brain tissues.

[0007] A light bead microscope can image large volumes ($\sim 2 \times 2 \times 0.5$ mm^3) at a relatively high speed (6.7 volumes per second). However, the number of Z planes is confined to 30 and z-axis scanning depth is limited due to theoretical and physical constraints. In light bead microscopes, a custom-made 4.7 MHz 60W source laser, which is 12-60 times more powerful than commercially available ultra-short pulse

lasers for 2p-LSM, is used to generate a single laser pulse that is then multiplied into 30 pulses in different z-axial locations by a multiplexing module. The pulse multiplications which theoretically achieve a 141 MHz stimulation rate cannot be increased further due to the limitation of fluorescence detection by a lifetime of fluorescence molecules such as green Calcium indicator fluorescent protein. If the laser repetition rate is increased (141 million pulses per second, 1 pulse per every 7 nanoseconds), the generated signal will significantly overlap with the previous signal thus the signals cannot be separately detected regardless of the acquisition rate.

[0008] Therefore, there exists a need for providing an improved system for *in vivo* imaging of biological phenomena in a large volume.

Summary

[0009] According to a first aspect of the present disclosure, a system for *in vivo* imaging biological phenomena is provided. The system may include an excitation module, configured to emit a light beam; an expansion module, configured to receive and adjust a width of the light beam; a Bessel-beam forming unit (BFU), configured to receive and convert the adjusted light beam by the expansion module into a Bessel beam; a beam splitter; and a microlens array, wherein the beam splitter is arranged between the BFU and an objective module, and is configured to transmit the adjusted light beam to the objective module and receive a returned light beam from the objective module, and to transmit the returned light beam to the microlens array; and the microlens array is configured to receive the returned light beam from the beam splitter.

Brief Description of the Drawings

[0010] In the drawings, like reference characters generally refer to like parts throughout the different views. The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the disclosure. In the following description, various embodiments of the disclosure are described with reference to the following drawings, in which:

[0011] FIG. 1A shows a schematic diagram of an example system for *in vivo* imaging biological phenomena, according to various embodiments.

[0012] FIG. 1B to FIG. 1E show exemplary Bessel-beam units, according to various embodiments.

[0013] FIG. 2 shows a schematic diagram of an example system for *in vivo* imaging biological phenomena, according to various embodiments.

[0014] FIG. 3 shows a schematic diagram of an example system for *in vivo* imaging biological phenomena, according to various embodiments.

[0015] FIG. 4 shows a schematic diagram of an example system for *in vivo* imaging biological phenomena, according to various embodiments.

[0016] FIG. 5 shows a schematic diagram of an example system for *in vivo* imaging biological phenomena, according to various embodiments.

[0017] FIG. 6 shows a schematic diagram of an example system for *in vivo* imaging biological phenomena, according to various embodiments.

[0018] FIG. 7A shows a schematic diagram of the principle of convention detection of fluorescence signals (prior art); FIG. 7B shows a schematic diagram of the principle of light-field detection of multiphoton fluorescence signals from the illuminated positions in an example system for *in vivo* imaging biological phenomena, according to various embodiments; and FIG. 7C shows a schematic diagram of the

inverse problem of light-field detection reconstruction of the light-field detection of multiphoton fluorescence signals as shown in FIG. 7B.

[0019] FIG. 8 shows a 2p-based volumetric imaging technique by an example system for *in vivo* imaging biological phenomena, according to various embodiments.

[0020] FIG. 9A shows an example image from convention detection of fluorescence signals (prior art); FIG. 9B shows an example image from light-field detection of multiphoton fluorescence signals from the illuminated positions in an example system for *in vivo* imaging biological phenomena, according to various embodiments; FIG. 9C shows a 3D reconstruction image from the example image of FIG. 9B.

[0021] FIG. 10 is a block diagram showing an example electronic device, according to an embodiment of the present disclosure.

Detailed Description

[0022] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details, and embodiments in which the disclosure may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the disclosure. Other embodiments may be utilized, and structural, logical, optical, and electrical changes may be made without departing from the scope of the disclosure. The various embodiments are not necessarily mutually exclusive, as some embodiments can be combined with one or more other embodiments to form new embodiments.

[0023] Embodiments described in the context of one of the methods or devices are analogously valid for the other methods or devices. Similarly, embodiments described in the context of a method are analogously valid for a device, and vice versa.

[0024] Features that are described in the context of an embodiment may correspondingly be applicable to the same or similar features in the other embodiments. Features that are described in the context of an embodiment may correspondingly be applicable to the other embodiments, even if not explicitly described in these other embodiments. Furthermore, additions and/or combinations and/or alternatives as described for a feature in the context of an embodiment may correspondingly be applicable to the same or similar feature in the other embodiments.

[0025] It should be understood that the terms “on”, “over”, “top”, “bottom”, “down”, “side”, “back”, “left”, “right”, “front”, “back”, “lateral”, “side”, “up”, “down”, “vertical”, “horizontal” etc., when used in the following description are used for convenience and to aid understanding of relative positions or directions, and not intended to limit the orientation of any device, or structure or any part of any device or structure. In addition, the singular terms "a", "an", and "the" include plural references unless context clearly indicates otherwise. Similarly, the “or” is intended to incude “and” unless the context clearly indicates otherwise.

[0026] It will be further understood that the terms “comprise” (and any form of comprise, such as “comprises” and “comprising”), “have” (and any form of have, such as “has” and “having”), “include” (and any form of include, such as “includes” and “including”), and “contain” (and any form of contain, such as “contains” and “containing”) are open-ended linking verbs. As a result, a method or device that “comprises,” “has,” “includes” or “contains” one or more steps or elements possesses those one or more steps or elements, but is not limited to possessing only those one or more steps or elements. Likewise, a step of a method or an element of a device that “comprises,” “has,” “includes” or “contains” one or more features possesses those one

or more features, but is not limited to possessing only those one or more features. Furthermore, a device or structure that is configured in a certain way is configured in at least that way, but may also be configured in ways that are not listed.

[0027] Approximating language, as used herein throughout the specification and claims, may be applied to modify any quantitative representation that could permissibly vary without resulting in a change in the basic function to which it is related. Accordingly, a value modified by a term or terms, such as "about," "substantially", is not limited to the precise value specified but within tolerances that are acceptable for the operation of the embodiment for an application for which it is intended. In some instances, the approximating language may correspond to the precision of an instrument for measuring the value.

[0028] The term "exemplary" may be used herein to mean "serving as an example, instance, or illustration". Any aspect or design described herein as "exemplary" is not necessarily to be construed as preferred or advantageous over other aspects or designs.

[0029] The terms "at least one" and "one or more" may be understood to include a numerical quantity greater than or equal to one (e.g., one, two, three, four, […], etc.). The term "a plurality" may be understood to include a numerical quantity greater than or equal to two (e.g., two, three, four, five, […], etc.). The phrase "at least one of" with regard to a group of elements may be used herein to mean at least one element from the group consisting of the elements. For example, the phrase "at least one of" with regard to a group of elements may be used herein to mean a selection of: one of the listed elements, a plurality of one of the listed elements, a plurality of individual listed elements, or a plurality of a multiple of listed elements.

[0030] The words “plural” and “multiple” in the description and the claims expressly refer to a quantity greater than one. Accordingly, any phrases explicitly invoking the aforementioned words (e.g., “a plurality of (objects)”, “multiple (objects)”) referring to a quantity of objects expressly refer to more than one of the said objects. The terms “group (of)”, “set (of)”, “collection (of)”, “series (of)”, “sequence (of)”, “grouping (of)”, etc., and the like in the description and in the claims, if any, refer to a quantity equal to or greater than one, i.e. one or more.

[0031] The term “first”, “second”, “third” detailed herein are used to distinguish one element from another similar element and may not necessarily denote order or relative importance, unless otherwise stated.

[0032] As used herein, the phrase of the form of “at least one of A or B” may include A or B or both A and B. Correspondingly, the phrase of the form of “at least one of A or B or C”, or including further listed items, may include any and all combinations of one or more of the associated listed items.

[0033] Various embodiments may provide a system for *in vivo* imaging biological phenomena. The system may include an excitation module, configured to emit a light beam; an expansion module, configured to receive and adjust the width of the light beam; Bessel-beam forming unit (BFU), configured to receive and convert the adjusted light beam by the expansion module into a Bessel beam; a tuning module, configured to receive and tune the Bessel beam in a manner that a diameter of the tuned light beam fits an objective module; a beam splitter; and a microlens assembly, wherein the beam splitter is arranged between the tuning module and the objective module, and is configured to transmit the tuned light beam to the objective module and receive a returned light beam from the objective module, and to transmit the returned light beam to the microlens assembly; and the microlens assembly is

configured to receive the returned light beam from the beam splitter. BFU also may convert the adjusted light beam into an Airy beam.

[0034] The proposed systems may be represented by SICLOPs (SIngle objective, sCanned Bessel beam, Light-field resOlved multi-Photon microscopy system) by combining long propagating (e.g. Bessel beam, Airy beam) multiphoton (e.g. 2-photon, 3-photon) light-sheet excitation with light-field-based signal detection strategy for a three-dimensional (3D) reconstruction. While traditional imaging captures focused images for objects lying in a single plane, "light field" captures perspectives from different angles within a single snapshot. The proposed imaging systems may enable brain Ca^{2+} imaging of up to 1.3 x 1.3 x 1 mm^3 volume at 29.89 Hz with a single 10x objective lens (NA 0.5), and the cellular resolution. Furthermore, the proposed SICLOPs may be implemented with 3-photon excitation and may be expanded to an even wider field-of-view—up to 6.5 x 6.5 x 1 mm^3 volume— at 5.7 Hz, using a 2x objective lens (NA 0.6). The proposed imaging systems may overcome a key barrier to understanding how neurons interact in networks within rodent brains or brain organoids derived from human pluripotent stem cells. A detailed understanding of these network-level processes may inform the design of new therapies for neuronal disease and disorders, such as Alzheimer's and Parkinson's diseases, in which these functions are compromised.

[0035] The proposed SICLOPs imaging systems may combine multiphoton light-sheet excitation/illumination with light-field detection using a single objective lens. The proposed system may provide sensitivity high enough to detect the low fluorescence signal generated by multi-photon (e.g. 2-photon, 3-photon) excitation.

[0036] The generation of Bessel beams may be achieved by providing a BFU (e.g. the pair of axicons, the lens-Axicon doublet, Spatial light modulator (SLM), or the

Axicon and relay lenses) into the optical path. The objective module may include a microscope objective lens with low magnification and a relatively high numerical aperture (NA). Quantum CMOS (qCMOS) camera, which has the sensitivity to resolve the number of photons in each pixel on the CCD, may detect relatively weak fluorescence generated by 2-photon excitation without signal accumulation. The microlens array (MA) may be consisted of multiple small lenses in a two-dimensional array on a substrate and refracts lights, converting the spatial location of sources to the dislocations on the CCD panel. Long Bessel beams may be generated to cover all layers of the mouse cerebral cortex or a whole brain organoid (~1 mm in thickness).

[0037] The BFU may convert a gaussian beam to a Bessel beam (e.g. a ring/annular-shaped light beam or a circular light beam). The size (e.g. a thickness) of the annular ring may be determined by the distance between the axicon and relay optics which convert diverging annular light to translational (e.g. the second axicon of the pair of the axicon, or a converging lens). Bessel beam may be scanned by an 8 kHz or 12 kHz resonant scanner to generate 2p-lightsheet (X-axis direction). 2p-lightsheet may be moved by Galvano-scanner non-continuously (e.g. every 10 μm) to cover all the neurons (Y-axis direction). The average diameter of mouse cortical neurons may be set as 10-15 μm to achieve a cellular resolution of ~2 μm in X-axis and ~5 μm PSF in Z-axis, which would minimize the time to scan the Y-axis without losing information. The fluorescence signal from the specimen will be collected by the same objective module (e.g. objective lens) and “unwoven” by light-field via the microlens array based on mathematical calculations (e.g. the inverse problem approach).

[0038] The microlens array may be tilted to maximize detection efficiency so that the 2p-Bessel lightsheet excitation looks like an oblique light-sheet from detection optics. The light-field fluorescence signal may be recorded by a CMOS camera, and the

inverse problem approach may be solved by software. By the inverse problem approach, the location of fluorescence emission may be determined, and 3D volumetric information may be reconstructed. The number of Y-axis frames and the resulting imaging speed for one volume may be dependent on the specifications of the CMOS Camera. The CMOS camera may be used to record 4096 x 64 pixels at 3,753 Hz, which is divided by the number of Y frames (e.g. volumes per second). The size of the imaging field in the proposed SICLOPs may be dependent on the magnification of the objective lens. A 2x lens (e.g. 6.5 x 6.5 x 1 mm^3 at 5.7 Hz) may be used for half hemisphere of the mouse brain. A 4x lens (e.g. 3.25 x 3.25 x 1 mm^3 at 11.5Hz) may be used for the hemisphere of the cerebral organoid. A 10x lens (e.g. 1.3 x 1.3 x 1 mm^3 at 28.9 Hz) may be used for columns in the cerebral cortex.

[0039] The proposed SICLOPs microscopy may be extended to 3-photon excitation as well. The SICLOPs may be applied for imaging rodent brains, intravital imaging, and high-content brain organoid imaging. The proposed SICLOPs microscopy may combine the principles of Bessel beam light-sheet excitation and light-field detection to overcome the spatial and temporal limitations of currently available techniques. Using this enhanced technique, imaging capability of ~1.3 x 1.3 x 1 mm^3 at 28.9 Hz and 6.5 x 6.5 x 1 mm^3 at 5.7 Hz may be achieved. The imaging speed of SICLOPs is dependent on the speed of detection camera, thus faster camera may enable faster imaging.

[0040] Light beads microscopy may image ~3 × 5 × 0.5 mm^3 at ~5 Hz or ~5.4 × 6 × 0.5 mm^3 at ~2 Hz. Light bead microscopy may split a single light pulse into 30 temporally differentiated pulses that are aligned at varied z-axis depths. Fluorescence signal from each Z-axis point may be recorded using a high-speed digitizer, which performs X-Z-Y imaging from 30 Z planes. However, light beads microscopy may be

limited by its inability to image the entire cortical layers along the Z-axis. Furthermore, it is not possible to increase the number of pulses to cover more Z-axis range due to physical constraint of fluorescence lifetime. Light beads microscopy may split excitation light into 30 temporally and spatially distributed "light beads", and image 30 different Z planes. The imaging may not be faster anymore nor extended more than 500 μm. The proposed SICLOPs may be faster than light beads microscopy to image entire cortex.

[0041] Extended Depth of Field (EDoF) imaging may utilize a Bessel beam, which is extended vertically, using an Axicon, a TAG lens, an Electrotunable Lens (ETL), or a Spatial light modulator (SLM). Using this technique, fluorescence from the vertical volume will be detected by Photomultiplier tubes (PMTs) and volumetric imaging may be performed with single scanning of the Bessel beam at 30-60 Hz. However, the image obtained may be a projection of the volume and lack information about Z depth, making it impossible to recover 3D volume or dissect overlapping signals.

[0042] To achieve fast volumetric imaging, light bead microscopy, EDoF and SICLOPs may utilize a combination of resonant scanners for X-axis scanning and Galvano scanner for Y-axis scanning. Z-axis movement may be significantly slower than XY movement in these microscopes, thus light bead microscope, EDoF, and SICLOPs may adopt additional mechanisms to image Z-axis faster. The light bead microscope may split one light pulse into 30 different pulses in the different z-axial positions. Since these pulses may be scanned and resolved, it may be difficult to extend the number of pulses to cover more Z volume due to theoretical and physical constraints (the signal may not be separated and resolved to the lifetime of fluorescence signals). EDoF may extend the PSF of excitation light using an optical or optomechanical apparatus (e.g. Axicon / TAG lens / ETL / SLM) to form a Bessel

beam. The image obtained in this technique may be a projection of the entire volume, although, axial information is lost and overlapping signals may not be resolved. The SICLOPs imaging platform may overcome this limitation of lost axial information faced by competing/existing techniques by use of light-field imaging scheme.

[0043] The following examples pertain to various aspects of the present disclosure.

[0044] Example 1 is a system for *in vivo* imaging biological phenomena, including: an excitation module, configured to emit a light beam; an expansion module, configured to receive and adjust a width of the light beam; a Bessel-beam forming unit (BFU), configured to receive and convert the adjusted light beam by the expansion module into a Bessel beam; a beam splitter; and a microlens array, wherein the beam splitter is arranged between the BFU and an objective module, and is configured to transmit the adjusted light beam to the objective module and receive a returned light beam from the objective module, and to transmit the returned light beam to the microlens array; and the microlens array is configured to receive the returned light beam from the beam splitter.

[0045] In Example 2, the subject matter of Example 1 may optionally include that the beam splitter is configured to transmit the adjusted light beam to the objective module in a manner that the adjusted light beam passes through the beam splitter by refraction, and the beam splitter is further configured to receive the returned light beam from the objective module and transmit by reflection the returned light beam to the microlens array.

[0046] In Example 3, the subject matter of Example 1 or Example 2 may optionally include a tuning module, configured to receive and tune the Bessel beam in a manner that a diameter of the tuned light beam fits the objective module, wherein the beam

splitter is arranged between the tuning module and the objective module and configured to transmit the tuned light beam to the objective module.

[0047] In Example 4, the subject matter of any one of Examples 1 to 3 may optionally include a reflector, arranged in a light path of the light beam emitted by the excitation module and oriented to reflect the light beam to be received by the expansion module;

[0048] In Example 5, the subject matter of Example 3 may optionally include a first adjustable reflector, arranged between BFU and the tuning module, and configured to receive the Bessel beam from BFU and reflect the Bessel beam from BFU to be received by the tuning module.

[0049] In Example 6, the subject matter of Example 5 may optionally include a second reflector, arranged between the first adjustable reflector and the tuning module, and configured to receive the reflected Bessel beam from the first adjustable reflector and reflect the reflected Bessel beam from the first adjustable reflector to be received by the tuning module.

[0050] In Example 7, the subject matter of Example 6 may optionally include that the first adjustable reflector and the second reflector are arranged in a manner that the arrangement of the first adjustable reflector and the second reflector reverses a light path of the Bessel beam from BFU.

[0051] In Example 8, the subject matter of any one of Examples 1 to 7 may optionally include that the light beam emitted by the excitation module travels in a light path being non-parallel to an observation axis of the objective module.

[0052] In Example 9, the subject matter of Example 3 may optionally include that the expansion module comprises a first lens and a second lens, both of the first lens and the second lens being converging lenses with a first focus length of the first lens

less than a second focus length of the second lens, wherein the first lens is spaced apart from the second lens by a distance equal to a summation of the first focus length and the second focus length.

[0053] In Example 10, the subject matter of Example 9 may optionally include that the tuning module comprises a third lens and a fourth lens, both of the third lens and the fourth lens are converging lenses, and a ratio of a third focus length of the third lens to a fourth focus length of the fourth lens is predetermined so as to tune by the ratio the diameter of the tuned light beam to fit the objective module, wherein the third lens is spaced apart from the fourth lens by a distance equal to a summation of the third focus length and the fourth focus length.

[0054] In Example 11, the subject matter of any one of Examples 1 to 10 may optionally include that an initial diameter of the Bessel beam is determined by a distance between the BFU.

[0055] In Example 12, the subject matter of any one of Examples 1 to 11 may optionally include that the objective module comprises a single objective lens for both illumination and acquisition.

[0056] In Example 13, the subject matter of any one of Examples 1 to 12 may optionally include that the returned light beam from the objective module comprises fluorescence signals emitted by a specimen imaging by the objective module, and wherein the system is configured as a fluorescence microscopy system.

[0057] In Example 14, the subject matter of Example 13 may optionally include that the excitation module comprises a titanium-sapphire laser that is tunable to emit red and near-infrared light in the range from 650 to 1600 nanometres and generates ultrashort pulses, and fluorophores stained in the specimen are excited to emit

multiple photons by the tuned light beam transmitted by the beam splitter to the objective module.

[0058] In Example 15, the subject matter of any one of Examples 1 to 14 may optionally include that the microlens array comprises a plurality of lenses in a 2-dimentional array, and is configured to refract the returned light beam received from the beam splitter.

[0059] In Example 16, the subject matter of Example 15 may optionally include a charge-coupled device (CCD), configured to receive the refracted light beam by the microlens array.

[0060] In Example 17, the subject matter of Example 16 may optionally include a complementary metal–oxide–semiconductor (CMOS) camera, configured to resolve fluorescence signals detected by the CCD.

[0061] In Example 18, the subject matter of Example 17 may optionally include fluorescence signals are resolved by an inverse problem approach in a manner that information relating a depth axis of the specimen is shown in images by the CMOS camera.

[0062] In Example 19, the subject matter of Example 15 may optionally include that the microlens array is tilted so as to be non-parallel to an incident direction of the returned light beam received from the beam splitter.

[0063] Example 20 is a method for *in vivo* imaging biological phenomena with the system of Example 1, the method including: (i) recording a returned light beam from the specimen by the system at a first position to generate a first lightsheet along a first axis; and preferably repeating step (i) for a second and optionally further position instead of the first position to generate a second and optionally further lightsheet, each

of the first, second and optionally the further position along a second axis perpendicular to the first axis.

[0064] In various embodiments, the systems for *in vivo* imaging biological phenomena will now be described by way of the following non-limiting examples.

[0065] FIG. 1A shows a schematic diagram of an example system 100 for *in vivo* imaging biological phenomena, according to various embodiments.

[0066] In the context of various embodiments, "*in vivo* imaging" may refer to imaging (e.g. investigating/testing/scanning using microscopy) within living things in which the images of various biological entities are scanned on whole, living organisms or cells, usually animals, including humans, and plants, as opposed to a tissue extract or dead organism.

[0067] In the context of various embodiments, "biological phenomena" may refer to processes and states that occur in the bodies and cells of living things. Some examples of biological processes are brain function, metabolism, digestion, cell growth, photosynthesis, and reproduction. It may be broadly understood as chemical reactions or transformations that occur in real time in humans, animals, microorganisms, and plants, and of the biosphere.

[0068] According to various non-limiting embodiments, the system 100 may include an excitation module 110, an expansion module 120, Bessel-beam forming unit (BFU) 140, a tuning module 160, a beam splitter 130, a microlens assembly 190, and an objective module 150. Some features shown in FIG. 1A may be optional to system 100, for example, the pockels cells. That is, the system 100 may optically include pockels cells, configured to modulate laser power from excitation module 110. System 100 may include further features that are not shown in FIG. 1A. For example,

the system 100 may include an X-Y scanner, as described below with reference to FIG. 2.

[0069] According to various non-limiting embodiments, the excitation module 110 may include a solid-state laser that emits a light beam with broadbands. The excitation module 110 may generate ultrashort pulses. In various embodiments, the excitation module 110 may include a titanium-sapphire (Ti:Sapphire) laser, a fiber laser and next-generation automated ultrafast tunable laser and an Optical parametric oscillator (OPO) that is tunable to emit a light beam in the range from 650 to 1600 nanometers. It should be appreciated that the excitation module 110 may include any other suitable laser, for example, other solid-state lasers (e.g. ruby lasers), semiconductor lasers may be used. The inset 111 of FIG. 1A shows a cross-section view of the light beam emitted by the excitation module 110.

[0070] According to various non-limiting embodiments, the expansion module 120 may include a first lens L1 and a second lens L2. In various embodiments, both of the first lens L1 and the second lens L2 may be converging lenses. For example, the first lens L1 may have a convex surface receiving the light beam emitted by the excited module 110 and a flat surfure transmitting the light beam to the second lens L2, that is, plano-convex. In another example, the first lens L1 may have two convex surfaces for receiving the light beam emitted by the excited module 110 and transmitting the light beam to the second lens L2, that is, double-convex. In another example, the first lens L1 may be an aspheric condenser lense that is for condensing or focusing incident light rays for illumination and collimation applications. Similarly, for example, the second lens L2 may have a convex surface receiving the light beam transmitted from the first Lens L1 and a flat surfure transmitting the light beam to BFU 140. In another example, the second lens L2 may have two convex surfaces for

receiving the light beam transmitted from the first Lens L1 and transmitting the light beam to BFU 140.

[0071] In various embodiments, the light beam emitted by the excitation module 110 (e.g. parallel beam) may travel to the expansion module 120 and be converged by the first lens L1 to a first focus of the first lens L1. The converging light beam from the first lens L1 may turn to diverging beam after passing through the first focus of the first lens L1. The diverging light beam may be further converged by the second lens L2 into parallel beam. A second focus of the second lens L2 may be arranged to be overlapped with the first focus of the first lens L1.

[0072] In various embodiments, a first focus length of the first lens L1 may be less than a second focus length of the second lens L2 in a manner that the light beam emitted by the excited module 100 is expanded (e.g. broadened, amplified, widened) by the expansion module 120 after passing through the first lens L1 and the second lens L2 in sequence. The term “focus length” used herein refers to the distance between the centre of a lens or curved mirror and its focus. In various embodiments, the first focus length of the first lens L1 may be greater than the second focus length of the second lens L2 in a manner that the light beam emitted by the excitation module 110 is contracted (e.g. shrunk, lessened, narrowed) by the expansion module 120 after passing through the first lens L1 and the second lens L2 in sequence. The inset 121 of FIG. 1A shows a cross-section view of the light beam expanded by the expansion module 120. Accordingly, by “expanded”, it may mean a diameter of the cross-section view of the light beam is increased; by “contracted”, it may mean a diameter of the cross-section view of the light beam is decreased. By adjusting the first focus length of the first lens L1, the second focus length of the second lens L2, a ratio of the first focus length of the first lens L1 to the second focus length of the

second lens L2, the expansion or contraction of the light beam may be adjusted. The ratio may depend on the dimension and focal length of microlens array, location of a CMOS detector as described herein.

[0073] In various embodiments, the first lens L1 may be spaced apart from the second lens L2 by a distance equal to a summation of the first focus length and the second focus length. The distance between the first Lens L1 and the second lens L2 may be adjusted according to the first focus length of the first lens L1 to the second focus length of the second lens L2.

[0074] It should be appreciated that the expansion module 120 may include any possible arrangements that are capable of providing a change in the diameter of the cross-section view of the light beam passing through the expansion module 120 according to requirements. For example, a diverging lens in combination with a converging lens while the diverging lens is configured to receive the light beam emitted from the excitation module 110 and the light beam emitted from the excitation module 110 may pass through the diverging lens and the converging lens in sequence. A distance between the diverging lens and the converging lens may be equal to a focus length of the converging lens.

[0075] According to various non-limiting embodiments, BFU 140 may be applied to convert the expanded light beam to form the Bessel beam (e.g. the ring-shaped light beam as shown in the inset 141 of FIG. 1A). BFU 140 may be composed of several modalities, such as a pair of axicons, a-lens axicon doublet, spatial light modulator or axicon and relay lenses. It should be appreciated that the BFU 140 may include any possible arrangements that are capable of converting the light beam into the Bessel beam, for example, a combination of multiple lens as shown in FIG. 1C, 1D and 1E.

[0076] FIG. 1B to FIG. 1E show exemplary BFUs 140, according to various embodiments. In various embodiments, the BFU 140 as shown in FIG. 1A may be replaced/substituted by any BFU 140 as shown in FIG. 1B to FIG. 1E so as to convert the expanded light beam from the expansion module 120 to the Bessel beam to be received by the tuning module 160 (or to be received by the beam splitter 130 if the tuning module 160 is omitted).

[0077] According to various non-limiting embodiments, BFU 140 may include a first axicon lens A1 and a scond axicon lens A2 as shown in FIG. 1B. The first and second axicon lenses A1 and A2 may include conical prisms. For example, the first axicon lens A1 may have a flat surface receiving the light beam expanded by the expansion module 120 and a conical surfure transmitting the light beam to the second axicon lens A2. Similarly, for example, the second axicon lens A2 may have a conical surface receiving the light beam transmitted from the first axicon Lens A1 and a flat surfure transmitting the resultant Bessel-beam to the tuning module 160.

[0078] In various embodiments, the light beam emitted by the expanded module 120 (e.g. parallel beam) may travel to BFU 140 and be converged by the first axicon lens A1 (e.g. by interference) to a first focus line aong the optical axis of the first axicon lens A1. Within the beam overlap region (called the depth of focus, DOF, i.e. a length of the focus line), the axicon may replicate the properties of a Bessel beam. The Bessel beam region may be thought of as the interference of conical waves formed by the axicon. The converging light beam from the first axicon lens A1 may turn to diverging beam (e.g. Bessel beam) after passing through the first focus line of the first axicon lens A1. The diverging light beam may be further converged by the second axicon lens A2 into parallel beam. A second focus line of the second axicon lens A2 may be arranged to be overlapped with the first focus line of the first axicon lens A1.

[0079] A Bessel beam is a special form of light that has self-regenerating properties and can be delivered long distance with coherence. It can be generated from a conventional Gaussian light-source like ultrashort pulse laser (commonly used light source for multi-photon microscope) using an axicon, spatial light modulator (SLM). During volumetric imaging, the z-axial information cannot be resolved if only a single objective lens is used for both illumination and acquisition. To address this, two objective lenses (one for illumination and the other for capturing lights from the sample illumination from side) may be typically used in multiphoton light-sheet microscopes, which enables it to resolve 3D information. However, the two objective lenses limit accessibility during live samples (e.g. animal brain) imaging due to physical constraints.

[0080] An axicon may be defined by its alpha angle (e.g. an angle formed by the bottom surface and the conical surface) and its apex angle (e.g. the top angle of the conical surface). In various embodiments, the alpha and apex angels of the first axicon lens A1 and the second axicon lens A2 are the same such that the first focus line of the first axicon lens A1 has the same length as the second focus line of the second axicon lens A2. The first axicon lens A1 may be spaced apart from the second axicon lens A2 in a manner that the first focus line of the first axicon lens A1 is overlapped with the second focus line of the second axicon lens A2. The distance between the first axicon lens A1 and the second axicon lens A2 may be adjusted according to the first focus line of the first axicon lens A1 and the second focus line of the second axicon lens A2. An initial diameter of the Bessel beam may be determined by the distance between BFU 140.

[0081] In various embodiments, the distance between BFU 140 may be equal to the length of the first focus line of the first axicon lens A1/ the lengh of the second focus

line of the second axicon lens A2 in a manner that the Bessel beam after passing through the first axicon lens A1 and the second axicon lens A2 in sequence has the initial diameter the same as the light beam expanded by the expansion module 120.

[0082] In various embodiments, the distance between BFU 140 may be greater than the length of the first focus line of the first axicon lens A1/ the lengh of the second focus line of the second axicon lens A2 in a manner that the Bessel beam after passing through the first axicon lens A1 and the second axicon lens A2 in sequence has the initial diameter greater than the diameter of the light beam expanded by the expansion module 120. In various embodiments, the distance between BFU 140 may be less than the length of the first focus line of the first axicon lens A1/ the lengh of the second focus line of the second axicon lens A2 in a manner that the Bessel beam after passing through the first axicon lens A1 and the second axicon lens A2 in sequence has the initial diameter less than the diameter of the light beam expanded by the expansion module 120. Hence, an initial diameter of the Bessel beam may be adjusted by the distance between BFU 140 such that the tuning module 160 may be omitted.

[0083] In various embodiments, the alpha and apex angels of the first axicon lens A1 and the second axicon lens A2 are different such that the first focus line of the first axicon lens A1 has the different length from the length of the second focus line of the second axicon lens A2.

[0084] According to various non-limiting embodiments, BFU 140 may include a first converging lens E1, an axicon lens A3 and a scond converging lens E2 forming a lens-axicon doublet as shown in FIG. 1C. The first converging lens E1 may be arranged in contact with the axicon lens A3. The first converging lens E1 may have a first convex surface receiving the light beam expanded by the expansion module 120, and a second convex surfure in contact with a flat surfact of the axicon lens A3 and

transmitting the light beam to the axicon lens A3. The axicon lens A3 may have the flat surface receiving the light beam transmitted from the first converging Lens E1 and a conical surfure transmitting the resultant Bessel beam to the second converging lens E2. The second converging lens E2 may in turn transmit the Bessel beam to the tuning module 160. Similarly as described with reference to FIG. 1B, an initial diameter of the Bessel beam may be adjusted by a distance between BFU 140 as shown in FIG. 1C such that the tuning module 160 may be omitted. For example, a distance between the axicon lens A3 and the second converging lens E2.

[0085] According to various non-limiting embodiments, BFU 140 may include a spatial light modulator (SLM) M3 and a converging lens E3 as shown in FIG. 1D. The SLM M3 may be programmable to generate Bessel beam or Airy beam. The SLM M3 may receive the light beam expanded by the expansion module 120, generate Bessel beam and transmit the Bessel beam to the converging lens E3. The converging lens E3 may in turn transmit the Bessel beam to the tuning module 160. Similarly as described with reference to FIG. 1B, an initial diameter of the Bessel beam may be adjusted by a distance between BFU 140 as shown in FIG. 1D such that the tuning module 160 may be omitted. For example, a distance between the SLM M3 and the converging lens E3.

[0086] According to various non-limiting embodiments, BFU 140 may include an axicon lens A4 and a converging lens E4 as relaying lens as shown in FIG. 1E. The axicon lens A4 may have a flat surface receiving the light beam expanded by the expansion module 120, and a conical surfure transmitting the resultant Bessel beam to the converging lens A4. The converging lens E4 may in turn transmit the Bessel beam to the tuning module 160. Similarly as described with reference to FIG. 1B, an initial diameter of the Bessel beam may be adjusted by a distance between BFU 140 as

shown in FIG. 1E such that the tuning module 160 may be omitted. For example, a distance between the axicon lens A4 and the converging lens E4.

[0087] According to various non-limiting embodiments, the tuning module 160 may include a third lens L3 and a fourth lens L4. The inset 161 of FIG. 1A shows a cross-section view of the light beam tuned by the tuning module 160. In various embodiments, both of the third lens L3 and the fourth lens L4 may be converging lenses, and a ratio of a third focus length of the third lens to a fourth focus length of the fourth lens is predetermined so as to tune by the ratio the diameter of the tuned light beam to fit the objective module, wherein the third lens is spaced apart from the fourth lens by a distance equal to a summation of the third focus length and the fourth focus length. The ratio may depend on the dimension and focal length of microlens array, location of a CMOS detector as described herein.

[0088] For example, the third lens L3 may have a flat surface receiving the light beam from BFU 140 and a convex surfure transmitting the light beam to the fourth lens L4. In another example, the third lens L3 may have two convex surfaces for receiving the light beam from BFU 140 and transmitting the light beam to the fourth lens L4. Similarly, for example, the fourth lens L4 may have a flat surface receiving the light beam transmitted from the third Lens L3 and a convex surfure transmitting the light beam to the beam splitter 130. In another example, the fourth lens L4 may have two convex surfaces for receiving the light beam transmitted from the third Lens L3 and transmitting the light beam to the beam splitter 130.

[0089] In various embodiments, the light beam from BFU (e.g. parallel, converging or diverging ring-shaped beam) may travel to the tuning module 160 and be converged by the third lens L3 to a third focus of the third lens L3. The converging light beam from the third lens L3 may turn to diverging beam after passing through the third

focus of the third lens L3. The diverging light beam may be further converged by the fourth lens L4 into parallel beam. A fourth focus of the fourth lens L4 may be arranged to be overlapped with the third focus of the third lens L3.

[0090] In various embodiments, a third focus length of the third lens L3 may be less than a fourth focus length of the fourth lens L4 in a manner that the light beam from BFU 140 is tuned (e.g. expanded, contracted) by the tuning module 160 after passing through the third lens L3 and the fourth lens L4 in sequence. The inset 161 of FIG. 1A shows a cross-section view of the light beam tuned (i.e. expanded) by the tuning module 160. By adjusting the third focus length of the third lens L3, the fourth focus length of the fourth lens L4, a ratio of the third focus length of the third lens L3 to the fourth focus length of the fourth lens L4, a diameter of the light beam may be tuned.

[0091] In various embodiments, the third lens L3 may be spaced apart from the fourth lens L4 by a distance equal to a summation of the third focus length and the fourth focus length. The distance between the third Lens L3 and the fourth lens L4 may be adjusted according to the third focus length of the third lens L3 to the fourth focus length of the fourth lens L4.

[0092] It should be appreciated that the tuning module 160 may include any possible arrangements that are capable of tuning the diameter of the cross-section view of the light beam pass through the tuning module 160 according to requirements. For example, a diverging lens in combination with a converging lens while the diverging lens is configured to receive the light beam from BFU 140 and the light beam from BFU 140 may pass through the diverging lens and the converging lens in sequence. A distance between the diverging lens and the converging lens may be equal to a focus length of the converging lens.

[0093] A beam splitter (or beamsplitter) is an optical component used for splitting light into two separate beams, usually by wavelength or polarity. The beam splitter 130 may include a plate beam splitter. A plate beam splitter may include a thin, flat glass plate that has been coated on a first surface of the substrate. In various embodiments, the beam splitter 130 may include a dichroic beam splitter (or dichroic mirror). The dichroic splitter beam may transmit selected wavelengths while reflecting others. It should be appreciated that the beam splitter 130 may include cube beam splitters in terms of contruction, polarizing beam splitters or non-polarizing beam splitters in terms of function.

[0094] According to various non-limiting embodiments, the beam splitter 130 may be placed between the tuning module 160 and the objective module 150. The beam splitter 130 may be configured to transmit the tuned light beam from the tuning module 160 to the objective module 150 in a manner that the tuned light beam passes through the beam splitter 130 by refraction. This may mean that the beam splitter 130 may be so oriented that the refracted light beam by the beam splitter 130 is directed to the objective module 150. For example, the beam splitter 130 may be placed at an orientation of 45 degrees to the incident light beam transmitted from the tuning module 160. The beam splitter 130 may be further configured to receive the returned light beam from the objective module 130 and transmit by reflection the returned light beam to the microlens array 190. This may mean that the beam splitter 130 may be so oriented that the reflected light beam by the beam splitter 130 is directed to the microlen array 190. The reflected light beam from the beam splitter 130 may make an angle of 90 degrees. FIG. 1A shows this arrangement.

[0095] According to various non-limiting embodiments, the objective module 150 may include a single objective lens for both illumination and acquisition. The returned

light beam from the objective module 150 may include fluorescence signals emitted by a specimen illuminated by the objective module 150. The system 100 may be configured as a fluorescence microscopy system. The fluorophores stained in the specimen may be excited to emit multiple photons by the tuned light beam transmitted by the beam splitter 130 to the objective module 150. The multiphoton excitation may include 2-photons or 3- photons excitation. As described above, the beam spliter 130 may be so designed that the returned light beam (e.g. fluorescence signals) from the objective module 150 may be reflected to the microlens array 190; or the beam spliter 130 may be so designed that the returned light beam (e.g. fluorescence signals) from the objective module 150 may be refracted (pass through the beam splitter 130) to the microlens array 190.

~~[0096]~~ According to various non-limiting embodiments, the microlens array 190 may include a plurality of micro lenses arranged in a 2-dimensional array. The microlens array 190 may be configured to refract the returned light beam received from the beam splitter to a charge-coupled device (CCD) 180.

[0097] In various embodiment, the beam splitter 130 may be so designed that selected light beam from the tuning module 160 is transmitted by the beam splitter 130 to the objective module 150 having the single objective lens. The selected light beam (e.g. the light field) may be used for both illumination of the specimen and acquisition of the returned light beam. The beam splitter 130 may be so designed that the returned light beam from the objective module 150 is transmitted (by fraction or reflection) to the microlens array 190. The returned light beam may include parallel light beam, converging or diverging light beam as scattered from the specimen and transmitted by the beam splitter 130.

[0098] In various embodiment, the microlens array 190 may be tilted so as to be non-parallel to an incident direction of the returned light beam received from the beam splitter 130.

[0099] According to various non-limiting embodiments, the system 100 may further include a complementary metal–oxide–semiconductor (CMOS) camera 170 (e.g. a quantum CMOS), configured to resolve fluorescence signals detected by the CCD 180. Fluorescence signals may be resolved by an inverse problem approach in a manner that information relating a depth axis of the specimen is shown in images by the CMOS or CCD camera 170.

[00100] Imaging of the system 100 will be described in more details with reference to FIGS. 7A-7C, 8, 9A-9C.

[00101] FIG. 2 shows a schematic diagram of an example system 200 for *in vivo* imaging biological phenomena, according to various embodiments. The system 200 may include the features of the system 100 as described above in connection to FIG. 1A, and therefore, the common features are labelled with the same reference numerals and need not be discussed. Features that are described in the context of the system 100 may correspondingly be applicable to the same or similar features in the system 200. Furthermore, additions and/or combinations and/or alternatives as described for a feature in the context of the system 100 may correspondingly be applicable to the same or similar feature in the system 200.

[00102] The system 200 may include the excitation module 110, the expansion module 120, BFU 140, the tuning module 160, the beam splitter 130, the objective module 150, the microlens array 190, the CCD 180 and the CMOS camera 170. The system 200 may further include a first adjustable reflector 220, arranged between BFU 140 and the tuning module 160. The first adjustable reflector 220 may be

configured to receive the Bessel beam from BFU 140 and reflect the Bessel beam from BFU 140 to be received by the tuning module 160. For example, an angle formed by an incident direction of the Bessel beam from BFU 140 and the reflected light beam by the first adjustable reflector 220 may be an obtuse angle, right angle or an acute angle, and in a range of between 150 degrees to 70 degrees, between 130 degrees to 80 degrees, or between 110 degrees to 90 degrees. In this arrangement, the excitation module 110 may be arranged in a direction non-parallel to an observation axis of the objective module 150 and the light beam emitted by the excitation module 110 may travel in a light path being non-parallel to the observation axis of the objective module 150.

[00103] The first adjustable reflector 220 may include an X-Y scanner so as to adjust in a X-Y plane of the specimen (e.g. a surface of the specimen). The first adjustable reflector 220 may include a Galvanometer optical scanners or resonant-Galvo scanners, configured to adjust a desirable 2-dimensional orientation of the reflected light beam by the first adjustable reflector 220. Hence, the system 200 may be used to image the specimen in a flexible manner that the objective module 150 may be placed on various positions of the specimen by adjusting the first adjustable reflector 220.

[00104] Imaging of the system 200 will be described in more details with reference to FIGS. 7A-7C, 8, 9A-9C.

[00105] FIG. 3 shows a schematic diagram of an example system 300 for *in vivo* imaging biological phenomena, according to various embodiments. The system 300 may include the features of the systems 100, 200 as described above in connection to FIGS. 1 and 2, and therefore, the common features are labelled with the same reference numerals and need not be discussed. Features that are described in the context of the systems 100, 200 may correspondingly be applicable to the same or

similar features in the system 300. Furthermore, additions and/or combinations and/or alternatives as described for a feature in the context of the systems 100, 200 may correspondingly be applicable to the same or similar feature in the system 300.

[00106] The system 300 may include the excitation module 110, the expansion module 120, BFU 140, the tuning module 160, the beam splitter 130, the objective module 150, the microlens array 190, the CCD 180, the CMOS camera 170, and the first adjustable reflector 220. The system 300 may further include a reflector M1, arranged between the excitation module 110 and the expansion module 120. The reflector M1 may be configured to receive the light beam emitted by the excitation module 110 and reflect the light beam emitted by the excitation module 110 to be received by the expansion module 120. For example, an angle formed by an incident direction of the light beam emitted by the excitation module 110 and the reflected light beam by the reflector M1 may be an obtuse angle, right angle or an acute angle, and in a range of between 150 degrees to 70 degrees, between 130 degrees to 80 degrees, between 110 degrees to 90 degrees, or 90 degrees. In this arrangement, the excitation module 110 may be arranged in a direction non-perpendicular to the observation axis of the objective module 150 and the light beam emitted by the excitation module 110 may travel in a light path being non-perpendicular to the observation axis of the objective module 150. The reflector M1 may include a mirror oriented to reflect the light beam emitted by the excitation module 110 to a receving direction of the expansion module 120.

[00107] Imaging of the system 300 will be described in more details with reference to FIGS. 7A-7C, 8, 9A-9C.

[00108] FIG. 4 shows a schematic diagram of an example system 400 for *in vivo* imaging biological phenomena, according to various embodiments. The system 300

may include the features of the systems 100, 200 as described above in connection to FIGS. 1 and 2, and therefore, the common features are labelled with the same reference numerals and need not be discussed. Features that are described in the context of the systems 100, 200 may correspondingly be applicable to the same or similar features in the system 400. Furthermore, additions and/or combinations and/or alternatives as described for a feature in the context of the systems 100, 200 may correspondingly be applicable to the same or similar feature in the system 400.

[00109] The system 400 may include the excitation module 110, the expansion module 120, BFU 140, the tuning module 160, the beam splitter 130, the objective module 150, the microlens array 190, the CCD 180, the CMOS camera 170, and the first adjustable reflector 220. The system 400 may further include a second non-adjustable or adjustable reflector M2 (e.g. a deformable mirror), arranged between the first adjustable reflector 220 and the tuning module 160. The second reflector M2 may be configured to receive the light beam reflected by the first adjustable reflector 220 and reflect the light beam reflected by the first adjustable reflector 220 to be received by the tuning module 160. For example, an angle formed by an incident direction of the light beam reflected by the first adjustable reflector 220 and the reflected light beam by the second reflector M2 may be an obtuse angle, right angle or an acute angle, and in a range of between 150 degrees to 70 degrees, between 130 degrees to 80 degrees, between 110 degrees to 90 degrees, or 90 degrees. In this arrangement, the excitation module 110 may be arranged in a direction non-perpendicular to the observation axis of the objective module 150, for example, parallel to the observation axis of the objective module 150, and the light beam emitted by the excitation module 110 may travel in a light path being non-perpendicular to the observation axis of the objective module 150. The second reflector M2 may include a mirror oriented to

reflect the light beam beam reflected by the first adjustable reflector 220 to a receving direction of the tuning module 160. The first adjustable reflector 220 and the second reflector M2 may be arranged in a manner that the arrangement of the first adjustable reflector 220 and the second reflector M2 reverses a light path of the Bessel beam from BFU 140.

[00110] Imaging of the system 400 will be described in more details with reference to FIGS. 7A-7C, 8, 9A-9C.

[00111] FIG. 5 shows a schematic diagram of an example system 500 for *in vivo* imaging biological phenomena, according to various embodiments. The system 500 may include the features of the systems 100, 200, 300, 400 as described above in connection to FIGS. 1, 2, 3 and 4, and therefore, the common features are labelled with the same reference numerals and need not be discussed. Features that are described in the context of the systems 100, 200, 300, 400 may correspondingly be applicable to the same or similar features in the system 500. Furthermore, additions and/or combinations and/or alternatives as described for a feature in the context of the systems 100, 200, 300, 400 may correspondingly be applicable to the same or similar feature in the system 500.

[00112] The system 500 may include the excitation module 110, the expansion module 120, BFU 140, the tuning module 160, the beam splitter 130, the objective module 150, the microlens array 190, the CCD 180, the CMOS camera 170, the first adjustable reflector 220, the reflector M1 and the second reflector M2. The reflector M1 may be configured to receive the light beam emitted by the excitation module 110 and reflect the light beam emitted by the excitation module 110 to be received by the expansion module 120. For example, an angle formed by an incident direction of the light beam emitted by the excitation module 110 and the reflected light beam by the

reflector M1 may be an obtuse angle, right angle or an acute angle, and in a range of between 150 degrees to 70 degrees, between 130 degrees to 80 degrees, between 110 degrees to 90 degrees, or 90 degrees. The second reflector M2 may be configured to receive the light beam reflected by the first adjustable reflector 220 and reflect the light beam reflected by the first adjustable reflector 220 to be received by the tuning module 160. For example, an angle formed by an incident direction of the light beam reflected by the first adjustable reflector 220 and the reflected light beam by the second reflector M2 may be an obtuse angle, right angle or an acute angle, and in a range of between 150 degrees to 70 degrees, between 130 degrees to 80 degrees, between 110 degrees to 90 degrees, or 90 degrees.

[00113] In this arrangement, the excitation module 110 may be arranged in a direction non-parallel to the observation axis of the objective module 150 and the light beam emitted by the excitation module 110 may travel in a light path being non-parallel to the observation axis of the objective module 150. The reflector M1 may include a mirror oriented to reflect the light beam emitted by the excitation module 110 to a receving direction of the expansion module 120. The second reflector M2 may include a mirror oriented to reflect the light beam beam reflected by the first adjustable reflector 220 to a receving direction of the tuning module 160.

[00114] Imaging of the system 500 will be described in more details with reference to FIGS. 7A-7C, 8, 9A-9C.

[00115] FIG. 6 shows a schematic diagram of an example system 600 for *in vivo* imaging biological phenomena, according to various embodiments. The system 600 may include the features of the system 100 as described above in connection to FIG. 1A, and therefore, the common features are labelled with the same reference numerals and need not be discussed.

[00116] The system 600 may include the excitation module 110, the expansion module 120, BFU 140, the tuning module 160, the beam splitter 130, the objective module 150, the microlens array 190, the CCD 180 and the CMOS camera 170. The system 600 may further include a further tuning module 630. According to various non-limiting embodiments, the further tuning module 630 may include a fifth lens L5 and a sixth lens L6. In various embodiments, both of the fifth lens L5 and the sixth lens L6 may be converging lenses, and a ratio of a fifth focus length L5 of the fifth lens to a sixth focus length of the sixth lens is predetermined so as to tune by the ratio a diameter of the retuned light beam from the beam splitter 130 according to a length of the microlens array 190, e.g. increase by the ratio (e.g. two times) the diameter of the retuned light beam from the beam splitter 130 to be half (1/2) of the length of the microlens array 190. In an example, the diameter of the retuned light beam from the beam splitter 130 may be tuned by the further tuning module 630 to match (e.g. be equal to) the length of the microlens array 190. The fifth lens L5 may be spaced apart from the sixth lens L6 by a distance equal to a summation of the fifth focus length of the fifth lens L5 and the sixth focus length of the sixth lens L6.

[00117] For example, the fifth lens L5 may have a flat surface receiving the light beam from the beam splitter 130 and a convex surfure transmitting the light beam to the sixth lens L6. In another example, the fifth lens L5 may have two convex surfaces for receiving the light beam from beam splitter 130 and transmitting the light beam to the sixth lens L6. Similarly, for example, the sixth lens L6 may have a flat surface receiving the light beam transmitted from the fifth Lens L5 and a convex surfure transmitting the light beam to the microlens array 190. In another example, the sixth lens L6 may have two convex surfaces for receiving the light beam transmitted from the fifth Lens L5 and transmitting the light beam to the CDC 180.

[00118] In various embodiments, the light beam from the beam splitter 130 (e.g. substantially parallel beam) may travel to the futher tuning module 630 and be converged by the fifth lens L5 to a fifth focus of the fifth lens L5. The converging light beam from the fifth lens L5 may turn to diverging beam after passing through the fifth focus of the fifth lens L5. The diverging light beam may be further converged by the sixth lens L6 into substantially parallel beam. A sixth focus of the sixth lens L6 may be arranged to be overlapped with the fifth focus of the fifth lens L5.

[00119] In various embodiments, a fifth focus length of the fifth lens L5 may be less than a sixth focus length of the sixth lens L6 in a manner that the light beam from the beam splitter 130 is tuned (e.g. expanded, contracted) by the further tuning module 630 after passing through the fifth lens L5 and the sixth lens L6 in sequence. By adjusting the fifth focus length of the fifth lens L5, the sixth focus length of the sixth lens L6, a ratio of the fifth focus length of the fifth lens L5 to the sixth focus length of the sixth lens L6, a diameter of the light beam may be tuned.

[00120] In various embodiments, the fifth lens L5 may be spaced apart from the sixth lens L6 by a distance equal to a summation of the fifth focus length and the sixth focus length. The distance between the fifth lens L5 and the sixth lens L6 may be adjusted according to the fifth focus length of the fifth lens L5 to the sixth focus length of the sixth lens L6.

[00121] Similarly as above, it should be appreciated that the further tuning module 630 may include any possible arrangements that are capable of tuning the diameter of the cross-section view of the light beam pass through the further tuning module 630 according to requirements. For example, a diverging lens in combination with a converging lens while the diverging lens is configured to receive the light beam from the beam splitter 130 and the light beam from beam splitter 130 may pass through the

diverging lens and the converging lens in sequence. A distance between the diverging lens and the converging lens may be equal to a focus length of the converging lens.

[00122] The system 600 may further include the features of the systems 200, 300, 400 and 500 as described above in connection to FIGS. 2 to 5, that is, the system 600 may further include a first adjustable reflector 220, a second reflector M2 and/or a reflector M1. Features that are described in the context of the systems 100, 200, 300, 400, 500 may correspondingly be applicable to the same or similar features in the system 600. Furthermore, additions and/or combinations and/or alternatives as described for a feature in the context of the systems 100, 200, 300, 400 and 500 may correspondingly be applicable to the same or similar feature in the system 600.

[00123] While the systems 100, 200, 300, 400, 500 and 600 described above is illustrated and described as a series of components or light transmission, it will be appreciated that any ordering of such components or light transmission are not to be interpreted in a limiting sense. For example, some components may be arranged in different orders and/or some light transmission may occur concurrently with other light transmission apart from those illustrated and/or described herein. In addition, not all illustrated components may be required to implement one or more aspects or embodiments described herein. Also, one or more of the light transmission depicted herein may be carried out in one or more separate acts and/or phases.

[00124] Imaging of the systems 100, 200, 300, 400, 500 and 600 will be described in more details with reference to FIGS. 7A-7C, 8, 9A-9C.

[00125] FIG. 7A shows a schematic diagram of the principle of convention detection of fluorescence signals (prior art); FIG. 7B shows a schematic diagram of the principle of light-field detection of multiphoton fluorescence signals from the illuminated positions in the systems (e.g. 100, 200, 300, 400, 500 or 600) for *in vivo*

imaging biological phenomena, according to various embodiments; and FIG. 7C shows a schematic diagram of the inverse problem of light-field detection reconstruction of the light-field detection of multiphoton fluorescence signals as shown in FIG. 7B.

[00126] In conventional fluorescence microscopy as shown in FIG. 7A, fluorescence signals emitted by the specimens (e.g. E_1, E_2, E_3), collected through the objective lens (e.g. 701) and relayed by the relay lens (e.g. 702) are detected by a CCD camera (e.g. 703). Light from the focal positions (e.g. rays 72 from E_2) may hit the relevant location on the CCD (e.g. the center pixel); however, light from the out-of-focus positions (e.g. rays 73 from E_3) may become diverged and result in a blurry image at the CCD camera. In addition, an axially overlapping signal may hit the same pixel on the CCD camera, in which case the z-information of the fluorescence signals may not be resolved. For example, rays 72a (e.g. three rays as shown in FIG. 7A) from the specimen E_2 and converged by the relay lens 702 hit a middle pixel 70; one of rays 73a (e.g. three rays as shown in FIG. 7A) from the specimen E_3 and converged by the relay lens 702 also hit the middle pixel 70. Hence, the pixel signal from the specimen E_2 overlaps with the pixel signal from the specimen E_3 which is axially overlapped with the specimen E_2.

[00127] Light-field microscopy is a technique used to extract 3D spatial information of light emissions using a microlens array and may be applied in biological imaging. Most of light-field microscopy techniques utilize the visible light for excitation, which cannot avoid phototoxicity and makes it difficult to image opaque tissues like live rodent brain or human brain organoids derived from human pluripotent stem cells due to heavy scattering and absorption. Currently, the application of light field microscopy is mostly limited to relatively transparent model

organisms like *C. elegans*, *Drosophila* embryos or *Danio rerio* embryos, or samples after tissue-clearing.

[00128] The systems may be configured as light field microscopies (LFMs) by utilizing the objective module 150 and the microlens array 190. The selected light beam from the beam splitter 130 may be used for both illumination of the specimen and acquisition of the returned light beam. A relay lens 750 may be additionally used as shown in FIG. 7B and FIG. 7C. The relay lens 750 may not be used.

[00129] A light field may be generated by placing the microlens array 190 at the rear focal plane of the optional relay lens 750 and further captured by placing the CCD 180 at the rear focal plane of the microlens array 190. Stated differently, a distance between the relay lens 750 and the microlens array 190 may be equal to a focus length of the relay lens and a distance between the relay lens 750 and the CCD 180 may be equal to a focus length of the microlens array 190. The relay lens 750 may be a converging lens including a double convex and a plano convex.

[00130] The specimens E_1 and E_2 may be placed on the front focus plane of the objective module 150 and the specimen E3 may be placed further from the front focus plane of the objective module 150. That is, the specimens E_1 and E_2 may be located at the focus of the objective module 150 and the specimen E3 may be located at the out-of-focus region of the objective module 150. Returned rays 701 from the specimen E_1 may be captured and transmited (e.g. refracted) by the objective module 150 to the relay lens 750. The relay lens 750 may transmit (e.g. converge) the returned rays 701 from the objective module 150 to one lens of the microlens array 190 (e.g. the converged rays 701a). That may mean that returned rays from specimens located at the focus of the objective module 150 may be focused on a single lens of the microlens arrary 190. The converged rays 701a may be transmitted (e.g. refracted) by

the one lens of the microlens array 190 to the CCD 180 (e.g. the transmitted rays 701b). The CCD 180 may captured the transmitted rays 701b by multiple pixles. Similarly, returned rays 702 from the specimen E_2 may be captured and transmited (e.g. refracted) by the objective module 150 to the relay lens 750. The relay lens 750 may transmit (e.g. converge) the returned rays 702 from the objective module 150 to one lens of the microlens arrary 190 (e.g. the converged rays 702a). The converged rays 702a (e.g. three rays as shown in FIG. 7B) may be transmitted (e.g. refracted) by the one lens of the microlens array 190 to the CCD 180 (e.g. the three transmitted rays 702b as shown in FIG. 7B). The CCD 180 may captured the transmitted rays 702b by multiple pixles. This is in contrast with the prior art as shown in FIG. 7A, wherein the transmitted rays by the relay lens are captured by one pixel of the CCD.

[00131] Returned rays 703 from the specimen E_3 may be captured and transmited (e.g. refracted) by the objective module 150 to the relay lens 750. The relay lens 750 may transmit (e.g. converge) the returned rays 703 from the objective module 150 to multiple lens of the microlens arrary 190 (e.g. the converged rays 703a). The converged rays 703a (e.g. three rays as shown in FIG. 7B) may be transmitted (e.g. refracted) by the mutiple lens of the microlens array 190 to the CCD 180 (e.g. the three transmitted rays 703b as shown in FIG. 7B). The CCD 180 may captured the transmitted rays 703b by multiple pixles.

[00132] As shown in FIG. 7B, fluorescence signals from the specimens E_1, E_2 and E_3 that hit the microlens array 190 (e.g. 701a, 702a, 703a) may be further refracted and eventually hit pixels on the CCD 180 (e.g. 701b, 702b, 703b). As the fluorescence signals from the specimens E_1, E_2 and E_3 may hit different positions on the CCD 180 according to the light paths, the exact spatial location of the fluorescence signal may be calculated based on an inverse problem approach using the location and signals on

pixels of the CCD 180, as shown in FIG. 7C. The signals from the specimen E_2 may be captured by at least one separate pixel (e.g. pixel 182) that is different from one separate pixel (e.g. pixel 183) by which signal from the specimen E_3 is captured. Stated differently, signals from axially overlapped specimens may be separated by the systems.

[00133] FIG. 8 shows a 2p-based volumetric imaging technique by the systems (e.g. 100, 200, 300, 400, 500 or 600) for *in vivo* imaging biological phenomena, according to various embodiments. The frame of reference 801 includes a first axis in a first direction (e.g., the X-direction), a second axis in a second direction (e.g., the Y-direction), and a third axis in a third direction (e.g., the Z-direction).

[00134] The 2p-microscopy may generate volumetric images through point-by-point, lateral raster scanning in XY plane while the z-axial position is moved either optically or mechanically. The Z-axis movement may be usually significantly slower than X- Y- axis scanning, mainly due to the inertia of the heavy objective lens or a specimen holder; this property significantly limits the speed of volumetric imaging, making it unideal to capture brain activities in physiologically relevant time scales. Bessel beam may be scanned by a 8 kHz or 12kHz resonant scanner to generate multiphoton lightsheet (X-axis direction). 2p-lightsheet may be moved by Galvano-scanner in non-continuous manner (e.g. every 10 μm) to cover all the neurons (Y-axis direction). The average diameter of mouse cortical neurons may be set as 10-15 μm to achieve a cellular resolution of ~2 μm in X-axis and ~5 μm PSF in Z-axis, which would minimize the time to scan the Y-axis without losing information. The fluorescence signal may be collected by the same objective module (e.g. the objective module 150) and “unwoven” by light-field via the microlens array based on mathematical calculations (e.g. the inverse problem approach).

[00135] Light Bead microscopy may achieve faster volumetric imaging. However, this technique requires a special laser (custom-made 4.7MHz 60W laser) and a pulse splitting/resolving mechanism. In addition, the number of acquired image planes is limited to 30 due to theoretical and physical constraints in pulse splitting/resolving. Another technique— extended depth of field (EDoF)—utilizes a laser with elongated point spread function (PSF), called Bessel beam, which is generated by either axicon, TAG lens, or SLM (Spatial Light Modulator). This system may image relatively large volumes at 30-60Hz (30-60 volumes per second). However, it cannot resolve the Z-axial information, meaning that fluorescence signals in the different z- axial positions in the specimen will not be distinguished but merged onto the same XY pixels.

[00136] The systems may rapidly image large volumes, such that physiological phenomena at cellular resolution are captured on all three axes. The systems may be based on a single-shot 3D reconstruction scheme from light-field detection using a microlens array. Furthermore, light-field detection with deconvolution may theoretically achieve improved axial point-spread-function (PSF) compared to light bead microscopy.

[00137] The performances of the systems are characterized and summarized in Table 1 as below.

Table 1. Performances of systems

Performances	Systems
X-Y scanning	Resonant-Galvo
Z scanning	No need
Z division	400
Axial PSF	2-5μm
Additional component	Axicon Microlens Arrary

	(400*400) qCMOS camera
3D reconstruction	Yes
3D imaging speed (volume per second)	29.89-50.80 (limited by camera)
Imaging Density: X	continuous
Imaging Density: Y	10μm step
Imaging Density: Z	continuous

[00138] Light field microscopes may utilize microlens array to distinguish the location of fluorescence and reconstruct volumetric information. The imaging speed of light field microscope may be dependent on the camera to be used; thus faster-volumetric imaging may be acheived with a camera with higher speed. Light field microscope may utilize the visible wavelength light which is absorbed by biological tissue so as to limit the imaging depth. In addition, visible wavelength light may cause phototoxicity so as to limit imaging time on biological tissue.

[00139] The imaging density in XZ directions may be continuous, but sparse in the Y direction (e.g. 10 μm step), as shown in Table 1. The imaging speed of the volume is done at 28.9 Hz with a 10x lens. The CMOS camera imaging speed is at 3,753 Hz at 4096 x 64 pixels. A faster camera with equivalent sensitivity may be used to increase imaging speed or density. The detection area may be decreased or increased. the imaging speed may be increased to 6,613 Hz to obtain 4096 x 32 pixels to reconstruct the light field. The imaging sizes of 1.3 x 1.3 x 1 mm^3 at 50.8 Hz may be achieved with a 10x lens.

[00140] The imaging size is ~1.3 x 1.3 x 1 mm^3 with a 10x lens. The size of the field of view may be limited by the magnification of the objective lens. An objective

lens with lower magnification (e.g. 2xNA0.6 objective lens), may be used to image 6.5 x 6.5 x 1 mm^3 at 5.7 Hz.

[00141] The Bessel beam and jumped Y-axis is used to cover a large volume. The thickness of the light-sheet, which is limited by the size of the Bessel beam, may be expected to be ~2 μm. A step size of 10 μm is adopted to fill the Y-axis as the average size of neurons is 10-15 μm. The Y-axis step size may be decreased to obtain information between each light-sheet. The Y-axis step size and imaging volume or repetition rate of volumetric imaging may be tightly associated. By reducing the step size to 5 μm, imaging speed may be 14.4 Hz for 1.3 x 1.3 x 1 mm^3. Planar illumination generated by a cylindrical lens may be adopted, which increases the thickness of the multiphoton Bessel beam light-sheet, and more information from the brain volume without modification of the detection mechanism may be obtained. An alternative strategy may be to tilt the microlens array, so that the imaging plane may look oblique. Oblique plane illumination may produce wider light field, allowing the extraction of additional information.

[00142] FIG. 9A shows an example image from convention detection of fluorescence signals (prior art); FIG. 9B shows an example image from light-field detection of multiphoton fluorescence signals from the illuminated positions in the systems 100, 200, 300, 400, 500 and 600 for *in vivo* imaging biological phenomena, according to various embodiments; FIG. 9C shows a 3D reconstruction image from the example image of FIG. 9B.

[00143] The example images as shown in FIGS. 9A and 9B are taken to image fluorescent beads (either 1 μm or 5μm in diameter) embedded in 4% agarose using a conventional 2-photon microscope and the systems 100, 200, 300, 400, 500 and 600, respectively. The conventional 2-photon microscope is equipped with Galvano

scanners and motorized Z-drive, which required a total of 254 seconds (> 6 min) to image 500 x 500 x 200 μm^3 volume with 1μm z-step. Scale bar = 50 μm. In the systems 100, 200, 300, 400, 500 and 600, the FWHM of the Bessel Beam was approximately 300 μm and 497 μSec (497 x 10^{-6} seconds) was required to take single light field image. Signals were accumulated 5.96 times with a 12kHz resonant scanner. As shown in FIG. 9C, from single light field imaging data obtained from the systems 100, 200, 300, 400, 500 and 600, the 3D volumetric image can be reconstructed.

[00144] FIG. 10 is a block diagram showing an example electronic device, according to an embodiment of the present disclosure. The electronic device 1000 may be a laptop computer, a desktop computer, a tablet computer, an automobile computer, a smart phone, a personal digital assistant, a server, or other electronic devices capable of running computer applications. In some implementations, the electronic device 1000 includes a processor 1002, an input/output (I/O) module 1004, memory 1006, a power unit 1008, and one or more network interfaces 1010. The processor 1002 may include a Graphics Processing Unit. The electronic device 1000 can include additional components. In some implementations, the processor 1002, input/output (I/O) module 1004, memory 1006, power unit 1008, and the network interface(s) 1010 are housed together in a common housing or other array.

[00145] The example processor 1002 can execute instructions, for example, to generate output data based on data inputs. The instructions can include programs, codes, scripts, modules, or other types of data stored in memory (e.g., memory 1006). Additionally, or alternatively, the instructions can be encoded as pre-programmed or re-programmable logic circuits, logic gates, or other types of hardware or firmware components or modules. The processor 1002 may be, or may include, a multicore

processor having a plurality of cores, and each such core may have an independent power domain and can be configured to enter and exit different operating or performance states based on workload. Additionally, or alternatively, the processor 1002 may be, or may include, a general-purpose microprocessor, as a specialized co-processor or another type of data processing apparatus. In some cases, the processor 1002 performs high-level operation of the electronic device 1000. For example, the processor 1002 may be configured to execute or interpret software, scripts, programs, functions, executables, or other instructions stored in the memory 1006.

[00146] The example I/O module 1004 may include a mouse, keypad, touch screen, scanner, optical reader, and/or stylus (or other input device(s)) through which a user of the electronic device 1000 may provide input to the electronic device 1000 and may also include one or more of a speaker for providing audio output and a video display device for providing textual, audiovisual, and/or graphical output.

[00147] The example memory 1006 may include computer-readable storage media, for example, a volatile memory device, a non-volatile memory device, or both. The memory 1006 may include one or more read-only memory devices, random-access memory devices, buffer memory devices, or a combination of these and other types of memory devices. In some instances, one or more components of the memory can be integrated or otherwise associated with another component of the electronic device 1000. The memory 1006 may store instructions that are executable by the processor 1002. In some examples, the memory 1006 may store instructions for an operating system 1012 and for application programs 1014. The memory 1006 may also store a database 1016.

[00148] The example power unit 1008 provides power to the other components of the electronic device 1000. For example, the other components may operate based on

electrical power provided by the power unit 1008 through a voltage bus or other connection. In some implementations, the power unit 1008 includes a battery or a battery system, for example, a rechargeable battery. In some implementations, the power unit 1008 includes an adapter (e.g., an AC adapter) that receives an external power signal (from an external source) and coverts the external power signal to an internal power signal conditioned for a component of the electronic device 1000. The power unit 1008 may include other components or operate in another manner.

[00149] The electronic device 1000 may be configured to operate in a wireless, wired, or cloud network environment (or a combination thereof). In some implementations, the electronic device 1000 can access the network using the network interface(s) 1010. The network interface(s) 1010 can include one or more adapters, modems, connectors, sockets, terminals, ports, slots, and the like. The wireless network that the electronic device 1000 accesses may operate, for example, according to a wireless network standard or another type of wireless communication protocol. For example, the wireless network may be configured to operate as a Wireless Local Area Network (WLAN), a Personal Area Network (PAN), a metropolitan area network (MAN), or another type of wireless network. Examples of WLANs include networks configured to operate according to one or more of the 802.11 family of standards developed by IEEE (e.g., Wi-Fi networks), and others. Examples of PANs include networks that operate according to short-range communication standards (e.g., BLUETOOTH®, Near Field Communication (NFC), ZigBee), millimeter wave communications, and others. The wired network that the electronic device 1000 accesses may, for example, include Ethernet, SONET, circuit-switched networks (e.g., using components such as SS7, cable, and the like), and others.

[00150] Some of the subject matter and operations described in this specification can be implemented in digital electronic circuitry, or in computer software, firmware, or hardware, including the structures disclosed in this specification and their structural equivalents, or in combinations of one or more of them. Some of the subject matter described in this specification can be implemented as one or more computer programs, i.e., one or more modules of computer program instructions, encoded on a computer storage medium for execution by, or to control the operation of, data-processing apparatus. A computer storage medium can be, or can be included in, a computer-readable storage device, a computer-readable storage substrate, a random or serial access memory array or device, or a combination of one or more of them. Moreover, while a computer storage medium is not a propagated signal, a computer storage medium can be a source or destination of computer program instructions encoded in an artificially generated propagated signal. The computer storage medium can also be, or be included in, one or more separate physical components or media (e.g., multiple CDs, disks, or other storage devices).

[00151] Some of the operations described in this specification can be implemented as operations performed by a data processing apparatus on data stored on one or more computer-readable storage devices or received from other sources.

[00152] The term “data processing apparatus” encompasses all kinds of apparatus, devices, and machines for processing data, including by way of example a programmable processor, a computer, a system on a chip, or multiple ones, or combinations, of the foregoing. The apparatus can include special purpose logic circuitry, e.g., an FPGA (field programmable gate array) or an ASIC (application specific integrated circuit). The apparatus can also include, in addition to hardware, code that creates an execution environment for the computer program in question,

e.g., code that constitutes processor firmware, a protocol stack, a database management system, an operating system, a cross-platform runtime environment, a virtual machine, or a combination of one or more of them.

[00153] A computer program (also known as a program, software, software application, script, or code) can be written in any form of programming language, including compiled or interpreted languages, declarative or procedural languages, and it can be deployed in any form, including as a stand-alone program or as a module, component, subroutine, object, or other unit suitable for use in a computing environment. A computer program may, but need not, correspond to a file in a file system. A program can be stored in a portion of a file that holds other programs or data (e.g., one or more scripts stored in a markup language document), in a single file dedicated to the program, or in multiple coordinated files (e.g., files that store one or more modules, sub programs, or portions of code). A computer program can be deployed to be executed on one computer or on multiple computers that are located at one site or distributed across multiple sites and interconnected by a communication network.

[00154] Some of the processes and logic flows described in this specification can be performed by one or more programmable processors executing one or more computer programs to perform actions by operating on input data and generating output. The processes and logic flows can also be performed by, and apparatus can also be implemented as, special purpose logic circuitry, e.g., an FPGA (field programmable gate array) or an ASIC (application specific integrated circuit).

[00155] While the disclosure has been particularly shown and described with reference to specific embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from

the spirit and scope of the disclosure as defined by the appended claims. The scope of the disclosure is thus indicated by the appended claims and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.

CLAIMS

What is claimed is:

1. A system for *in vivo* imaging biological phenomena, comprising:

an excitation module, configured to emit a light beam;

an expansion module, configured to receive and adjust a width of the light beam;

a Bessel-beam forming unit (BFU), configured to receive and convert the adjusted light beam by the expansion module into a Bessel beam;

a beam splitter; and

a microlens array,

wherein the beam splitter is arranged between the BFU and an objective module, and is configured to transmit the adjusted light beam to the objective module and receive a returned light beam from the objective module, and to transmit the returned light beam to the microlens array; and the microlens array is configured to receive the returned light beam from the beam splitter.

2. The system according to claim 1, wherein the beam splitter is configured to transmit the adjusted light beam to the objective module in a manner that the adjusted light beam passes through the beam splitter by refraction, and the beam splitter is further configured to receive the returned light beam from the objective module and transmit by reflection the returned light beam to the microlens array.

3. The system according to claim 1 or claim 2, further comprising:

a tuning module, configured to receive and tune the Bessel beam in a manner that a diameter of the tuned light beam fits the objective module,

wherein the beam splitter is arranged between the tuning module and the objective module and configured to transmit the tuned light beam to the objective module.

4. The system according to any one of claims 1 to 3, further comprising:

a reflector, arranged in a light path of the light beam emitted by the excitation module and oriented to reflect the light beam to be received by the expansion module;

5. The system according to claim 3, further comprising:

a first adjustable reflector, arranged between BFU and the tuning module, and configured to receive the Bessel beam from BFU and reflect the Bessel beam from BFU to be received by the tuning module.

6. The system according to claim 5, further comprising:

a second reflector, arranged between the first adjustable reflector and the tuning module, and configured to receive the reflected Bessel beam from the first adjustable reflector and reflect the reflected Bessel beam from the first adjustable reflector to be received by the tuning module.

7. The system according to claim 6, wherein the first adjustable reflector and the second reflector are arranged in a manner that the arrangement of the first adjustable reflector and the second reflector reverses a light path of the Bessel beam from BFU.

8. The system according to any one of claims 1 to 7, wherein the light beam emitted by the excitation module travels in a light path being non-parallel to an observation axis of the objective module.

9. The system according to claim 3, wherein the expansion module comprises a first lens and a second lens, both of the first lens and the second lens being converging lenses with a first focus length of the first lens less than a second focus length of the second lens, wherein the first lens is spaced apart from the second lens by a distance equal to a summation of the first focus length and the second focus length.

10. The system according to claim 9, wherein the tuning module comprises a third lens and a fourth lens, both of the third lens and the fourth lens are converging lenses, and a ratio of a third focus length of the third lens to a fourth focus length of the fourth lens is predetermined so as to tune by the ratio the diameter of the tuned light beam to fit the objective module, wherein the third lens is spaced apart from the fourth lens by a distance equal to a summation of the third focus length and the fourth focus length.

11. The system according to any one of claims 1 to 10, wherein an initial diameter of the Bessel beam is determined by a distance between the BFU.

12. The system according to any one of claims 1 to 11, wherein the objective module comprises a single objective lens for both illumination and acquisition.

13. The system according to any one of claims 1 to 12, wherein the returned light beam from the objective module comprises fluorescence signals emitted by a specimen imaging by the objective module, and wherein the system is configured as a fluorescence microscopy system.

14. The system according to claim 13, wherein the excitation module comprises a titanium-sapphire laser that is tunable to emit red and near-infrared light in the range from 650 to 1600 nanometres and generates ultrashort pulses, and fluorophores stained in the specimen are excited to emit multiple photons by the tuned light beam transmitted by the beam splitter to the objective module.

15. The system according to any one of claims 1 to 14, wherein the microlens array comprises a plurality of lenses in a 2-dimentional array, and is configured to refract the returned light beam received from the beam splitter.

16. The system according to claim 15, further comprising a charge-coupled device (CCD), configured to receive the refracted light beam by the microlens array.

17. The system according to claim 16, further comprising a complementary metal–oxide–semiconductor (CMOS) camera, configured to resolve fluorescence signals detected by the CCD.

18. The system according to claim 17, wherein fluorescence signals are resolved by an inverse problem approach in a manner that information relating a depth axis of the specimen is shown in images by the CMOS camera.

19. The system according to claim 15, wherein the microlens array is tilted so as to be non-parallel to an incident direction of the returned light beam received from the beam splitter.

20. A method for *in vivo* imaging biological phenomena with the system of claim 1, the method comprising:

(i) recording a returned light beam from the specimen by the system at a first position to generate a first lightsheet along a first axis; and

preferably repeating step (i) for a second and optionally further position instead of the first position to generate a second and optionally further lightsheet, each of the first, second and optionally the further position along a second axis perpendicular to the first axis.