

PARALLEL SCANNING CONFOCAL MICROSCOPE AND PARALLEL SCANNING
CONFOCAL MICROSCOPY METHOD

Cross-Reference To Related Application

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[0001] This application claims the benefit of priority of Singapore patent application No. 10202302433R, filed 29 August 2023, the content of it being hereby incorporated by reference in its entirety for all purposes.

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Technical Field

[0002] Various embodiments relate to a parallel scanning confocal microscope and a parallel scanning confocal microscopy method.

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Background

[0003] Laser scanning confocal microscopy (LSCM) is an imaging technique that may acquire 2D images at different depths, enabling 3D image formation in high-resolution. It has wide scientific and industrial applications in life sciences, semiconductor inspection and materials science.

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[0004] However, traditional free-space LSCM is usually bulky and expensive. The free-space confocal light path is also sensitive to environmental vibration. Recently, fiber optics have been incorporated in LSCM due to the miniaturized size, dynamic and customizable form factor with absolute measurement readouts, stability to electromagnetic interference, excellent resolution and range, portability, multiplex possibility and being economical in value. Many of the fiber-based LSCM uses fiber combiner, which may introduce interference among different ports. Moreover, conventional LSCM, either free-space or fiber-based, still depends on point-by-point scanning to reconstruct a 2D/3D image which requires long acquisition time. For example, a typical conventional LSCM with a fiber circulator may only illuminate a single point each time on a target. Even by extending such

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existing microscope with multi-fiber cable input, a 2D array charge-coupled device (CCD) may be required to collect data.

[0005] Thus, there is a need for a confocal microscope with a more robust confocal light path and being operable to significantly reduce the image acquisition time, thereby addressing at least the problems mentioned above and enabling more real-time applications.

Summary

10 [0006] According to an embodiment, a parallel scanning confocal microscope is provided. The parallel scanning confocal microscope may include an optical circulator including a first port, a second port and a third port; a light source optically coupled to the first port of the optical circulator; an optical arrangement in optical communication with the second port of the optical circulator; an focusing lens in optical communication with the optical arrangement; and a photodetector optically coupled to the third port of the optical circulator. The light source may be configured to emit light towards the optical arrangement through the first port and the second port along an illumination path. The optical arrangement may be configured to direct the light as multiple optical beams towards the focusing lens along the illumination path. The focusing lens may be configured to focus the multiple optical beams into multiple illumination points to confocally and simultaneously illuminate a sample under observation by the parallel scanning confocal microscope along the illumination path. The focusing lens may further be configured to collect the light reflected from the sample confocally along a reflection path. The optical arrangement may further be configured to direct the reflected light towards the photodetector in a form of reflected photons through the second port and the third port along the reflection path.

[0007] According to an embodiment, a parallel scanning confocal microscopy method is provided. The method may include emitting light through a first port and a second port of an optical circulator along an illumination path, directing the light as multiple optical beams along the illumination path, focusing the multiple optical beams into multiple illumination points to confocally and simultaneously illuminate a sample under observation along the

illumination path, collecting the light reflected from the sample confocally along a reflection path, and directing the reflected light in a form of reflected photons through the second port and a third port of the optical circulator along the reflection path.

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Brief Description of the Drawings

[0008] 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 invention. In the following description, various embodiments of the invention are described with reference to the following drawings, in which:

[0009] FIG. 1 shows a schematic representative view of a parallel scanning confocal microscope (PSCM), according to various embodiments.

[0010] FIG. 2 shows a flow chart illustrating a parallel scanning confocal microscopy method, according to various embodiments.

[0011] FIG. 3 shows a schematic representation illustrating a PSCM with a first parallel scanning scheme, according to one example.

[0012] FIG. 4 shows a schematic representation illustrating a PSCM with a second parallel scanning scheme, according to one example.

[0013] FIG. 5A shows a schematic cross-sectional view of a collimator with three fibers collimated together in close proximity used in the PSCM of FIG. 4, according to one example.

[0014] FIG. 5B shows a schematic cross-sectional view of a collimator with four fibers collimated together in close proximity used in the PSCM of FIG. 4, according to a different example.

[0015] FIG. 6A shows an image of resolution test targets used by the United States air force, according to an example.

[0016] FIG. 6B shows an image of a specific part of the resolution test targets of FIG. 6A, obtained by the PSCM.

[0017] FIG. 7 shows an image of red blood cells from human blood sample, as obtained by the PSCM.

[0018] FIG. 8 shows an image of white blood cells from human blood sample, as obtained by the PSCM.

Detailed Description

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[0019] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and embodiments in which the invention may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the invention. Other embodiments may be utilized and structural, logical, and electrical changes may be made without departing from the scope of the invention. The various embodiments are not necessarily mutually exclusive, as some embodiments can be combined with one or more other embodiments to form new embodiments.

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[0020] 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.

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[0021] 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.

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[0022] In the context of various embodiments, the articles “a”, “an” and “the” as used with regard to a feature or element include a reference to one or more of the features or elements.

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[0023] In the context of various embodiments, the phrase “at least” may include “exactly” and a reasonable variance.

[0024] In the context of various embodiments, the term “about”, or interchangeably “approximately”, as applied to a numeric value encompasses the exact value and a reasonable variance.

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[0025] As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0026] As used herein, the expression “configured to” may mean “constructed to” or “arranged to”.

[0027] Various embodiments may provide a fiber-optic parallel scanning confocal microscope (PSCM) with single-pixel detector. The PSCM is miniaturized and may include light source, e.g. laser diodes or light emitting diodes (LEDs) with driver boards, single-mode or multi-mode fibers, fiber collimators, objective, galvo mirror or spatial light modulator (SLM), as well as single-pixel photodetectors. To minimize interference from illumination laser and increase the imaging robustness, fiber circulators are employed to combine illumination, collection and detection ports. Attributing to the stability and flexibility of the circulators, the PSCM has a more robust confocal light path. The PSCM may operate with two different novel scanning schemes, namely parallel scanning or simultaneous multiple point scanning, to improve imaging speed by significantly reducing the image acquisition time. The resolutions may be as high as sub-micron levels, which make the PSCM suitable for real-time applications in life science, semiconductor, dermatology, material, amongst others.

[0028] FIG. 1 shows a schematic representative view of a parallel scanning confocal microscope 100, according to various embodiments. The parallel scanning confocal microscope 100 may include an optical circulator 102 including a first port 104, a second port 106 and a third port 108; a light source 110 optically coupled (as denoted by a line 118) to the first port 104 of the optical circulator 102; an optical arrangement 112 in optical communication (as denoted by a line 120) with the second port 106 of the optical circulator 102; a focusing lens 114 in optical communication (as denoted by a line 122) with the optical arrangement 112; and a photodetector 116 optically coupled (as denoted by a line 124) to the third port 108 of the optical circulator 102. The light source 110 may be configured to emit light towards the optical arrangement 112 through the first port 104 and the second port 106 along an illumination path. The optical arrangement 112 may be configured to direct the light as multiple optical beams towards the focusing lens 114 along the illumination path. The focusing lens 114 may be configured to focus the multiple optical beams into multiple illumination points to confocally and simultaneously illuminate a sample under observation (not shown in FIG. 1) by the parallel scanning confocal microscope 100 along the illumination path. The focusing lens 114 may further be

configured to collect the light reflected from the sample confocally along a reflection path. The optical arrangement 112 may further be configured to direct the reflected light towards the photodetector 116 in a form of reflected photons through the second port 106 and the third port 108 along the reflection path.

5 [0029] In the context of various embodiments, the term “coupled” may mean connected to or in communication with, directly or indirectly. The focusing lens 114 may be any type of focusing lens, including objectives (objective lens), convex lenses, metasurfaces, and the like.

[0030] In various embodiments, the photodetector 116 may be a single-pixel
10 photodetector. In other embodiments, the photodetector 116 may include array detectors.

[0031] The light source 110 may include a non-coherent light source, preferably a light emitting diode (LED).

[0032] The parallel scanning confocal microscope 100 may essentially involve single-wavelength light source(s) and mechanical scanning. No spatial disperser may be required
15 since spectral information is not considered. The setup of the parallel scanning confocal microscope 100 may also be much simpler without spectral or time encoding/decoding.

[0033] In an embodiment, the optical arrangement 112 may be a single spatial light modulator (SLM) configured to modulate the light in forming the multiple optical beams along the illumination path. Here, the parallel scanning confocal microscope 100 may
20 further include a data acquisition unit in electrical communication with the photodetector 116. The data acquisition unit may be configured to acquire and process information based on the reflected photons to generate a reconstructed image of the sample. The single spatial light modulator may be configured to generate a plurality of time-varying coded patterns based on the light reflected from the sample at pre-determined sampling intervals. At each
25 pre-determined sampling interval, the photodetector 116 may be configured to detect the reflected photons including correlated light intensities between each time-varying coded pattern of the plurality of time-varying coded patterns and a target image of the sample. More specifically, each time-varying coded pattern of the plurality of time-varying coded patterns may form a row of an encoded matrix F, the target image may be represented by a
30 target matrix having a plurality of elements, each element of the target matrix representing a light intensity at a location on the sample and the target matrix being expanded to a vector

X. For M number of pre-determined sampling intervals forming a complete sampling cycle, the reflected photons may be represented by a measurement vector S, where $S = FX$, to facilitate the generation of the reconstructed image of the sample. The parallel scanning confocal microscope 100 may be configured to perform compressive sensing, and the reflected photons may be represented by a modified measurement vector S', where $S' = FB\theta$, to facilitate the generation of the reconstructed image of the sample, B being a known basis of X, and θ being a sparse vector. This embodiment provides an implementation scheme of parallel scanning to improve the imaging speed significantly by employing structural illumination using a spatial light modulator and compressive sensing-based image reconstruction. The image reconstruction may be carried out with a specific decoding algorithm. For example, compressive sensing (or minimization) algorithms may include but are not limited to off-the-shelf algorithm, orthogonal matching pursuit, ridge regression, gradient projection for sparse reconstruction, Nestorov's algorithm.

[0034] In an embodiment, the parallel scanning confocal microscope 100 may further include one or more additional optical circulators, each including a first port, a second port and a third port; and one or more additional photodetectors, each optically coupled to the third port of each of the one or more additional optical circulators. The light source 110 may include multiple output ports, each output port optically coupled to the first port of each of the one or more additional optical circulators. The optical arrangement 112 may further be in optical communication with the second port of each of the one or more additional optical circulators. For each of the one or more additional optical circulators, the optically coupled output port may be configured to emit light towards the optical arrangement 112 through the first port (of each additional optical circulator) and the second port (of each additional optical circulator) along the illumination path. For each of the one or more additional optical circulators, the optical arrangement 112 may further be configured to direct the reflected light towards the optically coupled additional photodetector through the second port (of each additional optical circulator) and the third port (of each additional optical circulator), along the reflection path, forming a part of the reflected photons. In this embodiment, the light source 110 may include a light emitting diode (LED) or a laser diode.

[0035] In an embodiment, the parallel scanning confocal microscope 100 may further include one or more additional optical circulators, each including a first port, a second port and a third port; one or more additional light sources, each optically coupled to the first port of each of the one or more additional optical circulators; and one or more additional photodetectors, each optically coupled to the third port of each of the one or more additional optical circulators. The optical arrangement 112 may further be in optical communication with the second port of each of the one or more additional optical circulators. For each of the one or more additional optical circulators, the optically coupled additional light source may be configured to emit light towards the optical arrangement 112 through the first port and the second port along the illumination path. For each of the one or more additional optical circulators, the optical arrangement 112 may further be configured to direct the reflected light towards the optically coupled additional photodetector through the second port (of each additional optical circulator) and the third port (of each additional optical circulator), along the reflection path, forming a part of the reflected photons. The light source 110 and the one or more additional light sources may be configured to emit the light simultaneously. In this embodiment, the light source and the one or more additional light sources each may include a light emitting diode (LED) or a laser diode.

[0036] In the abovementioned two preceding embodiments, each additional optical circulator, each additional photodetector and each additional light source may be respectively described in similar context with the optical circulator 102, the photodetector 116 and the light source 110 of FIG. 1. The optical arrangement 112 may include a collimator configured to collimate the light from or to de-collimate the reflected light to the second port of each of the optical circulator and the one or more additional optical circulators; and one or more scanning mirrors in optical communication with the collimator. The one or more scanning mirrors may include one or more galvo mirrors, one or more micro-electrical-mechanical system (MEMS) mirrors, or one or more polygon scanning mirrors. The parallel scanning confocal microscope 100 may further include a data acquisition unit in electrical communication with the photodetector 116 and the one or more additional photodetectors. The data acquisition unit may be configured to acquire and process information based on the reflected photons to generate an image of the sample.

[0037] The one or more additional photodetectors may include one or more additional single-pixel photodetectors, or one or more additional array detectors.

[0038] The abovementioned two preceding embodiments provide an implementation scheme of parallel scanning to improve the imaging speed significantly by employing multi-foci confocal parallel scanning with customized fiber collimator. Here, the implementation scheme of parallel scanning may be used for single-wavelength imaging. It provides N illumination points simultaneously and may reduce imaging time to 1/N. With this configuration, the need for image reconstruction may be avoided.

[0039] FIG. 2 shows a flow chart illustrating a parallel scanning confocal microscopy method 240, according to various embodiments. As shown in FIG. 2, at Step 242, light may be emitted through a first port (e.g. 104, FIG. 1) and a second port (e.g. 106, FIG. 1) of an optical circulator (e.g. 102, FIG. 1) along an illumination path. At Step 244, the light may be directed as multiple optical beams along the illumination path. At Step 246, the multiple optical beams may be focused into multiple illumination points to confocally and simultaneously illuminate a sample under observation along the illumination path. At Step 248, the light reflected from the sample may be collected confocally along a reflection path. At Step 250, the reflected light may be directed in a form of reflected photons through the second port and a third port (e.g. 108, FIG. 1) of the optical circulator along the reflection path.

[0040] The parallel scanning confocal microscopy method 240 may include the same or like elements or components as used in the parallel scanning confocal microscope 100 of FIG. 1, and as such, the like elements may be as described in the context of the parallel scanning confocal microscope 100 of FIG. 1, and therefore some corresponding descriptions are omitted here.

[0041] The method 240 may further include acquiring and processing information based on the reflected photons to generate an image of the sample.

[0042] In various embodiments, directing the light (at Step 244) as multiple optical beams along the illumination path may include modulating the light and forming the multiple optical beams along the illumination path.

[0043] Prior to the step of directing the reflected light at Step 250, the method 240 may further include generating a plurality of time-varying coded patterns based on the light reflected from the sample at pre-determined sampling intervals.

[0044] The method 240 may further include detecting, at each pre-determined sampling interval, the reflected photons. The reflected photons may include correlated light intensities between each time-varying coded pattern of the plurality of time-varying coded patterns and a target image of the sample. Each time-varying coded pattern of the plurality of time-varying coded patterns forms a row of an encoded matrix F . The target image may be represented by a target matrix having a plurality of elements, each element of the target matrix representing a light intensity at a location on the sample and the target matrix being expanded to a vector X . For M number of pre-determined sampling intervals forming a complete sampling cycle, the reflected photons are represented by a measurement vector S , where $S = FX$, to facilitate the generation and reconstruction of the image of the sample.

[0045] The method 240 may further include performing compressive sensing. The reflected photons may be represented by a modified measurement vector S' , where $S' = FB\theta$, to facilitate the generation and reconstruction of the image of the sample, B being a known basis of X , and θ being a sparse vector. θ may be reconstructed through a minimization algorithm. For example, the minimization algorithm may include a convex optimization algorithm or a greedy algorithm.

[0046] The minimization algorithm may be defined by:

$$\text{minimize } \|X\|_1 \text{ subject to } \|S - FB\theta\|_2 \leq \delta$$

where δ is a noise boundary reflective of a real practical application.

[0047] In other embodiments, the method 240 may further include providing one or more additional optical circulators, each including a first port, a second port and a third port; emitting light through the first port and the second port of each of the one or more additional optical circulators along the illumination path; and directing the reflected light through the second port and the third port of each of the one or more additional optical circulators, along the reflection path, forming a part of the reflected photons. Emitting the light through the first port and the second port of each of the one or more additional optical circulators and emitting the light through the first port and the second port of the optical circulator (Step 242) may be carried out simultaneously.

[0048] Directing the light as multiple optical beams along the illumination path (Step 244) may include collimating the light from the second port of each of the optical circulator and the one or more additional optical circulators. Directing the reflected light along the reflection path (Step 250) may include de-collimating the reflected light to the second
5 port of each of the optical circulator and the one or more additional optical circulators. This configuration may avoid the need for reconstruction of the image.

[0049] While the method described above is illustrated and described as a series of steps or events, it will be appreciated that any ordering of such steps or events are not to be interpreted in a limiting sense. For example, some steps may occur in different orders
10 and/or concurrently with other steps or events apart from those illustrated and/or described herein. In addition, not all illustrated steps may be required to implement one or more aspects or embodiments described herein. Also, one or more of the steps depicted herein may be carried out in one or more separate acts and/or phases.

[0050] Examples of the parallel scanning confocal microscope (e.g. 100 of FIG. 1) and the
15 parallel scanning confocal microscopy method (e.g. 240 of FIG. 2) will be described in more details below.

[0051] Two schemes of parallel scanning confocal microscope (PSCM) based on fiber circulator may be provided. The feature of fiber circulator is that light source goes into port 1 and travels to port 2, then from port 2 to port 3. Light cannot travel from port 1 to port 3
20 directly, bypassing port 2, which minimizes the interference from the light source.

[0052] FIG. 3 shows a schematic representation illustrating a PSCM 300 with a first parallel scanning scheme, according to one example. The PSCM 300 may include the same or like elements or components as those of the parallel scanning confocal microscope 100 of FIG. 1, and as such, the same ending numerals are assigned and the like elements may
25 be as described in the context of the parallel scanning confocal microscope 100 of FIG. 1, and therefore the corresponding descriptions are omitted here.

[0053] As seen in FIG. 3, a small LED 310 (non-coherent) with a driver board may be used as illumination source whose output is connected to Port 1 304 of a fiber circulator 302 and delivered to a spatial light modulator (SLM) 328 via Port 2 306, as denoted by a directional
30 arrow 311. The fiber circulator 302 may be a multimode optical fiber circulator. The SLM 328 may modulate or encode the single beam light 313 to several points 315 by controlling

the phases and structurally illuminate the sample 322 through an objective 314. Reflected photons may then be collected by the same objective 314 in a confocal way. The objective 314 may be coupled to a z-axis motor 330 to facilitate z-axis adjustments. Those photons may go back to Port 2 306 and travel to Port 3 308, denoted by a directional arrow 317.

5 Port 3 308 is connected to a receiver 316. Unlike conventional structural illumination scheme using a camera, a single-pixel photodetector (PD) may be used as the receiver 316. A data acquisition unit (DAQ) 326 may be configured to digitalize the analog signals from the PD 316. The original image 336 may be reconstructed (as denoted by a directional arrow 334) from the several coded signals/patterns 332a, 332b, 332c from the single-pixel
10 PD 316.

[0054] The following describes how compressive coding and decoding may be implemented for the PSCM 300 to reconstruct the image 336 from the coded signals/patterns 332a, 332b, 332c.

[0055] In this single-pixel imaging (SPI) system, the target image may be considered as a
15 two-dimensional matrix, and each element in the matrix represents the light intensity information at its corresponding location. This matrix may be expanded to a vector $\mathbf{X}$ with N unknown light intensities, and the time-varying patterns may be expressed by a matrix $\mathbf{F}$ with dimension $M \times N$. Each row of the encoding matrix is the pattern $\mathbf{F}(j)$ generated by the SLM 328 at each sampling step, and thus the correlated light intensities between the
20 encoding pattern and the target image may be recorded and may be mathematically expressed by the inner products of $\mathbf{F}(j)$ with $\mathbf{X}$, where j is the serial number of the element in the matrix. The unknown light intensities may be the light intensities from the sample 332 via the objective 314 of the microscope 300.

[0056] After a complete sampling cycle, M measurements may be obtained to form the
25 measurement vector $\mathbf{S}$ as follows:

$$\mathbf{S} = \mathbf{F}\mathbf{X}.$$

[0057] A complete orthogonal matrix may be first employed to construct an encoding-based SPI system, such as Hadamard basis and Fourier basis. Despite the fact that this method may reduce the sampling noise, this type of system requires N measurements to
30 reconstruct an N -dimensional image, N being the pixel number of an image.

[0058] When the number of measurements is smaller than the signal dimension ($M < N$), compressive sensing (CS) may be used here to decode the patterns 332a, 332b, 332c and reconstruct the original image 336 with high fidelity. CS indicates that a signal may be accurately recovered with high-probability with much fewer measurements by using its sparsity in some transform bases, after the signal is modulated with pseudo-random patterns. The efficiency of CS depends on the assumption that the signal $\mathbf{X}$ tends to be sparse in a known basis $\mathbf{B}$, such as JPEG algorithms. Thus, the sampling process may be modeled as follows after representing the target image with a basis $\mathbf{B}$ and sparse vector θ :

$$\mathbf{S} = \mathbf{B}\mathbf{B}\theta,$$

where the θ is usually called a K-sparse vector, which means only K elements in the vector are nonzeros.

[0059] The sparse vector may be efficiently reconstructed through l_1 minimization algorithms with an adequate number of measurements (M), and there may be two types of approaches to solve this optimization problem, including the convex optimization algorithms or greedy algorithms. However, it has been proved that pure l_1 minimization algorithms are vulnerable to noise, and thus a more general approach is usually applied to increase its robustness by introducing an additional l_2 minimization term:

$$\text{minimize } \|\mathbf{X}\|_1 \text{ subject to } \|\mathbf{S} - \mathbf{B}\mathbf{B}\theta\|_2 \leq \delta,$$

where δ is the acceptable noise boundary in the real applications.

[0060] The reconstructed accuracy may be ensured by minimizing the l_2 norm function of the measured signals and the signals resulting from the predicted signals, and the l_1 norm guarantees the sparse solution to satisfy the mentioned assumption. An image 336 may be reconstructed by exploring its sparsity in the pixel domain using the minimization of the total variation (TV) or the total curvature (TC) of the images, at least M -coded patterns 332a, 332b, 332c may be needed to fully recover/reconstruct the image 336, where $N > M > 0.25N$ (N is pixel numbers of an image). Hence, imaging time of $[(N-M) \times \text{sampling rate}]$ may be saved.

[0061] FIG. 4 shows a schematic representation illustrating a PSCM 400 with a second parallel scanning scheme, according to one example. The PSCM 400 may include the same or like elements or components as those of the parallel scanning confocal microscope 100 of FIG. 1, and as such, the same ending numerals are assigned and the like elements may

be as described in the context of the parallel scanning confocal microscope 100 of FIG. 1, and therefore the corresponding descriptions are omitted here.

[0062] As seen in FIG. 4, the PSCM 400 may include three or more sets of fiber circulators 402a, 402b, 402c. For each set, Port 1 404a, 404b, 404c may be connected to the laser diode 410a, 410b, 410c as a light source, Port 2 406a, 406b, 406c may connect to a collimator 438, and the reflected photons may be collected to a single-pixel photodetector 416a, 416b, 416c via Port 3 408a, 408b, 408c. The collimator 438 may be customized to collimate light beams from Port 2 406a, 406b, 406c of the two or more (e.g. three in illustrative FIG. 4) fiber circulators 402a, 402b, 402c into parallel rays 413.

[0063] FIG. 5A shows a schematic cross-sectional view of the collimator 438 with three fibers 437 collimated together in close proximity, according to one example. The three fibers 437 accommodated by the the collimator 438 may be arranged vertically or along a same plane with one another.

[0064] FIG. 5B shows a schematic cross-sectional view of a collimator 438' with four fibers 437' collimated together in close proximity, according to a different example. The four fibers 437' accommodated by the the collimator 438' may be arranged in an array form. Other examples (not shown in FIGS. 5A and 5B) with various number of fibers collimated by the collimator as well as different arrangements of each of the fibers with respect to an adjacent fiber within the collimator may be appreciated.

[0065] With reference to FIG. 4, the two or more light beams 413 from the collimator 438 may be directed onto the sample 432 through an objective 414 by a galvo 439, forming two or more focus points 419 on the sample 432. The objective 414 may be coupled to a z-axis motor 430 to facilitate z-axis adjustments. The reflected photons may be collected by the same objective 414 in a confocal way and reflect to the original point of the collimator 438, then transmitted to Port 3 408a, 408b, 408c through individual fiber circulators 402a, 402b, 402c. Photons collected by the photodetectors 416a, 416b, 416c may be analysed in a DAQ 426. The two or more focus points 419 may enable parallel scanning of the sample 432. The imaging time may be shortened by two or more times and no complex algorithm may be needed for the construction of image. Moreover, the number of parallel scanning points may be highly customizable, based on different applications. In general, more parallel scanning points provides faster imaging speed but with higher system cost.

[0066] Preliminary trials were conducted to evaluate the performance of the PSCM (e.g. 300, 400 of FIGS. 3 and 4).

[0067] FIG. 6A shows an image of resolution test targets 601 used by the United States air force, according to an example. FIG. 6B show an image 603 of a specific part of the resolution test targets 601 of FIG. 6A, obtained by the PSCM 300 with the first parallel scanning scheme. The calculated lateral resolution is about 462 nm.

[0068] FIG. 7 shows an image 705 of red blood cells from human blood sample, as obtained by the PSCM 300 with the first parallel scanning scheme. FIG. 8 shows an image 807 of white blood cells from human blood sample, as obtained by the PSCM 300 with the first parallel scanning scheme.

[0069] The preliminary trials endorse the performances of the PSCM, which is miniaturized, robust and based on the use of fiber circulator with minimal cross interference. A majority of free-space optical components may be removed by using fiber circulator and fiber bundles. Using fiber circulator instead of fiber combiner/coupler advantageously improves signal quality and minimize interference, while achieving high speed parallel scanning through fiber components.

[0070] While the invention 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 invention as defined by the appended claims. The scope of the invention 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

1. A parallel scanning confocal microscope comprising:
 - an optical circulator including a first port, a second port and a third port;
 - 5 a light source optically coupled to the first port of the optical circulator;
 - an optical arrangement in optical communication with the second port of the optical circulator;
 - an focusing lens in optical communication with the optical arrangement;
 - and
 - 10 a photodetector optically coupled to the third port of the optical circulator, wherein
 - the light source is configured to emit light towards the optical arrangement through the first port and the second port along an illumination path,
 - 15 the optical arrangement is configured to direct the light as multiple optical beams towards the focusing lens along the illumination path,
 - the focusing lens is configured to focus the multiple optical beams into multiple illumination points to confocally and simultaneously illuminate a sample under observation by the parallel scanning confocal microscope along the illumination path,
 - 20 the focusing lens is further configured to collect the light reflected from the sample confocally along a reflection path, and
 - the optical arrangement is further configured to direct the reflected light towards the photodetector in a form of reflected photons through the
 - 25 second port and the third port along the reflection path.
2. The parallel scanning confocal microscope as claimed in claim 1, wherein the photodetector is a single-pixel photodetector.
- 30 3. The parallel scanning confocal microscope as claimed in claim 1, wherein the photodetector comprises array detectors.

4. The parallel scanning confocal microscope as claimed in any one of claims 1 to 3, wherein the light source comprises a non-coherent light source, preferably a light emitting diode.
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5. The parallel scanning confocal microscope as claimed in claim 4, wherein the optical arrangement is a single spatial light modulator configured to modulate the light in forming the multiple optical beams along the illumination path.
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6. The parallel scanning confocal microscope as claimed in claim 5, further comprising a data acquisition unit in electrical communication with the photodetector, wherein the data acquisition unit is configured to acquire and process information based on the reflected photons to generate a reconstructed image of the sample.
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7. The parallel scanning confocal microscope as claimed in claim 6, wherein the single spatial light modulator is configured to generate a plurality of time-varying coded patterns based on the light reflected from the sample at pre-determined sampling intervals.
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8. The parallel scanning confocal microscope as claimed in claim 7, wherein at each pre-determined sampling interval, the photodetector is configured to detect the reflected photons comprising correlated light intensities between each time-varying coded pattern of the plurality of time-varying coded patterns and a target image of the sample.
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9. The parallel scanning confocal microscope as claimed in claim 8, wherein each time-varying coded pattern of the plurality of time-varying coded patterns forms a row of an encoded matrix F,

the target image is represented by a target matrix having a plurality of elements, each element of the target matrix representing a light intensity at a location on the sample and the target matrix being expanded to a vector X ,

for M number of pre-determined sampling intervals forming a complete sampling cycle, the reflected photons are represented by a measurement vector S , where $S = FX$, to facilitate the generation of the reconstructed image of the sample.

10. The parallel scanning confocal microscope as claimed in claim 9, wherein the parallel scanning confocal microscope is configured to perform compressive sensing, and the reflected photons are represented by a modified measurement vector S' , where $S' = FB\theta$, to facilitate the generation of the reconstructed image of the sample, B being a known basis of X , and θ being a sparse vector.

11. The parallel scanning confocal microscope as claimed in any one of claims 1 to 3, further comprising:

one or more additional optical circulators, each including a first port, a second port and a third port; and

one or more additional photodetectors, each optically coupled to the third port of each of the one or more additional optical circulators,

wherein the light source comprises multiple output ports, each output port optically coupled to the first port of each of the one or more additional optical circulators,

wherein the optical arrangement is further in optical communication with the second port of each of the one or more additional optical circulators,

wherein for each of the one or more additional optical circulators, the optically coupled output port is configured to emit light towards the optical arrangement through the first port and the second port along the illumination path, and

wherein for each of the one or more additional optical circulators, the optical arrangement is further configured to direct the reflected light

towards the optically coupled additional photodetector through the second port and the third port, along the reflection path, forming a part of the reflected photons.

- 5 12. The parallel scanning confocal microscope as claimed in claim 11, wherein the light source comprises a light emitting diode or a laser diode.
13. The parallel scanning confocal microscope as claimed in any one of claims 1 to 3, further comprising:
- 10 one or more additional optical circulators, each including a first port, a second port and a third port;
- one or more additional light sources, each optically coupled to the first port of each of the one or more additional optical circulators; and
- one or more additional photodetectors, each optically coupled to the third
- 15 port of each of the one or more additional optical circulators,
- wherein the optical arrangement is further in optical communication with the second port of each of the one or more additional optical circulators,
- wherein for each of the one or more additional optical circulators,
- 20 the optically coupled additional light source is configured to emit light towards the optical arrangement through the first port and the second port along the illumination path,
- wherein for each of the one or more additional optical circulators,
- the optical arrangement is further configured to direct the reflected light
- 25 towards the optically coupled additional photodetector through the second port and the third port, along the reflection path, forming a part of the reflected photons, and
- wherein the light source and the one or more additional light sources are configured to emit the light simultaneously.

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14. The parallel scanning confocal microscope as claimed in claim 13, wherein the light source and the one or more additional light sources each comprises a light emitting diode or a laser diode.
- 5 15. The parallel scanning confocal microscope as claimed in any one of claims 11 to 14, wherein the one or more additional photodetectors comprise one or more additional single-pixel photodetectors, or one or more additional array detectors.
- 10 16. The parallel scanning confocal microscope as claimed in any one of claims 11 to 15, wherein the optical arrangement comprises
a collimator configured to collimate the light from or to de-collimate the reflected light to the second port of each of the optical circulator and the one or more additional optical circulators; and
one or more scanning mirrors in optical communication with the collimator.
- 15 17. The parallel scanning confocal microscope as claimed in claim 16, wherein the one or more scanning mirrors comprise one or more galvo mirrors, one or more micro-electrical-mechanical system (MEMS) mirrors, or one or more polygon scanning mirrors.
- 20 18. The parallel scanning confocal microscope as claimed in claim 16 or 17, further comprising a data acquisition unit in electrical communication with the photodetector and the one or more additional photodetectors, wherein the data acquisition unit is configured to acquire and process information based on the reflected photons to generate an image of the sample.
- 25 19. A parallel scanning confocal microscopy method comprising:
emitting light through a first port and a second port of an optical circulator along an illumination path,
30 directing the light as multiple optical beams along the illumination path,

focusing the multiple optical beams into multiple illumination points to confocally and simultaneously illuminate a sample under observation along the illumination path,

5 collecting the light reflected from the sample confocally along a reflection path, and

directing the reflected light in a form of reflected photons through the second port and a third port of the optical circulator along the reflection path.

20. The parallel scanning confocal microscopy method as claimed in claim 19, further
10 comprising acquiring and processing information based on the reflected photons to generate an image of the sample.

21. The parallel scanning confocal microscopy method as claimed in claim 20, wherein
15 the step of directing the light comprises modulating the light and forming the multiple optical beams along the illumination path.

22. The parallel scanning confocal microscopy method as claimed in claim 20 or 21,
further comprising prior to the step of directing the reflected light, generating a
20 plurality of time-varying coded patterns based on the light reflected from the sample at pre-determined sampling intervals.

23. The parallel scanning confocal microscopy method as claimed in claim 22, further
comprising detecting, at each pre-determined sampling interval, the reflected
25 photons, wherein the reflected photons comprise correlated light intensities between each time-varying coded pattern of the plurality of time-varying coded patterns and a target image of the sample.

24. The parallel scanning confocal microscopy method as claimed in claim 23, wherein
30 each time-varying coded pattern of the plurality of time-varying coded patterns forms a row of an encoded matrix F,

the target image is represented by a target matrix having a plurality of elements, each element of the target matrix representing a light intensity at a location on the sample and the target matrix being expanded to a vector X ,

for M number of pre-determined sampling intervals forming a complete sampling cycle, the reflected photons are represented by a measurement vector S , where $S = FX$, to facilitate the generation and reconstruction of the image of the sample.

25. The parallel scanning confocal microscopy method as claimed in claim 24, further comprising performing compressive sensing, wherein the reflected photons are represented by a modified measurement vector S' , where $S' = FB\theta$, to facilitate the generation and reconstruction of the image of the sample, B being a known basis of X , and θ being a sparse vector.

26. The parallel scanning confocal microscopy method as claimed in claim 25, wherein θ is reconstructed through a minimization algorithm.

27. The parallel scanning confocal microscopy method as claimed in claim 26, wherein the minimization algorithm comprises a convex optimization algorithm or a greedy algorithm.

28. The parallel scanning confocal microscopy method as claimed in claim 26 or 27, wherein the minimization algorithm is defined by:

$$\text{minimize } \|X\|_1 \text{ subject to } \|S - FB\theta\|_2 \leq \delta$$

where δ is a noise boundary reflective of a real practical application.

29. The parallel scanning confocal microscopy method as claimed in claim 19 or 20, further comprising:

providing one or more additional optical circulators, each including a first port, a second port and a third port;

emitting light through the first port and the second port of each of the one or more additional optical circulators along the illumination path; and

directing the reflected light through the second port and the third port of each of the one or more additional optical circulators, along the reflection path,
5 forming a part of the reflected photons,

wherein the step of emitting the light through the first port and the second port of each of the one or more additional optical circulators and the step of emitting the light through the first port and the second port of the optical circulator are carried out simultaneously.

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30. The parallel scanning confocal microscopy method as claimed in claim 29, wherein the step of directing the light as multiple optical beams along the illumination path comprises collimating the light from the second port of each of the optical circulator and the one or more additional optical circulators; and the step of directing the reflected light along the reflection path comprises de-collimating the reflected
15 lighted to the second port of each of the optical circulator and the one or more additional optical circulators.