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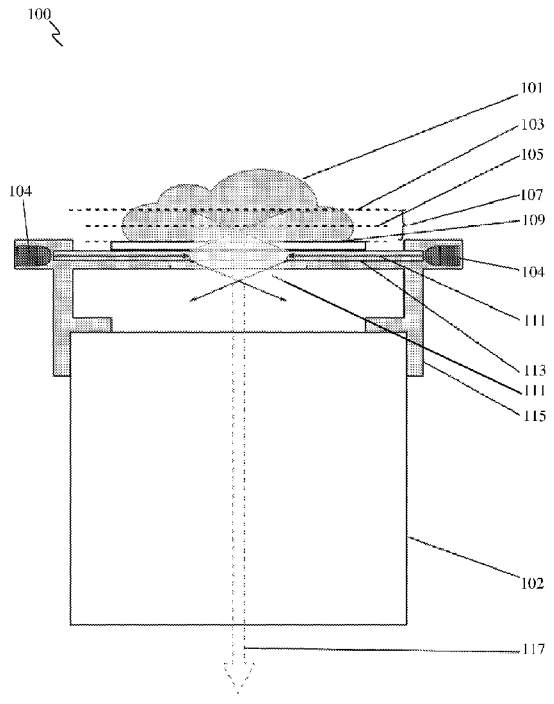

FIG. 1

(57) Abstract: According to embodiments of the present invention, a microscopy apparatus for superficial imaging of a sample is provided. The microscopy apparatus includes an objective lens; a surface illuminator configured to illuminate into the sample at a depth less than or equal to a depth of field of the objective lens; and a stage module configured to receive, adjust and position the sample for repeatable imaging. The surface illuminator includes a light source configured to emit light to illuminate the sample, the light source arranged adjacent to and on a same side as the objective lens; and an adjustment mechanism configured to adjust an angle and/or an intensity of the light directed at the sample. According to further embodiments, a method for superficial imaging of a sample, a surface illuminator, and a stage module are also provided.

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MICROSCOPY APPARATUS AND METHOD FOR SUPERFICIAL IMAGING OF A SAMPLE

Cross-Reference To Related Application

[0001] This application claims the benefit of priority of Singapore patent application No. 10202302761V, filed 28 September 2023, the content of it being hereby incorporated by reference in its entirety for all purposes.

Technical Field

[0002] Various embodiments relate to a microscopy apparatus and a method for superficial imaging of a sample. Other embodiments relate to a surface illuminator in operable communication with a stage module of a microscopy apparatus. Yet further embodiments relate to a stage module in operable communication with a surface illuminator of a microscopy apparatus.

Background

[0003] Microscopic imaging of thick biological samples at high levels of magnification is challenging. High magnification objectives (typically 40 times or greater) have a small depth of focus (under 1 μm) and small fields of view (0.2mm by 0.2mm).

[0004] Under naive illumination, the depth that the illumination penetrates the thick biological sample typically exceeds the depth of focus, and the signal returned by the out-of-focus light from the biological sample degrades the image quality. This is observed as a blurry image with out-of-focus spots obscuring parts of the desired image. Currently, these problems may be solved by physically cutting thick samples into very thin slices, scanning the object using confocal microscopy (e.g. imaging a single pixel at a time excluding out-of-focus light with a confocal pinhole), or restricting the depth of illumination to no more than the depth of focus. The first two mentioned methods are more typical but are complex, time-consuming, and rely on expensive equipment. The first method relies on physical

sectioning. The second method relies on optical sectioning, by removing out-of-focus light. The third method also utilises optical sectioning, such that the only part of the tissue that is illuminated is within sharp focus in the image. However, it is challenging to accurately reduce the depth of illumination in biological samples to be less than the small depth of field of a high-powered objective in a simple yet cost-efficient manner. For example, light-sheet microscopy is an existing optical sectioning method that forms the light into a sheet thinner than the depth of field of an imaging system and illuminates the sample perpendicular to the optical axis. It may require lasers and specific optical properties of the sample under test to work. Such requirements cause limitations to certain uses, and thus light-sheet microscopy may not be suitable for general use.

[0005] Further, imaging a sample requires accurately positioning the stage. Under high magnification, the field of view is small, and the required accuracy is correspondingly greater. Currently, this accuracy may be achieved through mechanical designs that are inherently accurate but expensive to produce. With robotic control, it is possible to achieve the required accuracy using repeatable mechanical design and correcting for the deviation in software.

[0006] Thus, there is a need for improved illumination and more economical approaches for stage positioning of a microscopy apparatus or system, that address at least the problems mentioned above.

Summary

[0007] According to an embodiment, a microscopy apparatus for superficial imaging of a sample is provided. The microscopy apparatus includes an objective lens; a surface illuminator configured to illuminate into the sample at a depth less than or equal to a depth of field of the objective lens; and a stage module configured to receive, adjust and position the sample for repeatable imaging. The surface illuminator includes a light source configured to emit light to illuminate the sample, the light source arranged adjacent to and on a same side as the objective lens; and an adjustment mechanism configured to adjust an angle and/or an intensity of the light directed at the sample.

[0008] According to an embodiment, a method for superficial imaging of a sample is provided. The method includes illuminating into the sample at a depth less than or equal to a depth of field of an objective lens of a microscopy apparatus; and adjusting and positioning the sample for repeatable imaging. Illuminating into the sample may include emitting light from a light source to illuminate the sample, the light source arranged adjacent to and on a same side as the objective lens; and adjusting an angle and/or an intensity of the light directed at the sample.

[0009] According to an embodiment, a surface illuminator in operable communication with a stage module of a microscopy apparatus for receiving, adjusting and positioning a sample is provided. The surface illuminator includes a light source configured to emit light to illuminate the sample, the light source adapted to be arranged adjacent to and on a same side as an objective lens of the microscopy apparatus; and an adjustment mechanism configured to adjust an angle and/or an intensity of the light directed at the sample. The surface illuminator may be configured to illuminate into the sample at a depth less than or equal to a depth of field of the objective lens.

[0010] According to an embodiment, a stage module in operable communication with a surface illuminator of a microscopy apparatus for illuminating into a sample at a depth less than or equal to a depth of field of an objective lens of the microscopy apparatus is provided. The stage module may be configured to receive, adjust and position the sample for repeatable imaging.

Brief Description of the Drawings

[0011] 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:

[0012] FIG. 1 shows a schematic side view of a microscope setup illustrating the basic components of an illumination path, according to an example.

[0013] FIG. 2 shows a schematic representation of a microscopy apparatus for superficial imaging of a sample, according to various embodiments.

[0014] FIG. 3 shows a flow chart illustrating a method for superficial imaging of a sample, according to various embodiments.

[0015] FIGS. 4A to 4F show schematic representations of a microscope setup (in parts), illustrating six degrees of freedom that may be required when adjusting an illuminator, according to an example.

[0016] FIG. 5 shows a schematic side view of a flexure mechanism design, according to one example.

[0017] FIG. 6 shows a schematic side view of a leaf-and-slider mechanism design, according to one example.

[0018] FIG. 7A shows a schematic side view of an exemplary slider with three sliding joints arranged spaced apart from each other along the slider, according to one example.

[0019] FIG. 7B shows a schematic side view of an exemplary slider with a sloped sliding joint, according to another example.

[0020] FIG. 7C shows a schematic side view of an exemplary slider with a wavy sliding joint, according to yet another example.

[0021] FIG. 8 shows a schematic side view of a leaf-and-slider mechanism design, according to another example.

[0022] FIG. 9 shows a schematic side view of a leaf-and-slider mechanism design of FIG. 6 with an actuator, according to a further example.

[0023] FIG. 10 shows a schematic side view of a leaf-and-slider mechanism design of FIG. 8 with a rotating slider operably using an actuator, according to an example.

[0024] FIG. 11 shows a schematic side view of a leaf-and-slider mechanism design of FIG. 8 with a rotating slider operably using an actuator, according to another example.

[0025] FIG. 12 shows a schematic top view of a leaf-and-slider mechanism using an annular ring and leaf mechanism to control the angle of the light source, according to an example.

[0026] FIG. 13 shows a schematic cross-sectional side view of FIG. 12 as seen from line A-A'.

[0027] FIGS. 14A and 14B show schematic side views of a microscope setup, comparatively illustrating over-penetrating illumination due to large exit angle and minimized-penetration illumination using depressed-angle illumination, respectively, according to an example.

[0028] FIG. 15 shows a schematic top view of a single-piece waveguide coupled to a light source, according to an example.

[0029] FIG. 16A shows a schematic side view of illuminating a sample placed on a sample holder or plate through a waveguide having a straight-wall exit feature, according to one example.

[0030] FIG. 16B shows a schematic side view of illuminating a sample placed on a sample holder or plate through a waveguide having an exit feature with a positive draft angle, according to one example.

[0031] FIG. 16C shows a schematic side view of illuminating a sample placed on a sample holder or plate through a waveguide having an exit feature with a negative draft angle for depressed-angled illumination, according to one example.

[0032] FIG. 17A shows a representative diagram illustrating the deviation varied across the span of a stage, i.e. before correction, according to an example.

[0033] FIG. 17B shows a representative diagram illustrating the compensation being carried on the deviation (errors) seen in FIG. 17A, i.e. after correction, according to an example.

[0034] FIG. 18 shows an exemplary mechanism to allow a pair of low-precision linear motion parts to be used in parallel without binding by rigidly affixing one rail, while permitting small and repeatable deflections of the other rail.

[0035] FIGS. 19A and 19B show exploded schematic top views of a detachable microscope stage design, according to an example.

[0036] FIG. 19C shows a schematic top view of the detachable microscope stage design of FIGS. 19A and 19B, when assembled.

Detailed Description

[0037] 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.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] In the context of various embodiments, the phrase "at least" may include "exactly" and a reasonable variance.

[0042] As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items.

[0043] As used herein, the expression "configured to" may mean "constructed to" or "arranged to".

[0044] Various embodiments provide methods for illumination and stage design for high magnification superficial imaging microscopy. More specifically, the microscope stage and the methods of illumination enable the inexpensive imaging of thick biological samples at high magnification. FIG. 1 shows a schematic side view of a microscope setup 100

illustrating the basic components of an illumination path 111, according to an example. As seen in FIG. 1, light may be delivered, via a waveguide 113, onto the surface of the biological sample 101 from outside the objective path on the same side as the objective (or interchangeably referred to as objective lens) 102. The sample 101 may be placed on a sample holder or plate 109 which may in turn be placed on top of or over the waveguide 113. The waveguide 113 and the light source(s) 104 may be coupled to an adaptor mount 115 placed over the objective 102. The adapter mount 115 may be part of a stage module (not shown in FIG. 1). The light source(s) 104 may be specifically positioned to ensure the depth of illumination is restricted to the most superficial layer of tissue of the sample 101 such that the depth of illumination is less than the depth of field 107 of the objective 102 (typically, only a few micrometres). The maximum depth of illumination 103 may be beyond the focal plane 105 of the objective 102. A clear and sharp high magnification image 117 may be obtained.

[0045] A series of opto-mechanical designs to precisely position the light source (e.g. 104) will be discussed in the various embodiments presented herein.

[0046] Further, positioning a stage to an accuracy sufficient for high-magnification imaging is expensive to achieve mechanically. Thus, a series of methods to make an inexpensive robotic microscope stage perform repeatable (but not necessarily accurate) imaging, in a way that may be corrected for in software, will also be discussed.

[0047] FIG. 2 shows a schematic representation of a microscopy apparatus 200 for superficial imaging of a sample (e.g. 101, FIG. 1), according to various embodiments. As shown in FIG. 2, the microscopy apparatus 200 includes an objective lens 202; a surface illuminator 208 configured to illuminate into the sample at a depth less than or equal to a depth of field (e.g. 107, FIG. 1) of the objective lens 202; and a stage module 210 configured to receive, adjust and position the sample for repeatable imaging (e.g. 117, FIG. 1). The surface illuminator 208 and the stage module 210 may work in co-operation with each other (as denoted by a line 216) to perform high magnification superficial imaging. The surface illuminator 208 includes a light source 204 configured to emit light to illuminate the sample, the light source 204 arranged adjacent to and on a same side as the objective lens 202; and an adjustment mechanism 206 configured to adjust an angle and/or an intensity of the light directed at the sample (as represented by a line 214). The

configuration of the microscopy apparatus 200 provides for light sheet-free optical sectioning which refers to optical sectioning in absence of (without using) light sheet. That is to say, in various embodiments, light sheet(s) are not used for optical sectioning. The light source and correspondingly, the light may be adjusted at multiple degrees of freedom. The objective lens 202 may be optically coupled to the surface illuminator 208, as represented by a line 212.

[0048] In other words, there is provided a method of obtaining sharp high magnification images of thick biological samples without the need for a confocal pinhole or physical sectioning of samples via controlled near-horizontal angled illumination from a light guide. Precise microscopy illumination control may be used to reduce the depth of illumination into the biological samples via optical sectioning, such that the depth of illumination is less than the depth of field of the in-use high magnification objective. This may involve cost-effective stage designs as well as methods to precisely position and direct the illumination source (e.g. light source 204), which is arranged on the same side as the objective lens with respect to a sample holder, thereby configured in a reflected illumination mode.

[0049] In the context of various embodiments, the expression "superficial imaging" may refer to surface imaging or near-surface imaging.

[0050] To "adjust an angle and/or an intensity of the light directed at the sample" may mean changing the angle of the light (or the light source), or changing the distance between the light source and the sample, or changing the orientation of the light source with respect to the sample, or any combinations thereof.

[0051] The microscopy apparatus 200 may include the same or like elements or components as those of the microscope setup 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 microscope setup 100 of FIG. 1, and therefore the corresponding descriptions may be omitted here.

[0052] In an aspect, various embodiments provide a surface illuminator. The surface illuminator may be in operable communication (or configured to work in cooperation) with a stage module (e.g. 210, FIG. 2) of a microscopy apparatus (e.g. 200, FIG. 2) for receiving, adjusting and positioning a sample (e.g. 101, FIG. 1). The surface illuminator may be described in a similar context with the surface illuminator 208 of FIG. 2, and include a light

source (e.g. 204, FIG. 2) configured to emit light to illuminate the sample, the light source adapted to be arranged adjacent to and on a same side as an objective lens (e.g. 202, FIG. 2) of the microscopy apparatus; and an adjustment mechanism (e.g. 206, FIG. 2) configured to adjust an angle and/or an intensity of the light directed at the sample, wherein the surface illuminator is configured to illuminate into the sample at a depth less than or equal to a depth of field of the objective lens. The configuration of the surface illuminator provides for light sheet-free optical sectioning.

[0053] It should be appreciated that the surface illuminator may or may not be used with the stage module.

[0054] In various embodiments, the light source 204 may include a light emitting diode, or a laser.

[0055] The adjustment mechanism 206 may include a waveguide.

[0056] In an embodiment, the waveguide may include a fibre-optic cable including a light-exiting end arrangeable towards the sample, and the adjustment mechanism 206 further may include one or more flexure mechanisms. Each flexure mechanism may include a clamp adapted to rigidly hold the fibre-optic cable towards (along or near) the light-exiting end; a base opposite to the clamp; a flexure segment extending between the clamp and the base; and an arm extending from the flexure segment. More specifically, a first end of the arm may be coupled to or attached to or shaped from the flexure segment, and a second end of the arm, that is opposite to the first end, may facilitate an actuating/adjustment member or a user of the microscopy apparatus to control deflection of the flexure segment. For example, the arm may be coupled to or linked to the base (via the actuating member) and configured to move towards or away from the base (by adjusting the actuating member) to facilitate deflection of the flexure segment. The actuating member may include an adjustment screw. The base may be a fixed base affixed to part of the microscopy apparatus 200.

[0057] Each flexure mechanism may be made of a single piece of material. For example, each flexure mechanism may be a unitary component or an integrated assembly of separate parts (e.g. clamp, base and arm).

[0058] The flexure segment may include a continuously curved flexure segment configured to produce deflection upon actuation (movement) of the arm. The deflection

may be preserved, reduced or magnified, depending on the geometry or construction of the arm.

[0059] Each flexure mechanism may further include an angular adjustment member arranged between the arm and the base operable to actuate the arm to deflect the flexure segment and adjust an angle of the clamp and consequently, the light directed at the sample.

[0060] Each flexure mechanism may further include a level adjustment member mountable to the base operable to adjust a level movement of the clamp, and collectively, the light-exiting end of the fibre-optic cable with respect to the sample.

[0061] One or more flexure mechanisms may include two or more flexure mechanisms arranged orthogonally or angularly apart from one another to provide adjustments along different axes or planes. For example, three flexure mechanisms may provide adjustments of the surface illuminator 208 in an x-axis direction, a y-axis direction and a z-axis direction. These adjustments may be made sequentially and in any order.

[0062] In a different embodiment, the adjustment mechanism 206 may include a leaf-slider mechanism. The leaf-slider mechanism may include a leaf having a first part and a second part spaced apart from the first part; and a slider being in contact with the leaf. The leaf may be coupled to the light source 204 at the first part in a manner such that when in operation, the light from the light source 204 is directed at the sample. The leaf may be pivotably coupled to an adjoining member at the second part. The adjoining member may be part of or provided by the leaf-slider mechanism. The slider may be coupled to the leaf via a preload force exerted therebetween.

[0063] The slider may be operable to translate or rotate along the leaf, allowing the leaf to pivot about the second part and consequently adjust a position and/or an orientation of the light source 204 relative to the sample.

[0064] For example, the slider may include one or more sliding joints arranged spatially apart from one another to form an arbitrary contour for translation along the leaf. Alternatively, the slider may include a sliding joint with a sloped geometry to form a smooth sloped contour for translation along the leaf. In yet another alternative, the slider may include a sliding joint with a wave geometry to form an irregular contour for translation along the leaf. In the context of various embodiments, the term “contour” may

mean an outline representing a polygon or a part thereof, optionally with curved edges of any shape.

[0065] The leaf-slider mechanism may further include a resilient member coupleable to the slider, the resilient member extending from the slider in a direction away from the leaf. For example, the resilient member may include a spring. The resilient member may be made of a shape-memory alloy.

[0066] In a different example, the slider may include an eccentric rotating element having a side cross-section with a circular shape, or an elliptical shape, or a polygonal shape for rotation along the leaf.

[0067] In another example, the adjoining member may include an inner annulus comprising outwardly extending threads; the slider may include an outer annulus comprising inwardly extending threads arranged in an interlocking manner with outwardly extending threads; and the leaf-slider mechanism may further include one or more further leaves arranged spatially apart from one another around the inner annulus and the outer annulus. Additional components may be configured to transmit motion from the outer annulus to the leaves. Such additional components may be arranged between the outer annulus and the leaves.

[0068] In these examples, the leaf-slider mechanism may further include an actuator coupleable to the slider and configured to operate the slider.

[0069] In an embodiment, the light source 204 may be configured to emit the light with an exit angle; and the adjustment mechanism 206 may be configured to adjust the light source 204 away from the sample with an azimuth angle less than the exit angle.

[0070] In a different embodiment, the waveguide may include one or more input features adapted to receive the light from the light source 204, the light being parallel to a major plane of the waveguide; and an exit feature adapted to direct the received light to exit onto the sample at a pre-determined angle. The pre-determined angle may be an appropriate angle to facilitate superficial imaging of the sample. The waveguide may further include one or more distribution features adapted to change a behaviour of the received light internally to correspondingly change an intensity and/or a distribution of the received light being directed to the exit feature. Each of the one or more distribution features may include an angled cut slot configured to facilitate internal reflection of the received light, or an

absorbent layer adapted to absorb a portion of the received light. For example, the waveguide may include a single plate of quartz, or polymethyl methacrylate. The waveguide may have curved edges or non-parallel edges.

[0071] In an aspect, various embodiments provide a stage module. The stage module may be in operable communication (or configured to work in cooperation) with a surface illuminator (e.g. 208, FIG. 2) of a microscopy apparatus (e.g. 200, FIG. 2) for illuminating into a sample (e.g. 101, FIG. 1) at a depth less than or equal to a depth of field (e.g. 107, FIG. 1) of an objective lens (e.g. 202, FIG. 2) of the microscopy apparatus.

[0072] The stage module may be described in a similar context with the stage module 210 of FIG. 2, and may be configured to receive, adjust and position the sample for repeatable imaging.

[0073] It should be appreciated that the stage module may or may not be used with the surface illuminator.

[0074] Multiple imprecise linear motion parts may be used in a microscope stage. In another example, the stage module 210 may include a rigid mount; a floating mount arranged spatially apart and alongside the rigid mount; a master rail affixed to the rigid mount; a slave rail coupled to the floating mount; a carriage plate coupled across the master rail and slave rail; and one or more pre-tensioner configured to exert a force on the slave rail in a direction substantially perpendicular or non-parallel to an axis of motion of the master rail, thereby allowing a repeatable deflection of the slave rail for each position of the carriage plate along the master rail and slave rail. Both the master rail and the slave rails may provide as a pair of low-precision linear motion parts.

[0075] For example, the rigid mount may include a steel shim stock. The floating mount may include a polytetrafluoroethylene shim stock. The low coefficient of friction between the slave rail and the polytetrafluoroethylene shim stock allows for the floating mount to float.

[0076] A design to repeatably position removable stages in parallel using kinematic couplings may be provided. In yet another embodiment, the stage module 210 may include a stage arranged between the sample and the objective lens 202; a static plate affixed to an effector arm of the stage; a replaceable plate configured to receive the sample and arrangeable parallel planarly over the static plate; at least two compressing mechanisms

configured to apply compression pressure between a parallel planar arrangement of the static plate and the replaceable plate; and a kinetic coupling mechanism configured to couple the static plate and the replaceable plate together under the compression pressure and facilitate repeatable positioning of the sample. The phrase "parallel planar" may mean the static plate and the replaceable plate inscribe planes that are substantially parallel and may or may not be displaced from each other.

[0077] The at least two compressing mechanisms may include at least two clasping mechanisms, without any clamping mechanism. In other examples, the at least two compressing mechanisms may include at least one clamping mechanism and at least one clasping mechanism.

[0078] The kinetic coupling mechanism may include a plurality of mating elements coupled to the static plate and a plurality of complementary elements coupled to the replaceable plate. The plurality of complementary elements may be configured to be correspondingly received by the plurality of mating elements.

[0079] The plurality of mating elements may be arranged spatially apart from one another, each mating element being positioned proximal to an edge of the static plate. The plurality of complementary elements may be arranged spatially apart from one another, each complementary element being positioned proximal to an edge of the replaceable plate. The plurality of mating elements and the plurality of complementary elements when mated may be each orientated with a first axis extending perpendicularly therefrom; and the at least two compressing mechanisms may be arranged spatially across the parallel planar arrangement, along a second axis coinciding with every first axis at a single point. The second axis may include a linear axis or a centroid axis When more than two compressing mechanisms are used, the sum of torques provided by the compressing mechanisms lies inside a convex hull of the assemblies of the plurality of mating elements and the plurality of complementary elements.

[0080] For example, the plurality of mating elements may include a plurality of rod pairs; or a plurality of bearings; or a mixture of rod pairs and bearings. the plurality of complementary elements may respectively include a plurality of complementary bearings; a plurality of complementary rod pairs; or a mixture of complementary bearings and complementary rod pairs.

[0081] A bearing may refer to a ball bearing or the like. A rod pair (or a complementary rod pair) refers to two elongate members placed longitudinally aside each other with a gap therebetween. The gap size may be substantially similar to the diameter of the ball bearing so that the ball bearing and rod pair may be releasably engaged and secured with each other. The gap is designed to form a kinematic coupling.

[0082] In various embodiments, the microscopy apparatus 200 may further include an image sensor coupleable to the objective lens 202 and configured to capture a superficial image of the sample; and a processor configured to receive the superficial image, wherein the received superficial image may be of repeatable positioning but not accurate positioning, estimate a positioning error based on the received superficial image, and correct the positioning error to obtain an accurate positioning.

[0083] FIG. 3 shows a flow chart illustrating a method 320 for superficial imaging of a sample (e.g. 101, FIG. 1), according to various embodiments. The microscopy apparatus used in the method 320 may include the same or like elements or components as those of the microscopy apparatus 200 of FIG. 2, and as such, the same numerals are assigned and the like elements may be as described in the context of the microscopy apparatus 200 of FIG. 2, and therefore the corresponding descriptions may be omitted here.

[0084] At Step 322, the sample may be illuminated into at a depth less than or equal to a depth of field of an objective lens 202 of a microscopy apparatus 200. At Step 324, the sample may be adjusted and positioned for repeatable imaging. Illuminating into the sample at Step 322 may include emitting light from a light source 204 to illuminate the sample, the light source arranged adjacent to and on a same side as the objective lens (Step 326); and adjusting an angle and/or an intensity of the light directed at the sample (Step 328). The method 320 performs light sheet-free optical sectioning of the sample.

[0085] In one embodiment, adjusting the angle and/or the intensity of the light directed at the sample at Step 328 may include rigidly holding a fibre-optic cable using a clamp of a flexure mechanism; arranging a light-exiting end of the fibre-optic cable towards the sample; and actuating deflection of a flexure segment of the flexure mechanism to adjust a position and/or an orientation of the light-exiting end.

[0086] The angle and/or the intensity of the light may be adjusted along different axes using two or more flexure mechanisms arranged orthogonally or angularly apart from one another.

[0087] In another embodiment, adjusting the angle and/or the intensity of the light directed at the sample at Step 328 may include providing a leaf-slider mechanism, which may include a leaf having a first part and a second part spaced apart from the first part, wherein the leaf may be coupled to the light source 204 at the first part and may be pivotably coupled to an adjoining member at the second part; and a slider being in contact with the leaf. The slider may be coupled to the leaf via a preload force exerted therebetween. The slider may be operated, e.g. translated or rotated along the leaf, to cause the leaf to pivot about the second part and consequently a position and/or an orientation of the light source may be adjusted relative to the sample. Optionally, operating the slider may be performed using an actuator coupled to the slider.

[0088] In a different embodiment, emitting the light from the light source at Step 326 may include emitting the light with an exit angle; and adjusting the angle and/or the intensity of the light directed at the sample at Step 328 may include adjusting the light source 204 away from the sample with an azimuth angle less than the exit angle.

[0089] The above discussed embodiments may be directed at free-space methods.

[0090] In yet another embodiment, adjusting the angle and/or the intensity of the light directed at the sample at Step 328 may include providing a waveguide including one or more input features, an exit feature and optionally, one or more distribution features; receiving, by the one or more input features, the light from the light source 204, the light being parallel to a major plane of the waveguide; directing, by the exit feature, the received light to exit onto the sample at a pre-determined angle; and optionally, changing, by the one or more distribution features, a behaviour of the received light internally to correspondingly change an intensity and/or a distribution of the received light being directed to the exit feature.

[0091] Changing the behaviour of the received light internally may include facilitating internal reflection of the received light by an angled cut slot of each distribution feature or absorbing a portion of the received light by an absorbent layer of each distribution feature. The waveguide may include a planar waveguide that provides for illumination to arrive

substantially parallel to a major surface of the waveguide. The shape of the waveguide need not have parallel sides. Curved edges or other shapes are possible for the waveguide.

[0092] In an embodiment, adjusting and positioning the sample for repeatable imaging at Step 324 may include adjusting and positioning the sample using a stage module including commodity parts mass-manufactured with standard dimensions; and custom-made parts affixed to the commodity parts, wherein the custom-made parts may include planar parts including two-dimensional features, or non-planar parts with lower rigidity as compared to the planar parts.

[0093] In a different embodiment, adjusting and positioning the sample for repeatable imaging at Step 324 may include using a stage module, which may include a rigid mount; a floating mount arranged spatially apart and alongside the rigid mount; a master rail affixed to the rigid mount; a slave rail coupled to the floating mount; a carriage plate coupled across the master rail and slave rail; and one or more pre-tensioner. A force may be exerted, via the one or more pre-tensioner, on the slave rail in a direction substantially perpendicular or non-parallel to an axis of motion of the master rail, thereby allowing a repeatable deflection of the slave rail for each position of the carriage plate along the master rail and slave rail.

[0094] In an alternative embodiment, adjusting and positioning the sample for repeatable imaging at Step 324 may include using a stage module, which may include a stage arranged between the sample and objective lens 202; a static plate affixed to an effector arm of the stage; a replaceable plate arrangeable parallel planarly over the static plate; at least two compressing mechanisms; and a kinetic coupling mechanism. The sample may be received on the replaceable plate. Compression pressure may be applied, by the at least two compressing mechanisms, between a parallel planar arrangement of the static plate and the replaceable plate. The static plate and the replaceable plate may be coupled together under the compression pressure, via the kinetic coupling mechanism, to facilitate repeatable positioning of the sample.

[0095] When coupling via the kinetic coupling mechanism, a plurality of mating elements coupled to the static plate may be provided, and a plurality of complementary elements coupled to the replaceable plate may be correspondingly received with or by the plurality of mating elements. The plurality of mating elements and the plurality of complementary

elements may be mated or coupled in a manner such that each mated mating element and complementary element may be orientated with a first axis extending perpendicularly therefrom; and the at least two compressing mechanisms may be arranged spatially across the parallel planar arrangement, along a second axis coinciding with every first axis at a single point. The second axis may include a linear axis or a centroid axis.

[0096] For example, adjusting and positioning the sample for repeatable imaging at Step 324 may include translating the stage module 210 along three axes, and rotating the light source 204; and adjusting the angle and/or the intensity of the light directed at the sample at Step 328 may include rotating the light along one rotational axis or two different rotational axes.

[0097] In various embodiments, the method 320 may further include capturing a superficial image of the sample; estimating a positioning error based on the captured superficial image; and correcting the positioning error.

[0098] 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 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.

[0099] Examples of illumination and stage movements for high magnification superficial imaging microscopy will be respectively described in more detail in Parts I and II below.

Part I - Surface illumination for superficial imaging

[0100] The stage described herein (e.g. the stage module 210 of FIG. 2) may be used for microscopy with surface illumination. All illumination may come from a light emitting didoe (LED), a laser source, or some other source and may be carried by a waveguide (such as a fibre-optic cable), free-space optical path, or some other mechanism to the sample (101, FIG. 1). Illumination may contain one or more wavelengths of light, including but not limited to ultraviolet (for MUSE microscopy). The final part of the assembly before free-space propagation to the target (sample) may be referred to as the illuminator (e.g. the

surface illuminator 208 of FIG. 2). A microscope (e.g. the microscopy apparatus 200 of FIG. 2) may contain one or more such illuminators. The surface illuminator described herein may be rotated in 1 axis or 2 axes, where in the case of multiple illuminators, the axes of rotation of each light may be different.

[0101] FIGS. 4A to 4F show schematic representations of a microscope setup (in parts) 400, illustrating six degrees of freedom that may be required when adjusting an illuminator 408, according to an example. The microscope setup 400 may include the same or like elements or components as those of the microscopy apparatus 200 of FIG. 2, and as such, the same ending numerals are assigned and the like elements may be as described in the context of the microscopy apparatus 200 of FIG. 2, and therefore the corresponding descriptions are omitted here.

[0102] The goal is for the illuminator 408 to be positioned such that the depth of illumination 403 where the illumination, that the illuminator 408 casts, extends into the sample 401 placed on a sample holder 425 only as far as the depth of field 407 of the lens 402 (see e.g. FIG. 4C).

[0103] More specifically, FIGS. 4A and 4B respectively show a side schematic view of the microscope setup 400, with excess penetration of the illumination, and a top schematic view of FIG. 4A showing the illumination missing the target (marked X). As seen in FIG. 4A, the pitch 421 and up-down movement 423 of the illuminator 408 may be adjusted. As seen in FIG. 4B, the yaw 427 and left-right movement 429 of the illuminator 408 may also be adjusted.

[0104] FIGS. 4C and 4D respectively show a side schematic view of the microscope setup 400, and a top schematic view of FIG. 4C with optimal illumination. The illuminator 408 centres its illumination on the spot (target X) being imaged with minimal spill over so as to minimize or avoid photobleaching, drying, or other thermal effects.

[0105] FIGS. 4E and 4F respectively show a side schematic view of the microscope setup 400, with excess spread, and a top schematic view of FIG. 4F showing polishing/surface treatment alignment. As seen in FIG. 4E, the forward-backward movement 431 of the illuminator 408 may be adjusted. As seen in FIG. 4F, the roll 433 (i.e. rotation about the center axis 435) of the illuminator 408 may also be adjusted.

[0106] In surface-illumination microscopy, the illuminator 408 may require any or all these degrees of freedom for optimal illumination.

[0107] The following specific examples describe a series of designs for manual and automated adjustment of the illuminator 408 in subsets of the axes (shown in FIGS. 4A to 4F), with the intention that these designs may be stacked to provide the required degrees of freedom for a particular embodiment.

Easy manual adjustment of illuminator positioning for surface illumination using a flexure mechanism with minimal mechanical hysteresis

[0108] Easy manual adjustment of fibre-optic cables may be provided for UV surface illumination using a flexure mechanism. FIG. 5 showing a schematic side view of a flexure mechanism 540 design that may include a clamp 544 that rigidly holds the fibre 542, a long continuously curved flexion segment 546 connecting it to a fixed base 548, a rigid arm 550 with an attached screw 552 allowing for angular adjustment by deflecting the flexure segment 546, and a vertical adjustment screw 554 mounted in the fixed base 548. The design may be manufactured as a single piece of some material with high tolerance for elastic deformation, such as plastic.

[0109] The flexure mechanism 540 controls the pitch angle of the fibre path using the adjustment screw 552, with the vertical adjustment screw 554 to control the and up/down position of the fibre 542. The long, continuously curved flexure segment 546 may allow the slight flexibility of the material to produce a relatively large deflection. By continuously curving the segment 546, the formation of stress concentration zones leading to premature failure of the part may be prevented or at least minimized. Further, the segment 546 may act as a spring, providing a balancing force against the screw 552 that greatly reduces mechanical hysteresis in adjustment, allowing for fine adjustment without static friction (i.e., "stiction"). The design may be inexpensively produced as a single piece through 3D-printing or similar manufacturing methods.

[0110] A similar mechanism may be constructed to provide for the other axes.

Automatic adjustment of illuminator angle for surface illumination using a leaf-and-slider mechanism

[0111] A leaf-and-slider mechanism may be provided for automated and motorized adjustment of the angular displacement of illuminator assemblies for the purpose of surface illumination. FIGS. 6 and 8 each show a schematic side view of the leaf-and-slider mechanism 660, 660' design, according to different examples. The leaf-and-slider mechanism 660, 660' design may include a rigidly affixed pivot joint 666 (e.g. coupled to a leaf support 670), a leaf 662 that pivots about that joint 666, some preload 661 that exerts a torque about that joint 666, and a slider 664 that translates 663 or a slider 664' that rotates 665. As the slider 664 translates 663, the slider joint 668 moves along the leaf 662, causing the leaf 662 to rotate about the pivot joint 666, and thus adjust the position of the light source 604 coupled to the leaf 662. In FIG. 8, as the slider 664' (in a form of an eccentric rotating element or a cam) rotates 665, the contact point moves along the slider 664' and/or the leaf 662, causing the leaf 662 to rotate about the pivot joint 666, and similar to FIG. 6, the position and/or angle of the light source 604 coupled to the leaf 662 may be adjusted. The slider 664, 664' may also translate 663 or rotate 665 along other axes, including out-of-plane axes. In other words, the sliding motion may include out-of-plane motion.

[0112] FIG. 7A to 7C shows schematic side view of exemplary sliders 664a, 664b, 664c, with different slider geometries for different effects. By adjusting the geometries of the leaf 662 and/or slider 664, 664a, 664b, 664c, a range of desired effects may be archieved, including but not limited to greater angular precision about some desired angles, pseudo-discrete angles (i.e. angles constrained to steps), amongst others.

[0113] For example, FIG. 7A shows a schematic side view of the exemplary slider 664a with three sliding joints 668a, 668b, 668c arranged spaced apart from each other along the slider 664a to provide an undulating contour for translation 663 along the leaf 662. FIG. 7B shows a schematic side view of another exemplary slider 664b with a sloped sliding joint 668' to provide a smooth sloped contour for translation 663 along the leaf 662. FIG. 7C shows a schematic side view of yet another exemplary slider 664b with a wavy sliding joint 668'' to provide an irregular contour for translation 663 along the leaf 662.

[0114] FIG. 9 shows a schematic side view of a leaf-and-slider mechanism 660 design of FIG. 6 with an actuator 672, according to a further example. The actuator 672 may include

a spring made from a shape memory alloy (such as nitinol) inside a tube to actuate the slider 664. The actuator 672 may be coupled to the slider 664 and when in operation, the slider 664 moves or translates, causing a preload spring force 661' to be experienced at the pivot joint 666. This mechanism is extremely compact, uses a significantly compact actuator, and allows for multiple leaves (not shown in FIG. 9) to be independently controlled.

[0115] FIGS. 10 and 11 each show a schematic side view of a leaf-and-slider mechanism 660' design of FIG. 8 with a rotating slider 664',664'' (e.g. cam) that may be operated using an actuator 674 coupled thereto, according to alternative examples. The rotating slider 664 of FIG. 10 and the rotating slider 664' of FIG. 11 provide non-limiting examples of arbitary slider geometries that may be employed. The actuator 674 may include an inexpensive off-the-shelf servo motor to provide the desired movement and may produce significantly precise angular deflection from extremely cheap parts. This may be less compact than the leaf-and-slider mechanism 660 design of FIGS. 6 and 9, but mulitple leaves may still be independently controlled.

[0116] FIG. 12 shows a schematic top view of a leaf-and-slider mechanism 1260 using an annular ring and leaf mechanism to control the angle of the light source 604, according to an example. FIG. 13 shows a schematic cross-sectional side view of FIG. 12 as seen from line A-A'. The leaf-and-slider mechanism 1260 may be similar to that as shown in FIG. 6 and some similar components or elements are referred to with the same numeral references. The leaf-and-slider mechanism 1260 may include a pair of annuluses (hollow tubes) 1264, 1270 with matching threads 1276. The outer annulus 1264 (i.e. the slider) may be rotated about the inner annulus 1270 (e.g. the leaves support), which also moves the outer annulus 1264 vertically with respect to the inner annulus 1270. Each of the one or more leaves 1262a, 1262b, 1262c, 1262d may be attached to the inner annulus 1270 by a pivot 1266 and slides freely against the outer annulus 1264. The imaging objective 1202 may be placed inside the entire mechanism 1260.

[0117] As the outer annulus 1264 is rotated about the inner annulus 1270, it extends or recedes, and the contact point between the leaves 1262a, 1262b, 1262c, 1262d and the sliding joint 668 with the outer annulus 1264 moves up or down. This changes the angle at which the leaves 1262a, 1262b, 1262c, 1262d are held with respect to the imaging plane.

The design 1260 may be completed by a rotating actuator 1274 to allow for automated adjustment of the angle of the light source 604 makes with a vertical axis 1267.

Use of depressed illuminator angles for grazing illumination of sample

[0118] A method for illumination that exploits the divergence inherent in illuminators to provide for illumination along a surface with minimal depth (i.e. grazing illumination) may be provided. FIGS. 14A and 14B show schematic side views of a microscope setup 1400, comparatively illustrating over-penetrating illumination due to large exit angle and minimized-penetration illumination using depressed-angle illumination, respectively, according to an example. The microscope setup 1400 may include the same or like elements or components as those of the microscopy apparatus 200 of FIG. 2, and as such, the same ending numerals are assigned and the like elements may be as described in the context of the microscopy apparatus 200 of FIG. 2, and therefore the corresponding descriptions are omitted here.

[0119] This method may include an illuminator 1404 with a nominal exit axis and a known or measured exit angle which characterizes the divergence of light exiting the illuminator 1404. As seen in FIG. 14B, the exit axis may be pointed downwardly away from the sample 1401, placed on a sample holder or plate 1409, with an azimuth angle not exceeding the exit angle such that only a small portion of the total light illuminates the sample 1401, and only at an extremely shallow angle. The illumination depth may be less than the depth of field 1407 of the objective lens 1402. Here, a depressed angle illumination strategy may be used for achieving the desired shallow depth of illumination.

[0120] The microscope setup 1400 may be embodied by bare LEDs, LEDs with optical elements, or fibre-optic cables or other waveguides. Optionally, light baffles or reflectors may be used to direct excess energy away from the sample 1401 and the imaging pathway, reducing heating and possible interference in the imaging pathway.

Use of single-piece waveguide with internal features for illumination delivery

[0121] A method for illumination which uses a single-piece waveguide to deliver light from one or more sources to the sample may be provided. The light may include UV light and/or non-UV light. FIG. 15 shows a schematic top view of a single-piece waveguide

1580 coupled to a light source 1502, according to an example. The waveguide 1580 may be placed below the stage (not shown in FIG. 15) so that the illumination is delivered to the sample (not shown in FIG. 15) placed over an exit feature 1584. The waveguide 1580 may be produced from a single plate of quartz, polytetrafluoroethylene (PMMA), or similar material and may include the exit feature 1584 which directs light exiting onto the sample at an appropriate angle, one or more input features 1582 which receive illumination from the light source 1502, and optionally, some distribution features 1586 that change the behaviour of light internally to change the intensity and distribution of illumination delivered from the exit feature 1584. The exit feature 1584 may include an orifice or a hole formed in the waveguide 1580, thereby allowing free-space propagation to complete "last-mile" delivery. The exit feature 1584 may be centrally positioned in the waveguide 1580.

[0122] As seen in FIG. 15, the distribution feature 1586 may be an angled cut slot that encourages internal reflection in a strategic position to provide illumination through the exit feature 1584. Two possible rays of light are illustrated to show the effect of the distribution feature 1586.

[0123] Any of the abovementioned features may contain additional surface treatment or ancillary features to provide desired effects: e.g. the input feature 1582 may use optical glue to couple with the light source 1502, or the distribution feature (e.g. 1586) may use black paint to absorb light.

[0124] FIGS. 16A to 16C show schematic side views of illuminating the sample 1601 placed on a sample holder or plate 1609 through different exit features 1584a, 1584b, 1584c, according to different examples. Illumination below the plate 1609 is not shown here. More specifically, FIGS. 16A, 16B and 16C show the waveguide 1680 having a straight-wall exit feature 1584a, an exit feature with positive draft angle 1584b, and an exit feature with negative draft angle for depressed-angled illumination 1584c, respectively. In this, it may be illustrated how it is possible to use the draft angle of the wall to change the depth of illumination. In other words, the exit feature (e.g. 1584a, 1584b, 1584c) includes setting the draft angle of the walls to change the distribution of the output light. The draft angle of the exit feature may be set at any angle, including being substantially parallel with the shape of the input feature.

[0125] The illumination setup described in FIGS. 16A to 16C may include the same or like elements or components as those used in the microscopy apparatus 200 of FIG. 2, and as such, the same ending numerals are assigned and the like elements may be as described in the context of the microscopy apparatus 200 of FIG. 2, and therefore the corresponding descriptions are omitted here.

Part II - Stage movements for high magnification imaging

[0126] The desired magnification requires significantly accurate positioning of the stage for complete coverage over the sample. Existing microscopes are typically designed to provide this mechanically, which generally drives up the cost and the complexity of producing such devices. Here, a stage design may be provided that overcomes or addresses the requirement for accuracy by instead achieving repeatability and numerically correcting for the resultant deviation, thereby reducing cost and complexity. The stage design may be described in similar context with the stage module 210 in FIG. 2, and as such, the corresponding descriptions are omitted here.

[0127] Existing robotic stages tend to favour mechanical designs that are inherently accurate, and so when the robotic stage is instructed to move to the target position, the final achieved positions are always close to the target. However, these designs are complex, require complex production steps, and are correspondingly expensive.

[0128] However, a stage that is designed to be mechanically inaccurate so long as the inaccuracy is the same each time may be provided. When requested to move to a particular position, the stage may have some systematic error, which is compensated for later. Preferably, the microscope (e.g. the microscopy apparatus 200 of FIG. 2) embodying this stage design may include an image sensor, and the output of the image sensor may be used to estimate the positioning error and correct for it; otherwise, alternative sensors may be used to estimate the deviation.

[0129] FIG. 17A shows a representative diagram 1701 illustrating the deviation varied across the span of the stage, i.e. before correction, according to an example. FIG. 17B shows a representative diagram 1703 illustrating the compensation carried on the deviation (errors) seen in FIG. 17A, i.e. after correction, according to an example. In FIGS. 17A and 17B, the fields of view (denoted by the squares) of an optical sensor around evenly spaced

points (denoted by X) on the stage. After calibration step(s) to determine these errors (see FIG. 17A), the systematic but varying deviations may be compensated over the span of the stage, thereby ensuring accurate positioning (FIG. 17B) over the span of the stage. As shown in FIG. 17B, the entire wide-angle view may be presented, without cropping, thereby allowing users to examine all overlapping areas as well.

[0130] The design features that allow the production of such a stage, which may be translated in 3 axes where Z axis may be separated from the X and Y axes, may include the following.

Use of multiple low-precision linear motion parts in parallel

[0131] There may be provided a design for multiple low-precision linear motion parts in parallel.

[0132] A low-precision linear motion part refers to a commodity part that permits movement (and possibly rotation) along its axis of movement while constraining translations and rotations along perpendicular axes. These commodity parts may be embodied as linear rails, optical axes, amongst others.

[0133] Stage designs preferably use a pair of linear motion parts in parallel to adequately constrain each axis. By using a pair in parallel, the rails and carriages are each rigidly affixed to each other so that the resultant arrangement permits translation (but not rotation) along the axis of movement. This arrangement may be repeated for both horizontal axes.

[0134] These components are available in various precision standards, with cost and complexity of production scaling exponentially with the precision.

[0135] A key difference between high- and low-precision linear motion parts is that of the perpendicular deflection.

[0136] High-precision rails exhibit minimal lateral movement, while low-precision rails display much greater lateral movement.

[0137] In an exemplary design, a pair of low-precision parts instead of a pair of high-precision parts may be used. However, doing this naively introduces the chance for mechanical binding, the tendency for a mechanical system to jam due to its component tolerances being violated when it should operate normally.

[0138] When the two rails and carriages are rigidly affixed to each other, the perpendicular deflection of each rail deviates the gap between carriages from the nominal amount. This gap may exceed the design tolerances of the system, and thus cause mechanical binding at some positions.

[0139] FIG. 18 shows an exemplary mechanism to allow a pair of low-precision linear motion parts 1801a, 1801b (or linear rails) to be used in parallel without binding by rigidly affixing one rail (henceforth called the "master" rail 1801a), while permitting small (typically, no more than 0.05 mm) and repeatable deflections of the other rail (henceforth called the "slave" rail 1801b). This mechanism may include the following three components in addition to the pair of linear motion parts 1801a, 1801b to provide linear motion: a rigid mount 1817 for the master rail 1801a, a floating mount 1819 for the slave rail 1801b allowing it to move perpendicular to the axis of motion of the master rail 1801a, and one or more pre-tensioners 1821 each exerting a force on the slave rail 1801b perpendicular to the axis of motion of the master rail 1801a, which ensures that the deflection of the slave rail 1801b is repeatable for each position of the carriage 1803.

[0140] For example, the rigid mount 1817 for the master rail 1801a may be a sheet of steel shim stock placed between the master rail 1801a and a component below (not shown in FIG. 18). The slave rail 1801b may be mounted in a manner that allows for slight perpendicular motion by affixing it to the component below the mechanism using the floating mount 1819, which may be a polytetrafluoroethylene (PTFE) shim stock and a well-specified bolt tension. The low coefficient of friction provided by PTFE and the use of an appropriate bolt tension allow some slight movement of the slave rail 1801b. Optionally, if deemed necessary, some other design feature may be used to prevent the slave rail 1801b from moving parallel to the axis of movement of the master rail 1801a. Finally, the use of thin springs (i.e. one or more pre-tensioners 1821) between the master 1801a and slave rail 1801b provides the perpendicular tension required to ensure the movement of the slave rail 1801b is repeatable.

Detachable microscope stages with repeatable parallel planarity using a kinetic coupling design

[0141] A common existing feature offered is the support for replaceable sample holders of different shapes for imaging on the same stage. This need for versatility may be typically addressed in existing microscopes by offering a rigid stage with inserts used to change the presented geometry. However, this solution proves unsatisfactory for arbitrary sample geometry and the possibility of further robotic automation with sample insertion.

[0142] A design for detachable microscope stages with repeatable parallel planarity may be provided, where each installed stage rests as parallel to the ideal imaging plane as possible, even if its position along that plane were to vary slightly. In the earlier examples presented above, the importance of repeatability instead of accuracy has been emphasized. The proposed post-hoc correction mechanism may correct for the change in the plane of the stage, and it is expected that this property greatly reduces the cost of calibration.

[0143] FIGS. 19A and 19B show exploded schematic top views of a detachable microscope stage design 1910, according to an example. FIG. 19C shows a schematic top view of the detachable microscope stage design 1910 when assembled. The detachable microscope stage design 1910 may include a static plate 1910a that is permanently attached to the effector arm of the stage (not shown in FIGS. 19A to 19C), a replaceable plate 1910b with the desired geometry to hold samples, a series of components that form a kinetic coupling attached to the static plate 1910a and the replaceable plate 1910b to provide repeatable positioning, and a clamping mechanism 1923 to compress the static plate 1910a and the replaceable plate 1910b together.

[0144] The detachable microscope stage design 1910 embodies the kinetic coupling as a Maxwell coupling. The hardened precision-ground rods 1925 are attached to one plate 1910a (see FIG. 19A) and the ball bearings 1927 are attached to another plate 1910b (see FIG. 19B) such that under compression the two plates 1910a, 1910b are parallel. For the coupling to work correctly, a consistent compression force is required to drive the two plates 1910a, 1910b together. See FIG. 19C. This may be provided by the clamping mechanism.

[0145] As seen in the exemplary detachable microscope stage design 1910, a Maxwell coupling may include three sets of pairs of rods 1925 and ball bearings 1927 to provide the repeatable mounting, and a pair of clamps 1923, 1923' for the necessary compression force.

[0146] In another example, for ease of insertion and removal, one clamp may be replaced with a clasping mechanism 1923' that may be released without any tools. Crucial to the presented embodiment of the design 1910 is that the lines 1929 perpendicular to the pairs of rods 1925 all pass through a single point 1931 that coincides with (or is close to) the line 1933 between the two clamping jaws. This ensures that the desired configuration is locally stable, and the system returns to that configuration after any small deflection, as shown in FIG. 19C.

[0147] 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 microscopy apparatus for superficial imaging of a sample, the microscopy apparatus comprising:

an objective lens;

a surface illuminator configured to illuminate into the sample at a depth less than or equal to a depth of field of the objective lens; and

a stage module configured to receive, adjust and position the sample for repeatable imaging,

wherein the surface illuminator comprises:

a light source configured to emit light to illuminate the sample, the light source arranged adjacent to and on a same side as the objective lens; and

an adjustment mechanism configured to adjust an angle and/or an intensity of the light directed at the sample.

2. A surface illuminator in operable communication with a stage module of a microscopy apparatus for receiving, adjusting and positioning a sample, the surface illuminator comprising:

a light source configured to emit light to illuminate the sample, the light source adapted to be arranged adjacent to and on a same side as an objective lens of the microscopy apparatus; and

an adjustment mechanism configured to adjust an angle and/or an intensity of the light directed at the sample,

wherein the surface illuminator is configured to illuminate into the sample at a depth less than or equal to a depth of field of the objective lens.

3. The microscopy apparatus as claimed in claim 1 or the surface illuminator as claimed in claim 2, wherein the light source comprises a light emitting diode, or a laser.

4. The microscopy apparatus as claimed in claim 1 or 3, or the surface illuminator as claimed in claim 2 or 3, wherein the adjustment mechanism comprises a waveguide.

5. The microscopy apparatus as claimed in claim 4, or the surface illuminator as claimed in claim 4,

wherein the waveguide comprises a fibre-optic cable comprising a light-exiting end arrangeable towards the sample, and

wherein the adjustment mechanism further comprises one or more flexure mechanisms, each flexure mechanism comprising:

a clamp adapted to rigidly hold the fibre-optic cable towards the light-exiting end;

a base opposite to the clamp;

a flexure segment extending between the clamp and the base; and

an arm extending from the flexure segment, wherein the arm is configured to move towards or away from the base to facilitate deflection of the flexure segment.

6. The microscopy apparatus as claimed in claim 5, or the surface illuminator as claimed in claim 5, wherein each flexure mechanism is made of a single piece of material.

7. The microscopy apparatus as claimed in claim 5 or 6, or the surface illuminator as claimed in claim 5 or 6, wherein the flexure segment comprises a continuously curved flexure segment configured to produce deflection upon actuation of the arm.

8. The microscopy apparatus as claimed in any one of claims 5 to 7, or the surface illuminator as claimed in any one of claims 5 to 7, wherein each flexure mechanism further comprises:

an angular adjustment member operable to actuate the arm to deflect the flexure segment and adjust an angle of the clamp and consequently, the light directed at the sample.

9. The microscopy apparatus as claimed in any one of claims 5 to 8, or the surface illuminator as claimed in any one of claims 5 to 8, wherein each flexure mechanism further comprises:

a level adjustment member operable to adjust a level movement of the clamp, and collectively, the light-exiting end of the fibre-optic cable with respect to the sample.

10. The microscopy apparatus as claimed in any one of claims 5 to 9, or the surface illuminator as claimed in any one of claims 5 to 9, wherein the one or more flexure mechanisms comprises two or more flexure mechanisms arranged orthogonally or angularly apart from one another to provide adjustments along different axes.

11. The microscopy apparatus as claimed in claim 1 or 3, or the surface illuminator as claimed in claim 2 or 3, wherein the adjustment mechanism comprises a leaf-slider mechanism comprising:

a leaf having a first part and a second part spaced apart from the first part,

wherein the leaf is coupled to the light source at the first part in a manner such that when in operation, the light from the light source is directed at the sample, and the leaf is pivotably coupled to an adjoining member at the second part; and

a slider being in contact with the leaf,

wherein the slider is coupled to the leaf via a preload force exerted therebetween.

12. The microscopy apparatus as claimed in claim 11, or the surface illuminator as claimed in claim 11, wherein the slider is operable to translate or rotate along the leaf, allowing the leaf to pivot about the second part and consequently adjust a position and/or an orientation of the light source relative to the sample.

13. The microscopy apparatus as claimed in claim 12, or the surface illuminator as claimed in claim 12, wherein the slider comprises one of the following:

- one or more sliding joints arranged spatially apart from one another to form an arbitrary contour for translation along the leaf; or

- a sliding joint with a sloped geometry to form a smooth sloped contour for translation along the leaf; or

- a sliding joint with a wave geometry to form an irregular contour for translation along the leaf.

14. The microscopy apparatus as claimed in claim 13, or the surface illuminator as claimed in claim 13, wherein the leaf-slider mechanism further comprises a resilient member coupleable to the slider, the resilient member extending from the slider in a direction away from the leaf.

15. The microscopy apparatus as claimed in claim 14, or the surface illuminator as claimed in claim 14, wherein the resilient member comprises a spring.

16. The microscopy apparatus as claimed in claim 14 or 15, or the surface illuminator as claimed in claim 14 or 15, wherein the resilient member is made of a shape-memory alloy.

17. The microscopy apparatus as claimed in claim 12, or the surface illuminator as claimed in claim 12, wherein the slider comprises an eccentric rotating element having a side cross-section with a circular shape, or an elliptical shape, or a polygonal shape for rotation along the leaf.

18. The microscopy apparatus as claimed in any one of claims 11 to 17, or the surface illuminator as claimed in any one of claims 11 to 17, wherein the leaf-slider mechanism further comprises an actuator coupleable to the slider and configured to operate the slider.

19. The microscopy apparatus as claimed in any one of claims 11 to 17, or the surface illuminator as claimed in any one of claims 11 to 17, wherein

the adjoining member comprises an inner annulus comprising outwardly extending threads;

the slider comprises an outer annulus comprising inwardly extending threads arranged in an interlocking manner with outwardly extending threads; and

the leaf-slider mechanism further comprises one or more further leaves arranged spatially apart from one another around the inner annulus and the outer annulus.

20. The microscopy apparatus as claimed in claim 1 or 3, or the surface illuminator as claimed in claim 2 or 3, wherein

the light source is configured to emit the light with an exit angle; and

the adjustment mechanism is configured to adjust the light source away from the sample with an azimuth angle less than the exit angle.

21. The microscopy apparatus as claimed in claim 1, 3 or 4, or the surface illuminator as claimed in any one of claims 2 to 4, wherein the waveguide comprises:

one or more input features adapted to receive the light from the light source, the light being parallel to a major plane of the waveguide; and

an exit feature adapted to direct the received light to exit onto the sample at a pre-determined angle.

22. The microscopy apparatus as claimed in claim 21, or the surface illuminator as claimed in claim 21, wherein the waveguide further comprises one or more distribution features adapted to change a behaviour of the received light internally to correspondingly change an intensity and/or a distribution of the received light being directed to the exit feature.

23. The microscopy apparatus as claimed in claim 21 or 22, or the surface illuminator as claimed in claim 21 or 22, wherein each of the one or more distribution features comprises an angled cut slot configured to facilitate internal reflection of the received light, or an absorbent layer adapted to absorb a portion of the received light.

24. The microscopy apparatus as claimed in any one of claims 21 to 23, or the surface illuminator as claimed in any one of claims 21 to 23, wherein the waveguide comprises a single plate of quartz, or polymethyl methacrylate.

25. The microscopy apparatus as claimed in any one of claims 21 to 24, or the surface illuminator as claimed in any one of claims 21 to 24, wherein the waveguide has curved edges or non-parallel edges.

26. A stage module in operable communication with a surface illuminator of a microscopy apparatus for illuminating into a sample at a depth less than or equal to a depth of field of an objective lens of the microscopy apparatus, wherein the stage module is configured to receive, adjust and position the sample for repeatable imaging.

27. The microscopy apparatus as claimed in claim 1 or any one of claims 3 to 25, or the stage module as claimed in claim 26, wherein the stage module comprises:

a rigid mount;

a floating mount arranged spatially apart and alongside the rigid mount;

a master rail affixed to the rigid mount;

a slave rail coupled to the floating mount;

a carriage plate coupled across the master rail and slave rail; and

one or more pre-tensioner configured to exert a force on the slave rail in a direction substantially perpendicular or non-parallel to an axis of motion of the master rail, thereby allowing a repeatable deflection of the slave rail for each position of the carriage plate along the master rail and slave rail.

28. The microscopy apparatus as claimed in claim 27, or the stage module as claimed in claim 27, wherein the rigid mount comprises a steel shim stock.

29. The microscopy apparatus as claimed in claim 27 or 28, or the stage module as claimed in claim 27 or 28, wherein the floating mount comprises a polytetrafluoroethylene shim stock.

30. The microscopy apparatus as claimed in claim 1 or any one of claims 3 to 25, or the stage module as claimed in claim 26, wherein the stage module comprises:

a stage arranged between the sample and the objective lens;

a static plate affixed to an effector arm of the stage;

a replaceable plate configured to receive the sample and arrangeable parallel planarly over the static plate;

at least two compressing mechanisms configured to apply compression pressure between a parallel planar arrangement of the static plate and the replaceable plate; and

a kinetic coupling mechanism configured to couple the static plate and the replaceable plate together under the compression pressure and facilitate repeatable positioning of the sample.

31. The microscopy apparatus as claimed in claim 30, or the stage module as claimed in claim 30, wherein the at least two compressing mechanisms comprise one of the following:

at least two clasping mechanisms, or

at least one clamping mechanism and at least one clasping mechanism.

32. The microscopy apparatus as claimed in claim 30 or 31, or the stage module as claimed in claim 30 or 31, wherein the kinetic coupling mechanism comprises:

a plurality of mating elements coupled to the static plate and a plurality of complementary elements coupled to the replaceable plate, wherein the plurality of complementary elements is configured to be correspondingly received by the plurality of mating elements.

33. The microscopy apparatus as claimed in claim 32, or the stage module as claimed in claim 32,

wherein the plurality of mating elements comprises:

a plurality of rod pairs; or

a plurality of bearings; or

a mixture of rod pairs and bearings; and

wherein the plurality of complementary elements respectively comprises:

a plurality of complementary bearings;

a plurality of complementary rod pairs; or

a mixture of complementary bearings and complementary rod pairs.

34. The microscopy apparatus as claimed in claim 32 or 33, or the stage module as claimed in claim 32 or 33, wherein

the plurality of mating elements is arranged spatially apart from one another, each mating element being positioned proximal to an edge of the static plate;

the plurality of complementary elements is arranged spatially apart from one another, each complementary element being positioned proximal to an edge of the replaceable plate;

the plurality of mating elements and the plurality of complementary elements when mated are each orientated with a first axis extending perpendicularly therefrom; and

the at least two compressing mechanisms are arranged spatially across the parallel planar arrangement, along a second axis coinciding with every first axis at a single point.

35. The microscopy apparatus as claimed in claim 34, or the stage module as claimed in claim 34, wherein the second axis comprises a linear axis or a centroid axis.

36. The microscopy apparatus as claimed in claim 1 or any one of claims 3 to 35, further comprising

an image sensor configured to capture a superficial image of the sample; and

a processor configured to receive the superficial image, estimate a positioning error based on the received superficial image, and correct the positioning error.

37. A method for superficial imaging of a sample, the method comprising:

illuminating into the sample at a depth less than or equal to a depth of field of an objective lens of a microscopy apparatus; and

adjusting and positioning the sample for repeatable imaging,

wherein illuminating into the sample comprises:

emitting light from a light source to illuminate the sample, the light source arranged adjacent to and on a same side as the objective lens; and

adjusting an angle and/or an intensity of the light directed at the sample.

38. The method as claimed in claim 37, wherein adjusting the angle and/or the intensity of the light directed at the sample comprises:

rigidly holding a fibre-optic cable using a clamp of a flexure mechanism;

arranging a light-exiting end of the fibre-optic cable towards the sample; and

actuating deflection of a flexure segment of the flexure mechanism to adjust a position and/or an orientation of the light-exiting end.

39. The method as claimed in claim 38, wherein adjusting the angle and/or the intensity of the light directed at the sample comprises adjusting the angle and/or the intensity of the light along different axes using two or more flexure mechanisms arranged orthogonally apart from one another.

40. The method as claimed in claim 37, wherein adjusting the angle and/or the intensity of the light directed at the sample comprises:

providing a leaf-slider mechanism comprising:

a leaf having a first part and a second part spaced apart from the first part, wherein the leaf is coupled to the light source at the first part and is pivotably coupled to an adjoining member at the second part; and

a slider being coupled to the leaf via a preload force exerted therebetween;

operating the slider to cause the leaf to pivot about the second part and consequently adjust a position and/or an orientation of the light source relative to the sample.

41. The method as claimed in claim 40, wherein operating the slider comprises translating or rotating the slider along the leaf.

42. The method as claimed in claim 40 or 41, wherein operating the slider is performed using an actuator coupled to the slider.

43. The method as claimed in claim 37, wherein

emitting the light from the light source comprises emitting the light with an exit angle; and

adjusting the angle and/or the intensity of the light directed at the sample comprises adjusting the light source away from the sample with an azimuth angle less than the exit angle.

44. The method as claimed in claim 37, wherein adjusting the angle and/or the intensity of the light directed at the sample comprises:

providing a waveguide comprising one or more input features, an exit feature and optionally, one or more distribution features;

receiving, by the one or more input features, the light from the light source, the light being parallel to a major plane of the waveguide;

directing, by the exit feature, the received light to exit onto the sample at a pre-determined angle; and

optionally, changing, by the one or more distribution features, a behaviour of the received light internally to correspondingly change an intensity and/or a distribution of the received light being directed to the exit feature.

45. The method as claimed in claim 44, wherein changing the behaviour of the received light internally comprises facilitating internal reflection of the received light by an angled cut slot of each distribution feature or absorbing a portion of the received light by an absorbent layer of each distribution feature.

46. The method as claimed in any one of claims 37 to 45, wherein adjusting and positioning the sample for repeatable imaging comprises adjusting and positioning the sample using a stage module comprising commodity parts mass-manufactured with standard dimensions; and custom-made parts affixed to the commodity parts, wherein the custom-made parts comprise planar parts comprising two-dimensional features, or non-planar parts with lower rigidity as compared to the planar parts.

47. The method as claimed in any one of claims 37 to 45, wherein adjusting and positioning the sample for repeatable imaging comprises:

using a stage module comprising:

a rigid mount;

a floating mount arranged spatially apart and alongside the rigid mount;

a master rail affixed to the rigid mount;

a slave rail coupled to the floating mount;

a carriage plate coupled across the master rail and slave rail; and

one or more pre-tensioner, and

exerting a force, via the one or more pre-tensioner, on the slave rail in a direction substantially perpendicular or non-parallel to an axis of motion of the master rail, thereby allowing a repeatable deflection of the slave rail for each position of the carriage plate along the master rail and slave rail.

48. The method as claimed in any one of claims 37 to 45, wherein adjusting and positioning the sample for repeatable imaging comprises:

using a stage module comprising:

a stage arranged between the sample and objective lens;

a static plate affixed to an effector arm of the stage;

a replaceable plate arrangeable parallel planarly over the static plate;

at least two compressing mechanisms; and

a kinetic coupling mechanism,

receiving the sample on the replaceable plate,

applying, by the at least two compressing mechanisms, compression pressure between a parallel planar arrangement of the static plate and the replaceable plate, and

coupling, via the kinetic coupling mechanism, the static plate and the replaceable plate together under the compression pressure to facilitate repeatable positioning of the sample.

49. The method as claimed in claim 48, wherein coupling, via the kinetic coupling mechanism, the static plate and the replaceable plate together under the compression pressure comprises:

providing a plurality of mating elements coupled to the static plate; and

correspondingly receiving a plurality of complementary elements coupled to the replaceable plate with the plurality of mating elements.

50. The method as claimed in claim 49,

wherein correspondingly receiving the plurality of complementary elements with the plurality of mating elements comprises mating the plurality of mating elements and the plurality of complementary elements in a manner such that each mated mating element and complementary element is orientated with a first axis extending perpendicularly therefrom; and

arranging the at least two compressing mechanisms spatially across the parallel planar arrangement, along a second axis coinciding with every first axis at a single point.

51. The method as claimed in claim 50, wherein the second axis comprises a linear axis or a centroid axis.

52. The method as claimed in any one of claims 46 to 51, wherein

adjusting and positioning the sample for repeatable imaging comprising translating the stage module along three axes, and rotating the light source; and

adjusting the angle and/or the intensity of the light directed at the sample comprises rotating the light along one rotational axis or two different rotational axes.

53. The method as claimed in any one of claims 37 to 52, further comprising:

capturing a superficial image of the sample;

estimating a positioning error based on the captured superficial image; and

correcting the positioning error.

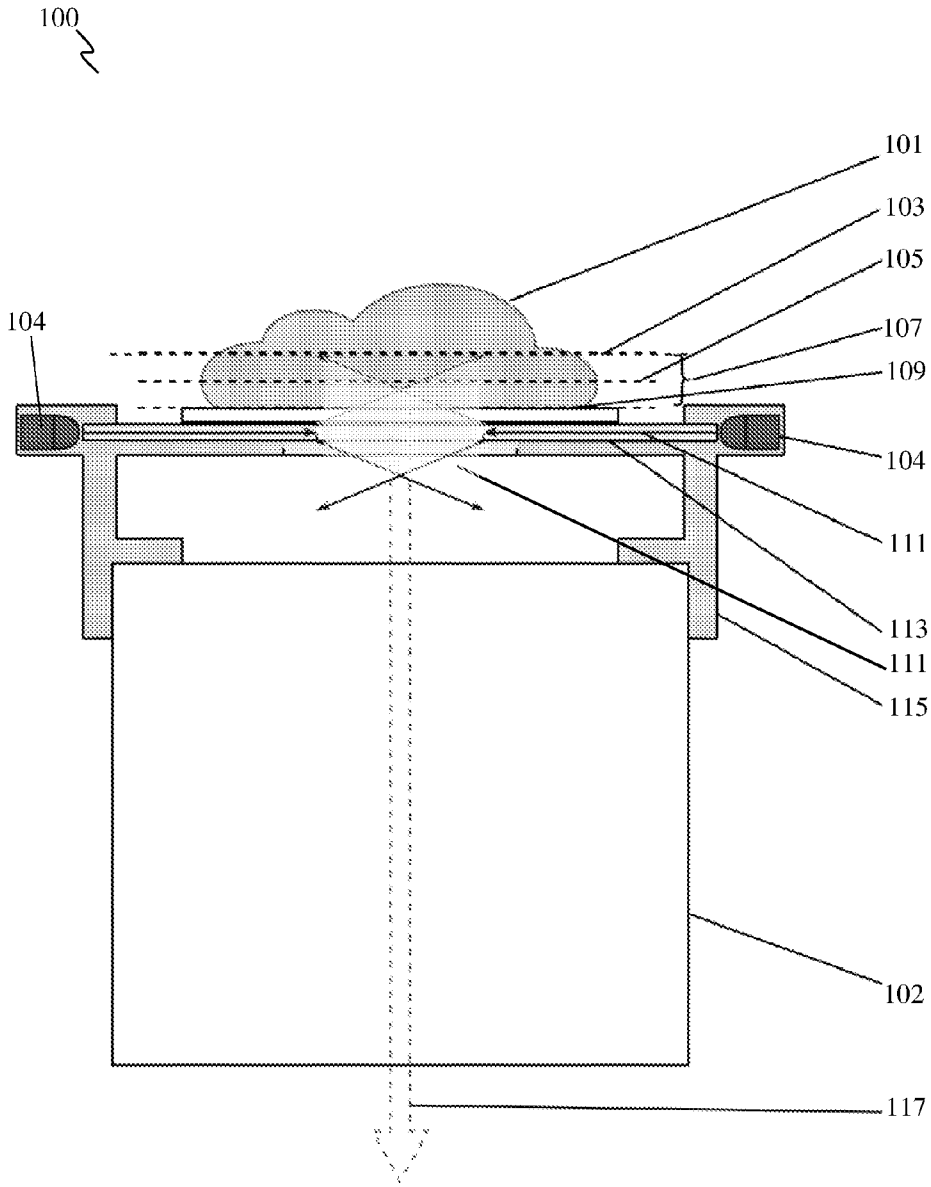

FIG. 1

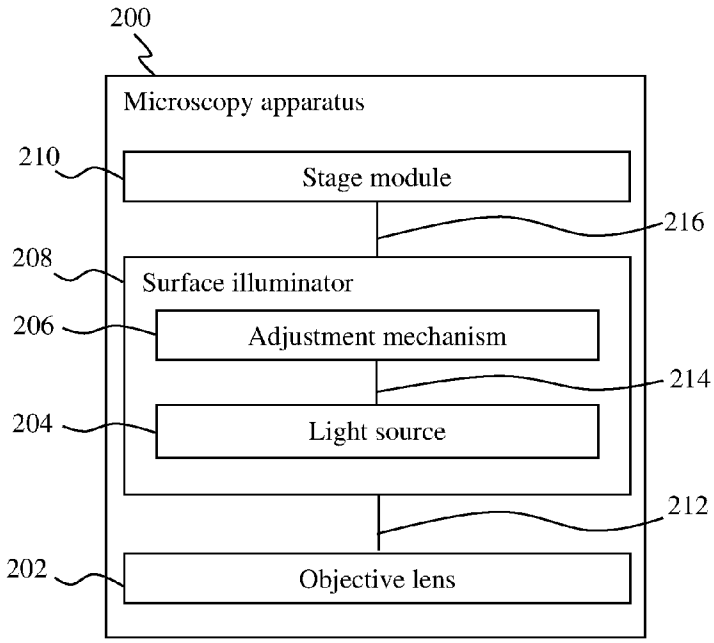
200
Microscopy apparatus
210
Stage module
216
208
Surface illuminator
206
Adjustment mechanism
214
204
Light source
212
202
Objective lens

FIG. 2

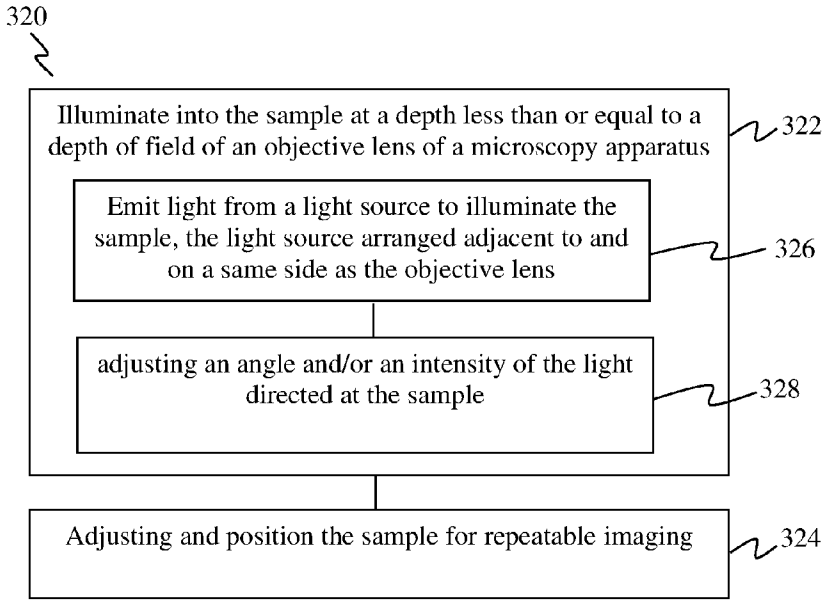
320
Illuminate into the sample at a depth less than or equal to a depth of field of an objective lens of a microscopy apparatus
322
Emit light from a light source to illuminate the sample, the light source arranged adjacent to and on a same side as the objective lens
326
adjusting an angle and/or an intensity of the light directed at the sample
328
Adjusting and position the sample for repeatable imaging
324

FIG. 3

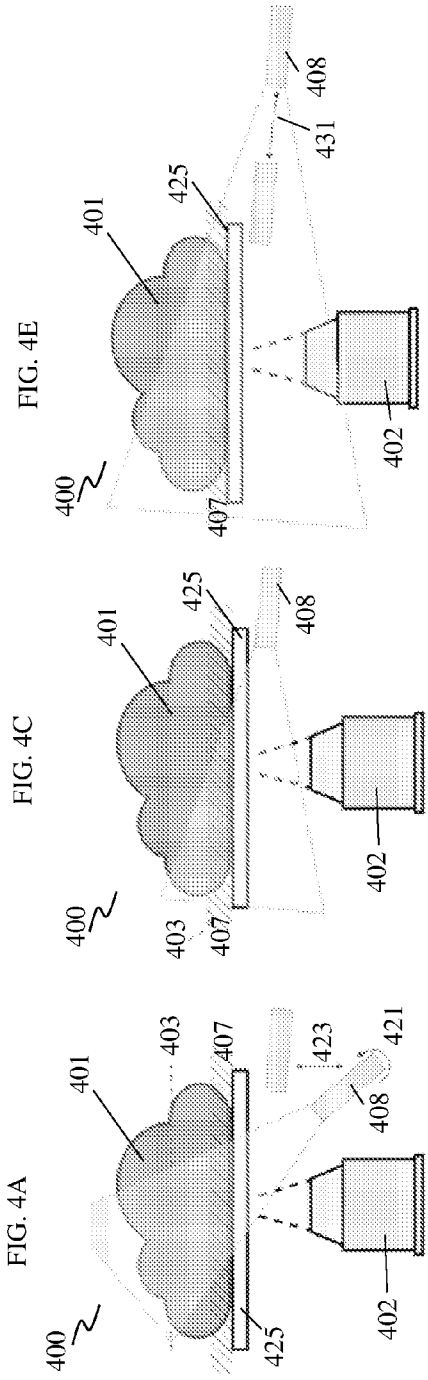

FIG. 4A

FIG. 4C

FIG. 4E

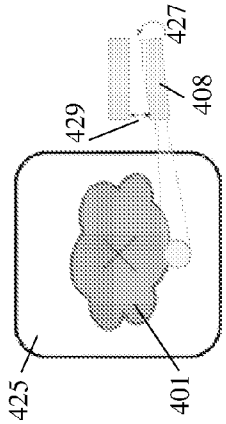

FIG. 4B

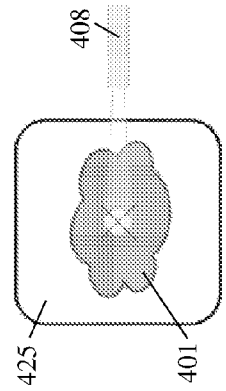

FIG. 4D

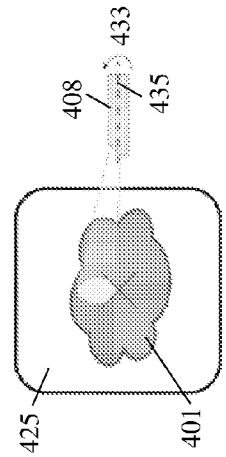

FIG. 4F

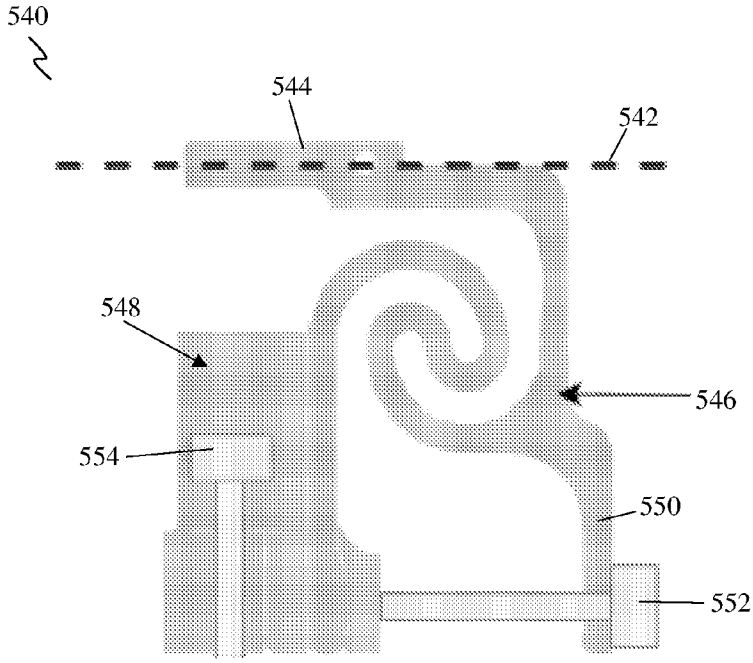

FIG. 5

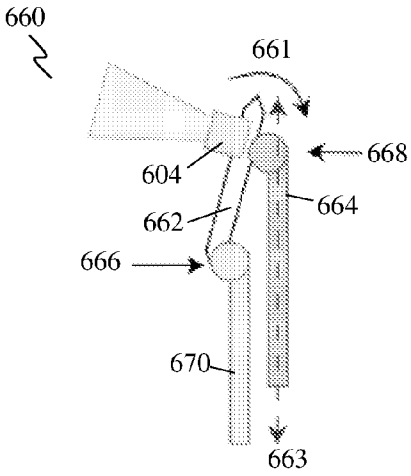

FIG. 6

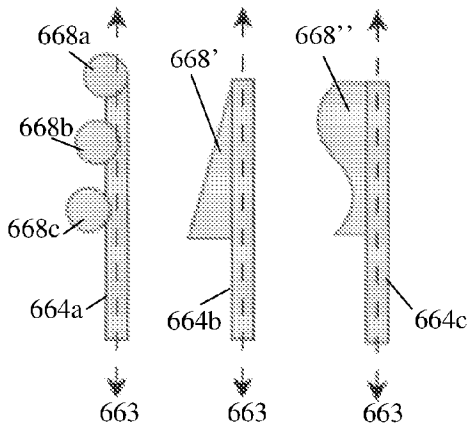

FIG. 7A FIG. 7B FIG. 7C

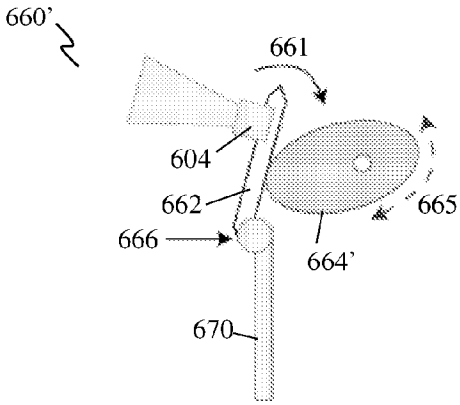

FIG. 8

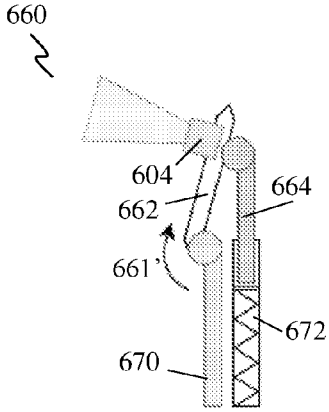

FIG. 9

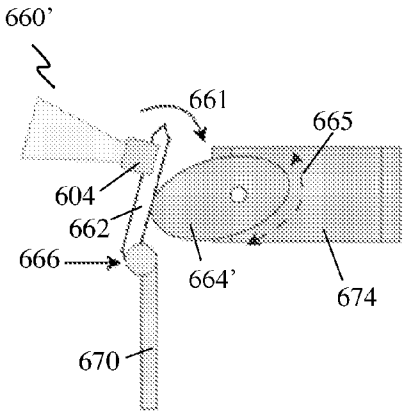

FIG. 10

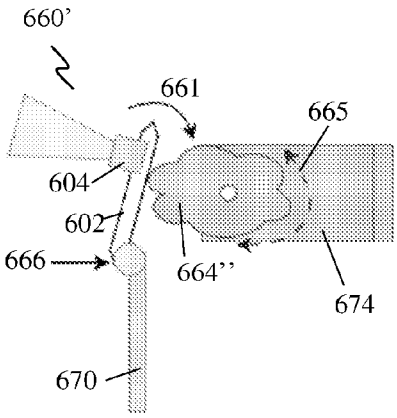

FIG. 11

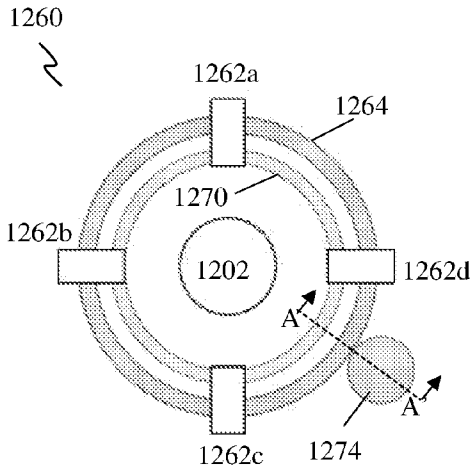

FIG. 12

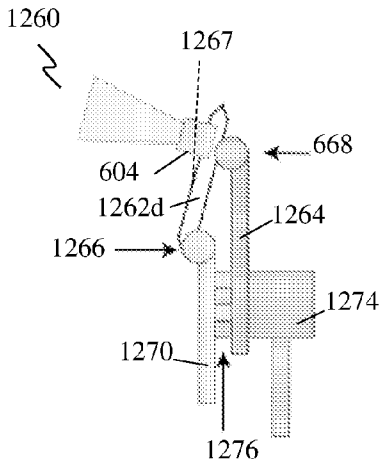

FIG. 13

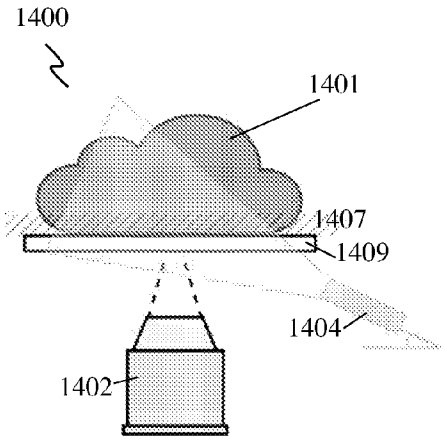

FIG. 14A

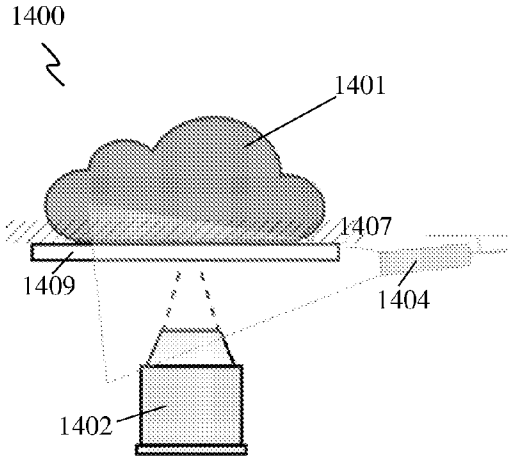

FIG. 14B

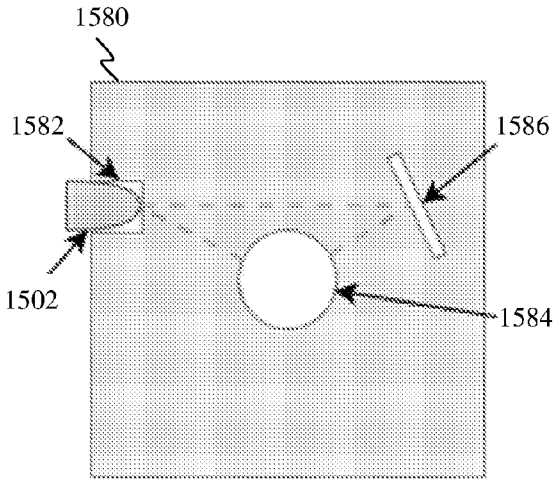

FIG. 15

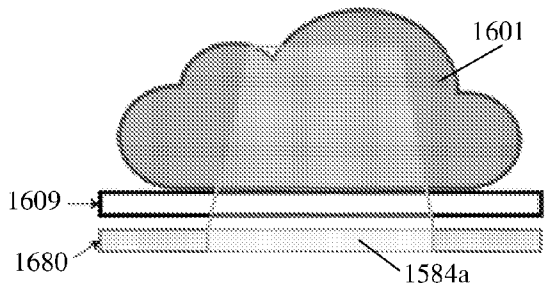

FIG. 16A

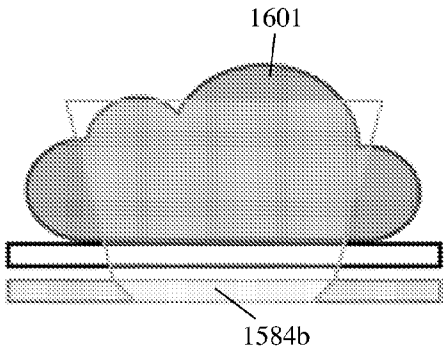

FIG. 16B

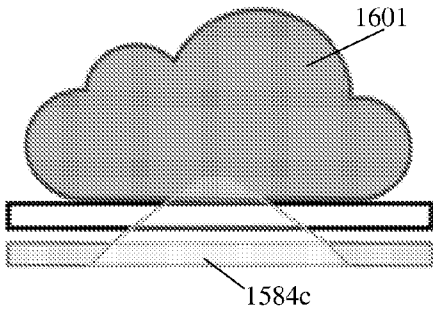

FIG. 16C

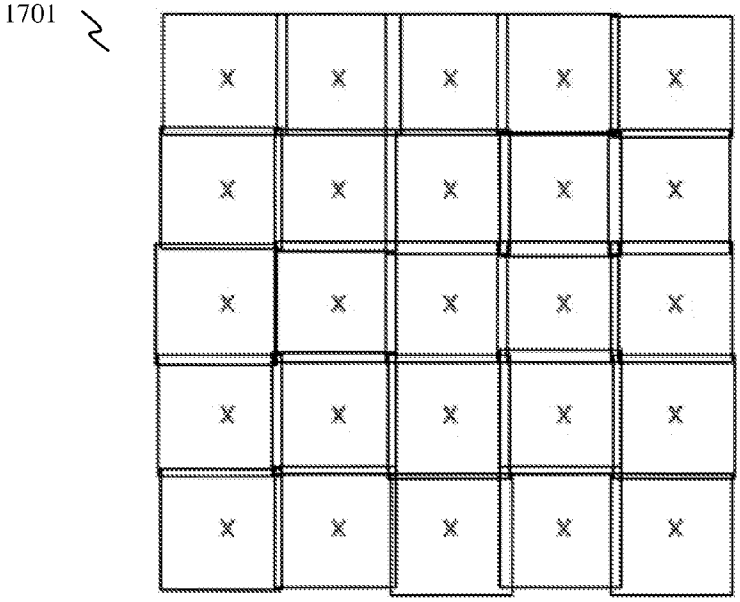

FIG. 17A

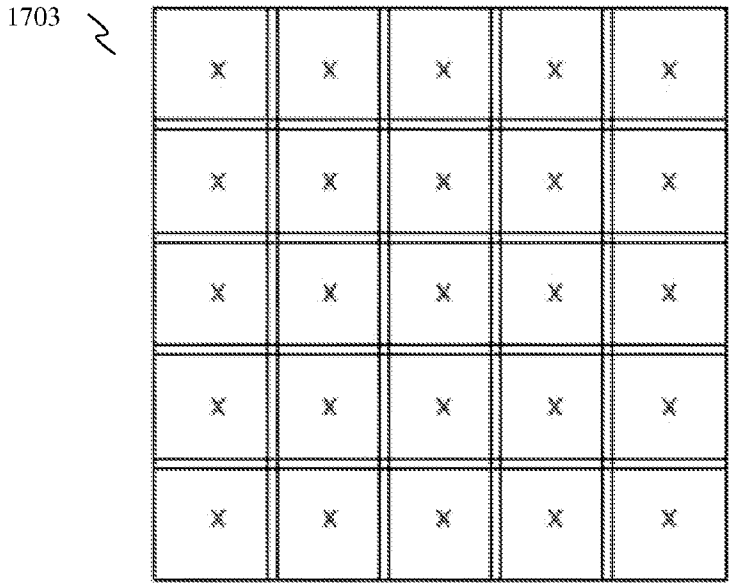

FIG. 17B

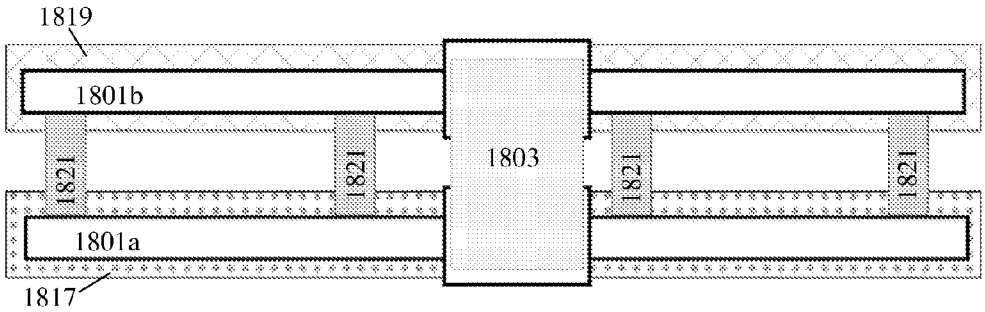

FIG. 18

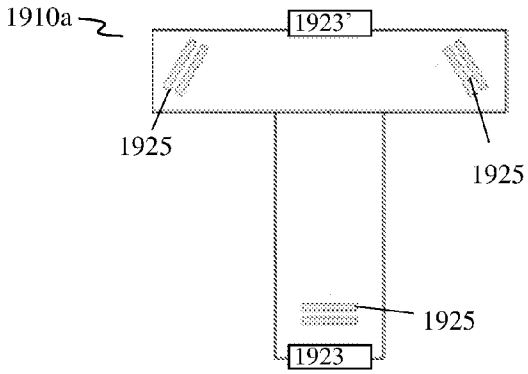

FIG. 19A

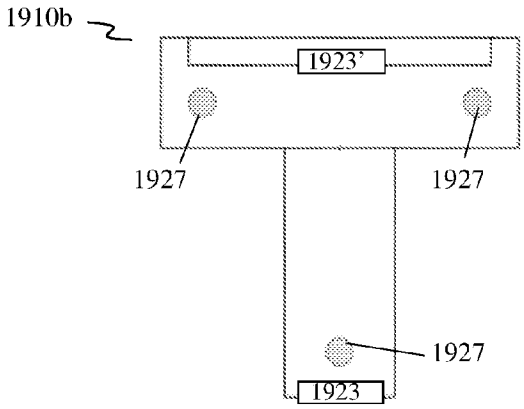

FIG. 19B

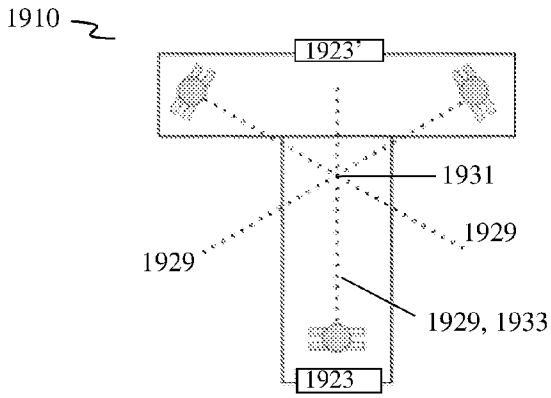

FIG. 19C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2024/050574

A. CLASSIFICATION OF SUBJECT MATTER

G02B 21/06 (2006.01) *G02B 21/26 (2006.01)* *G01N 21/00 (2006.01)*

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G02B, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

FAMPAT: microscopy, superficial, light source, illumination, optical, angle, direction, spatial, intensity, brightness, adjust, change, alter, modulate, depth of field, focus, stage, mount, carriage, clamp, hold, flexure, fiber, waveguide, rail, slider, and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2023/075695 A2 (AGENCY SCIENCE TECH & RES) 4 May 2023 Pg. 4, ln. 10-18, pg. 10, ln. 17-29, pg. 12, ln. 8-25, pg. 17, ln. 21-25, Fig. 2	1-4, 20-25, 36-37, 43-46, 52-53
A		5-19, 27-35, 38-42, 47-51
X	US 2016/0195705 A1 (BETZIG, R. E. ET AL.) 7 July 2016 Para. [0067], [0073]-[0080], [0134]-[0135], [0208], Fig. 5, 9, 19	1-4, 20-25, 36-37, 43-46, 52-53
A	US 2017/0191937 A1 (LEVENSON, R. ET AL.) 6 July 2017 Whole document	
A	US 2003/0234979 A1 (POO, M. ET AL.) 25 December 2003 Whole document	

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

*Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

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

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

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29/11/2024 *(day/month/year)*	03/12/2024 *(day/month/year)*

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2024/050574

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

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

1. ☐ Claims Nos.:

   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:

   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:

   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

Please refer to Supplemental Box (Continuation of Box No. III).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   1-25 (in full), 27-35 (in part), 36-53 (in full)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2024/050574

Supplemental Box
(Continuation of Box No. III)

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

Group I: claims 1-25 (in full), 27-35 (in part), 36-53 (in full) are directed to a microscopy apparatus for superficial imaging of a sample, the microscopy apparatus comprising: an objective lens; a surface illuminator configured to illuminate into the sample at a depth less than or equal to a depth of field of the objective lens; and a stage module configured to receive, adjust and position the sample for repeatable imaging, wherein the surface illuminator comprises: a light source configured to emit light to illuminate the sample, the light source arranged adjacent to and on a same side as the objective lens; and an adjustment mechanism configured to adjust an angle and/or an intensity of the light directed at the sample.

Group II: claims 26 (in full), 27-35 (in part) are directed to a stage module in operable communication with a surface illuminator of a microscopy apparatus for illuminating into a sample at a depth less than or equal to a depth of field of an objective lens of the microscopy apparatus, wherein the stage module is configured to receive, adjust and position the sample for repeatable imaging.

Please refer to **Box No. IV** of Written Opinion of The International Searching Authority (Form PCT/ISA/237) for detailed explanation.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2024/050574

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 218158545 U (AVE SCIENCE & TECHNOLOGY CO LTD) 27 December 2022 Whole document of the original non-English language document (a machine translation is enclosed **only** for your reference)	
A	CN 115882085 A (UNIV SOUTHERN SCI & TECH) 31 March 2023 Whole document of the original non-English language document (a machine translation is enclosed **only** for your reference)	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SG2024/050574

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2023/075695 A2	04/05/2023	EP 4423556 A2	04/09/2024
US 2016/0195705 A1	07/07/2016	NONE	
US 2017/0191937 A1	06/07/2017	NONE	
US 2003/0234979 A1	25/12/2003	CN 1391121 A	15/01/2003
CN 218158545 U	27/12/2022	NONE	
CN 115882085 A	31/03/2023	NONE	