

External Illuminator, A Microscope, and A Microscopy Method

The present application claims priority to Singapore patent application number 10202112066Q titled "Fluorescence microscope illuminator" filed on 29 October 5 2021 and is incorporated by reference herein in its entirety.

Technical Field of Invention

The present invention relates to an illuminator for use in fluorescence imaging.

10 Background of Invention

A fluorescence microscope is needed in almost all biological labs and generally starts from USD 5,000 and can go up to USD 500,000 depending on the customisation, resolution, and magnification of the fluorescence microscope.

15 Assessing tissue during surgery is typically done with a standard microscope, but this process is slow and inaccurate. A fluorescence microscope may provide an alternative option, but the prohibitive cost likely prevents widespread usage by hospitals.

20 The use of Deep ultraviolet (DUV, which has a wavelength of less than 300 nm) illumination of a biological sample 5 can enable optically sectioned fluorescence microscopy but is difficult to implement with a cheap standard high magnification objective lens used with standard microscopes. As the glasses used in standard microscopic objective lenses 10 do not permit the transmittance of DUV light. the delivery path 25 of the ultraviolet light (may also be termed illumination path) onto the sample 5 must be separate from the optical detection path (as shown in FIG. 1). Hence, specially modified microscopes are needed for DUV illumination and increases the costs of the microscope for DUV illumination.

30 The working distance 20 of the objective lens 10 generally decreases with an increase in magnification and numerical aperture of the lens 10. The higher the numerical aperture, the better the resolution of the image obtained. A standard high magnification microscope objective lens 10 may be available from USD 100 but has a short working distance of 2mm or less. The delivery of illumination light onto the

sample 5 is challenging when the objective lens 10 has a short working distance 20 of 1-2 millimetres or less and requires the light to be delivered at an extreme oblique angle (for example, that may be larger than 0° but less than or equal to 30° as measured from the horizontal plane i.e. the horizontal surface of the sample holder
5 7) such that it falls outside of the acceptance cone of the objective lens 10 to avoid obstructing the image field of view.

A high magnification objective lens with a long working distance (3mm and greater) avoids the problems associated with a working distance of 2mm or less but is
10 expensive (typically in the range of USD 1000 to 10000, which is 10 to 100 times more expensive than the standard high magnification objective lens with a working distance of 2mm or less) and adds substantially to the cost of a DUV fluorescence microscope and is a significant hurdle in making DUV microscopy more widely available. Hence, the working distance of the objective lens 10 greatly affects the
15 cost, the operation of the microscope and the required features.

Further, direct illumination from a bulky light source such as a light emitting diode (LED) is inefficient and very challenging to position close enough to the sample 5. The positioning of LEDs close to the sample 5 generally does not allow adequate
20 heat sinking, which is particularly critical for DUV LEDs which are energy-inefficient and rapidly overheat and degrade without thermal management, which leads to downtime of the microscope and increased maintenance costs.

Summary

25 In a first aspect, there is provided an external illuminator for a microscope, the external illuminator comprising a light source; at least one optical fibre optically coupled to the light source, the at least one optical fibre configured to deliver and direct light from the light source along an illumination path to a sample being observed through an objective lens of the microscope, the objective lens having a
30 working distance of 2mm or less; and an adjustment module couplable to a portion of the microscope and arranged to support the at least one optical fibre, wherein the adjustment module is configured to adjust the at least one optical fibre spatially and/or angularly relative to the objective lens in a manner such that the illumination path is separate from a detection path of the objective lens, and the objective lens

is movable to the working distance of the objective lens. Advantageously, the optical fibre may be adjusted to illuminate the sample within the confines of the short working distance of the objective lens and provide a compact and low-profile illumination path.

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Preferably, the adjustment module comprises a mount configured to releasably couple to the portion of the microscope; at least one pillar extending from the mount; at least one tubular holder holding the at least one optical fibre; and a hinge connector assembly arranged between the at least one pillar and the at least one
10 tubular holder, wherein the at least one pillar and the at least one tubular holder are pivotally coupled to each other via the hinge connector assembly. Preferably, the at least one pillar comprises a distal end attached to the mount, and a proximal end opposite to the distal end; and wherein the hinge connector assembly is disposed at the proximal end, wherein preferably the at least one pillar is extendable.

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Preferably, the mount comprises a ring mount configured to releasably couple around a housing of the objective lens. The ring mount may be provided with a non-mechanical fastening mechanism to couple releasably around the housing.

20 Preferably, the mount configured to releasably couple to the portion of the microscope comprises a non-mechanical coupling of the mount to the portion of the microscope. More preferably, the mount includes a first mount mating member to mate with an objective lens mating member of a housing of the objective lens. Even more preferably, the mount includes a second mating member to mate with a focal
25 tube mating member of the microscope.

Preferably, the at least one tubular holder comprises a first end, a second end opposite the first end, and a body extending between the first end and the second end; and wherein the hinge connector assembly is disposed along a part of the
30 body.

Preferably, the mount includes a slot for receiving a removable optical filter, and preferably the removable optical filter. Advantageously, undesired wavelengths may be filtered out.

Preferably, the illumination path is arrangeable with respect to the detection path in a manner to facilitate observation of the sample in a reflective mode.

- 5 Preferably, the light source has a wavelength of 300 nm or less, preferably from 100 nm to 300 nm, more preferably from 200 nm to 300 nm. More preferably, the light source is configured to emit light of another wavelength, and wherein a difference between the wavelength and the other wavelength is 50 nm or less.
- 10 Preferably, the external illuminator further comprises a subsequent light source configured to optically couple to the at least one optical fibre, wherein the subsequent light source has a predetermined wavelength, preferably a difference between the predetermined wavelength of the subsequent light source and the wavelength of the light source is 50 nm or less. More preferably, the subsequent
- 15 light source is a light emitting diode or a laser. Advantageously, the use of two or more wavelengths to illuminate the sample may provide images of the sample with different contrasts which may be combined to provide a more informative image of the sample.
- 20 Preferably, the external illuminator further comprises a non-mechanically fastened mechanism operable to switch between the light source and the subsequent light source to allow the light source and the subsequent light source to each be coupled independently to the at least one optical fibre for illuminating the sample. More preferably, the non-mechanically fastened mechanism is a magnetic fastened
- 25 mechanism. Advantageously, this allows for a quick and easy switching between light sources.

- Preferably, the at least one optical fibre comprises a light-entry end configured to receive the light from the light source or the subsequent light source; and a light-
- 30 emitting end opposite to the light-entry end, the light-emitting end configured to deliver the light to the sample, wherein the non-mechanically fastened mechanism comprises a mating member coupled towards the light-entry end of the at least one optical fibre; and a complementary mating member configured to couple to the light source for a first period of time and to the subsequent light source for a second

period of time, and wherein the mating member and the complementary mating member are configured to mate each other to provide optical coupling between the at least one optical fibre and the light source for the first period of time, and between the at least one optical fibre and the subsequent light source for the second period
5 of time.

Preferably, there are at least two optical fibres coupled to the light source which are arranged in at least one of the following conditions: in contact side by side, in a three-sided arrangement, and in a polygonal arrangement. More preferably, the
10 adjustment module is configured to independently adjust the at least two optical fibres spatially and/or angularly relative to the objective lens such that the illumination path is separate from a detection path of the objective lens.

Preferably, the at least two optical fibres are configurable to allow each optical fibre
15 to illuminate the sample independently.

Preferably, the light source is a light emitting diode or a laser.

Preferably, the adjustment module is configured to adjust the at least one optical
20 fibre spatially and/or angularly relative to the objective lens to illuminate the sample outside of an acceptance cone of the objective lens, preferably at an acute angle measured from a central axis of each optical fibre and a horizontal plane of a sample holder for holding the sample when being observed through the objective lens of the microscope, the acute angle being greater than 0° but less than or equal to 30° .
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In a second aspect, there is provided a microscope comprising an external illuminator as described in the first aspect, the external illuminator configured to provide light via an illumination path to illuminate a sample being observed by the microscope; and a detection module comprising an objective lens configured to
30 receive the light from the sample via a detection path to observe the sample, wherein the illumination path is separate from the detection path, and the objective lens is movable to the working distance of the objective lens.

Preferably, the detection module further comprises at least one of the following: a viewer, an image detector, and a camera. Preferably, the microscope is a fluorescence microscope.

- 5 In a third aspect, there is provided a microscopy method comprising: delivering and directing light from a light source, via at least one optical fibre, along an illumination path to a sample; adjusting the at least one optical fibre spatially and/or angularly relative to an objective lens in a manner such that the illumination path is separate from a detection path of the objective lens; adjusting the objective lens if needed to
10 its working distance, the objective lens having a working distance of 2mm or less; and viewing and/or imaging the sample via the detection path.

Preferably, delivering and directing the light further comprises switching, via a non-mechanically fastened mechanism, the light source and a subsequent light source
15 to deliver and direct the light. More preferably, each of the light source and the subsequent light source has a wavelength of 300 nm or less, preferably from 100 nm to 300 nm, more preferably from 200 nm to 300 nm. Preferably, a difference between the wavelength of the light source and the wavelength of the subsequent light source is 50 nm or less.

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Preferably, the illumination path is arrangeable with respect to the detection path in a manner to facilitate observation of the sample in a reflective mode.

Preferably, adjusting the at least one optical fibre comprises adjusting the at least
25 one optical fibre in a manner to illuminate the sample outside of an acceptance cone of the objective lens, preferably at an acute angle measured from a central axis of each optical fibre and a horizontal plane of a sample holder for holding the sample when being observed through the objective lens, the acute angle being greater than 0° but less than or equal to 30°.

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Preferably, there are two or more optical fibres grouped into two or more sets of optical fibres, wherein delivering and directing light from a light source comprises delivering and directing light sequentially from each set in the two or more sets of

optical fibres. In other words, the illumination of the sample may be provided in any specific sequence of illumination involving a subset of at least two optical fibres to provide any specific sequence of unique images from a single imaged field of view for analysis or processing to obtain additional information. Advantageously, the additional information may include, but is not limited to, depth and surface topography, contributing to the 3D visualisation of the sample.

In a fourth aspect, there is provided an adjustment module couplable to a portion of a microscope and arranged to support at least one optical fibre for delivering and directing light via an illumination path to illuminate a sample observed by the microscope, the adjustment module comprising a mount configured to releasably couple to the portion of the microscope; at least one pillar extending from the mount; at least one tubular holder, each configured to hold the at least one optical fibre; and a hinge connector assembly arranged between the at least one pillar and the at least one tubular holder, wherein the at least one pillar and the at least one tubular holder are pivotally coupled to each other via the hinge connector assembly, wherein the adjustment module is configured to adjust the at least one optical fibres at least spatially or angularly relative to an objective lens of the microscope in a manner such that the illumination path is separate from the detection path, and wherein the objective lens has a working distance of 2 mm or less.

Preferably, the at least one pillar comprises a distal end attached to the mount, and a proximal end opposite to the distal end; and wherein the hinge connector assembly is disposed at the proximal end, wherein preferably the at least one pillar is extendable.

Preferably, the mount comprises a ring mount configured to releasably couple around a housing of the objective lens. Preferably, the mount is non-mechanically couplable to the portion of the microscope.

Preferably, the mount includes a first mount mating member to mate with an objective lens mating member of a housing of the objective lens. More preferably, the mount includes a second mating member to mate with a focal tube mating member.

Preferably, the at least one tubular holder comprises a first end, a second end opposite the first end, and a body extending between the first end and the second end; and wherein the hinge connector assembly is disposed along a part of the
5 body.

Preferably, the mount includes a slot for receiving a removable optical filter, and preferably the removable optical filter.

10 Advantageously, the aspects and embodiments described herein provide a relatively low-cost external illuminator that may be used with a standard microscope objective lens with a short working distance of 2mm or less. The illuminator may be used with a DUV light source to allow DUV microscopy to be more widely performed without requiring specialised and expensive DUV microscopes.

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Detailed Description

Figure (FIG.) 1 shows a schematic drawing of an existing microscope setup used in fluorescence imaging;

FIG. 2 shows a schematic drawing of an embodiment of the external illuminator attached to a microscope tube and objective lens;
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FIG. 3A shows a picture of an embodiment of the external illuminator attached to a microscope tube and objective lens, and FIG. 3B is an enlarged view of the external illuminator attached to the microscope tube; and

FIGs. 4A and 4B show a thick slice of a rat brain and the inner wall of a rat stomach respectively.
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FIG. 5 shows a schematic drawing of a disassembled light source with the magnetic fastening mechanism;

FIG. 6 shows a picture of an embodiment of the light source of the external illuminator where the light source is optically coupled to two optical fibres via a non-mechanically magnetically fastened mechanism;
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FIG. 7 shows a schematic drawing of the external illuminator non-mechanically coupled to the objective lens and focal tube;

FIG. 8 shows a schematic drawing of an embodiment of the external illuminator with an optical filter;

FIG. 9 shows a schematic drawing of the external illuminator with the optical filter removed;

FIG. 10 shows a picture of an embodiment of the external illuminator holding the objective lens connected to the focal tube, with a partially inserted filter along the optical signal detection path;

FIG. 11 shows a top-down schematic view of an embodiment of the external illuminator with four optical fibres for sequential illumination by each optical fibre;

FIG. 12 shows pictures of the same region of a thick slice of rat heart illuminated by sequential direction of illumination. Panels A, B, C, and D show illumination from the top, right, bottom and left of the picture respectively;

FIG. 13 shows a picture of a 3D rendered model of the thick slice of rat heart obtained from the four pictures in FIG. 12.

Whilst there has been described in the following description various embodiments of the invention, it will be understood by those skilled in the field concerned that many variations or modifications in details of design or construction may be made without departing from the present invention. 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.

Where a combination is disclosed, each subcombination of the elements of that combination is also specifically disclosed and is within the scope of the invention. Conversely, where different elements or groups of elements are disclosed, combinations thereof are also disclosed. Where any element of an invention is disclosed as having a plurality of alternatives, examples of that invention in which each alternative is excluded singly or in any combination with the other alternatives are also hereby disclosed; more than one element of an invention can have such exclusions, and all combinations of elements having such exclusions are hereby disclosed.

Unless defined otherwise or the context clearly dictates otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The terms “about”, “approximately”, “substantially” must be read with reference to the context of the application as a whole, and have regard to the meaning a particular technical term qualified by such a word usually has in the field concerned.

- 5 For example, it may be understood that a certain parameter, function, effect, or result can be performed or obtained within a certain tolerance, and the skilled person in the relevant technical field knows how to obtain the tolerance of such term.

- 10 The phrase “at least one of A and B” means it requires only A alone, B alone, or A and B, i.e. only one of A or B is required. The phrase “A and/or B” includes A alone, B alone, and A and B.

- Terms such as “coupled”, “connected”, “attached”, “conjugated and “linked” are used interchangeably herein and encompass direct as well as indirect connection,
15 attachment, linkage or conjugation unless the context clearly dictates otherwise.

- FIG. 2 shows an embodiment of an external illuminator 100 (may also be termed illumination device) that delivers light externally for the illumination of a biological sample 5 for the microscopic imaging of optical fluorescence signals. The light from
20 a light source 150 is delivered and directed by at least one optical fibre 125 or waveguide that have a separate illumination path outside of the optical signal detection path. The embodiment shown in FIG. 2 has two optical fibres 125 and provides advantages as further described below. In an embodiment, the illumination path may be arranged with respect to the detection path for the sample 5 to be
25 observed in a reflective mode (i.e. the optical fibre 125 and objective lens 10 are on the same side with respect to sample 5 and correspondingly the illumination path and the detection path). This embodiment is different from other microscope illumination devices where the sample is illuminated from the back of the sample, where the illumination path is on the opposite side of the detection path.

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In another embodiment (not shown in the figures), the external illuminator 100 may be used as a backlight illumination of a sample target. However, in this other embodiment, the samples that may be observed in a transmission mode may be limited to extremely thin sample targets for the light to penetrate through.

Additionally, there is the advantage of the light being blocked by the objective lens 10, providing an exceptional sample signal to light source noise ratio. Thus, the use of the illuminator as a backlight illumination may still be considered as having an illumination path separate from the detection path of the objective lens. However, 5 the limitations of the thickness of the samples that may be observed may make the backlight illumination by the illuminator 100 less useful or practical.

The light may be of wavelengths including, but not limited to, deep-ultraviolet wavelengths (300 nanometres (nm) or less), in particular 100 nm to 300 nm, or 200 10 nm to 300 nm. The 200 nm to 300 nm wavelength range is a common range used in DUV fluorescence microscopy. The spatial and angular positioning of light delivery towards the sample 5 is controlled by adjusting the optical fibre 125 along at least 3 axes of freedom. The light is coupled into the optical fibre 125 from one or more light sources 150 such as a light emitting diode 155 or laser. In an 15 embodiment, one or more optical fibres 125 may be used and the light may be coupled into one or more of the optical fibres 125. Multiple optical fibres 125 may be coupled to each light source 150, and may be rapidly decoupled/switched ('hot swapped') from that source 150 and coupled to a different light source 150 via a non-mechanically fastened mechanism such as by magnetic force. In an 20 embodiment, a light source 150 with multiple switchable variable or pre-determined wavelengths may be used. In an embodiment, multiple light sources 150 with a single pre-determined wavelength or with multiple pre-determined or variable wavelengths may be used. In an embodiment, a light source 150 with a single pre-determined wavelength may be used. Each of the light sources 150 may 25 independently produce light of wavelengths including but not limited to deep-ultraviolet wavelengths (300 nanometres (nm) or less), in particular 100 nm to 300 nm, or 200 nm to 300 nm. Some of the light sources 150 may have a small difference between them in centre wavelength of at most 50 nanometres or be tuneable over a range of at most 50 nanometres.

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The illumination device 100 delivers deep ultraviolet or other wavelength illumination to a biological sample 5 for imaging via a separate illumination path outside of the optical detection path of the objective lens 10. The device 100 uses one or more optical fibres 125 to deliver and direct light from a light source 150 onto

the sample 5. In an embodiment, at least one optical fibre 125 may be used to couple light from one or more light sources 150. The optical fibre/s 125 provide a compact and low-profile oblique illumination path that reaches the sample 5 unencumbered by the short working distance 20 of the objective lens 10 and produces a uniform illumination over the imaging field of view. The standard high magnification and relatively low-cost objective lens 10 used in the embodiments herein may have a working distance of 2 millimetres or less to provide the required magnification and resolution. The objective lens 10 and the housing 15 may be moved to the required working distance 20 from the sample 5 or sample holder 7 to provide a sharp and focused image. If the sample 5 is positioned at a distance different from the working distance 20, the image may be out of focus and blurred.

The optical fibre/s 125 illuminate the sample 5 at an acute angle (may be termed illumination angle) measured from a central axis of each optical fibre 125 and a horizon or horizontal axis of the sample holder 7, the acute angle being greater than 0° but less than or equal to 30° . Alternatively, the angle may be measured from a central axis of the optical fibre and a central axis of the objective lens 10, and such an angle being more than or equal to 60° but less than 90° (as the central axis of the objective lens 10 is preferably perpendicular to the horizon). An adjustment module 105 is coupled to a portion of the microscope, for example the housing 15 of the objective lens 10, which may be standalone or part of an existing microscope. The adjustment module 105 is arranged to support the optical fibre 125 and is configured to adjust the optical fibre 125 spatially and/or angularly relative to the objective lens 10 to ensure that the illumination path is separate from the detection path of the objective lens 10. When there are at least two optical fibres 125, the adjustment module 105 is arranged to support the optical fibres 125 and is configured to independently adjust each of the optical fibres 125 spatially and/or angularly relative to the objective lens 10. Thus, the adjustment module 105 allows for independent triaxial movement of each of the optical fibre/s 125 for illumination of a sample 5 placed in a sample holder 7 of the microscope. The illumination path is separate from a detection path of the objective lens 10 and may operate in a reflective mode of sample illumination and detection to facilitate observation of the sample 5. The adjustment module 105 allows the position and angle of each optical fibre 125 to be independently adjusted along at least 3 axes of freedom (spatial and

angular movement). The use of optical fibres 125 avoids the problem of direct broad illumination from the source in many existing devices, which unnecessarily illuminates a large area and bleaches or damages the sample.

- 5 In an embodiment shown in FIG. 2, the adjustment module 105 includes a mount 107 configured to be releasably secured to the housing 15 of the objective lens 10 with at least one pillar 110 extending from the mount 107. Each pillar 110 is pivotally coupled by a hinge connector assembly 120 to a tubular holder 115 at the end away from the mount 107. Hence, the adjustment module 105 has at least one tubular
- 10 holder 115 with each holding one optical fibre 125. When there are two or more pillars, the position of the pillars 110 relative to each other may be adjustable, for example the two pillars 110 may be arranged directly opposite to each other (at an angle of 180°), or other desired angles.
- 15 In an embodiment, the mount 107 may be a ring mount 107 configured to releasably couple or secure around the housing 15, non-limiting examples include a clamp mechanism, a magnetic mechanism, nuts and bolts, and fasteners. Thus, each pillar 110 has a distal end attached to the mount and a proximal end pivotally coupled to the tubular holder 115 by the hinge connector assembly 120 (may be termed hinged
- 20 connection). In an embodiment, each pillar 110 may be extendable (for example telescopic) to allow the vertical position of the tubular holder 115 and hence the optical fibre 125 to be independently adjusted. The adjustment module 107 allows each optical fibre 125 to be independently positioned by adjusting at least one of the following:
- 25 (i) the position of the optical fibre 125 along the axis of the tubular holder 115,
(ii) the angle of the hinged connection 120,
(iii) the vertical height of the adjustment module, for example by moving the ring mount 107 on the lens housing 15 and/or by extending (or adjusting) the at least one pillar 110 that may be telescopic,
- 30 (iv) the rotational position of the ring mount 105 around the microscope tube housing 15, and
(v) the rotational position of each pillar 110.

The flexibility of positioning the optical fibres 125 provides versatility to adapt to objective lenses 10 of various small working distances 20.

The fibre 125 receive light from the light source/s 150 with their end-faces (the light-entry ends) in direct contact with the light-emitting surface for maximal light coupling. 5 The light-emitting end of the optical fibre 125 are directed to illuminate the sample 5. Two or more fibres 125 may be concurrently coupled to a single light source 150. The fibre/s 125 may be coupled (mounted) to the light source 150 by a non-mechanical fastening mechanism such as magnetic force, enabling rapid mating and hot swapping to alternate light sources 150 as required. Other examples of non-mechanical fastening mechanisms including, but not limited to, velcro strips, adhesives, stickers, clips, dowels, sliding dovetail, box joints, through dovetail, half-blind dovetail, a mortise-and-tenon joint, and the like may be used. In an embodiment, the light source 150 may be placed in a light source mount 160 with 10 the non-mechanical fastening mechanism. In an embodiment, the non-mechanically fastened mechanism may have a mating member coupled towards the light-entry ends of each optical fibre 125 and a complementary mating member configured to couple the optical fibre 125 to the light source 150. The mating member and complementary mating member allows for easy and rapid optical coupling of the 15 optical fibres 125 to the light source 150, for example by a magnetic mechanism. When there are two or more light sources 150, the mating member of the optical fibre 125 may be rapidly coupled and decoupled with the complementary mating member of each light source 150 and allows for the quick switching ("hot swapping") between the two or more light sources 150. This allows for the optical fibres 125 to 20 be optically coupled to a light source for a first period of time, after which it may be switched and optically coupled to another light source for a second period of time. Some of the light sources 150 may have centre wavelengths very close to each other and separated by at most 50 nanometres, to generate microscopic images with contrast for specific molecular components such as DNA. For example, two 25 light sources may have wavelengths of 260 nm and 270 nm. 30

FIG. 5 shows a schematic diagram of an embodiment of the light source mount 160 with the non-mechanical fastening mechanism disassembled. The embodiment in FIG. 5 uses two pairs of magnets and a mortise-and-tenon joint as the non-

mechanical fastening mechanism. Alternatively, either one alone may be used. The light source mount 160 may be made up of a base 170 with a groove and a cover 175 which is insertable into the groove of the base 170. The base 170 and cover 175 each have two magnets 165 that are used to couple the base 170 and cover 175. The magnets may be permanent magnets or temporary magnets that may be controlled by a switch. The groove of the base 170 as shown in FIG. 5 may have a mortise hole 180 which is adapted to receive a complementary tenon tongue 185 (a projection) disposed in the cover 175 forming the mortise-and-tenon joint. A bidirectional arrow 50 in FIG. 5 indicates the insertion of the tenon tongue 185 into the mortise hole 180. The combination of the magnets 165 and mortise-and-tenon joint provides a secure but releasable non-mechanical coupling mechanism to couple the optical fibre 125 to the light source 150, e.g. as shown in FIG. 6. The centre of the tenon tongue 185 may be provided with a through channel 190 to allow the at least one optical fibre 125 to pass through the cover 175 and tenon tongue 185 while being secured by the light source mount 160. The light source 150 may be placed near or at the mortise hole 180 to be coupled to the optical fibre 125 thereby allowing transmission of light from the light source 150 to the at least one optical fibre 125.

FIG. 6 shows a picture of the light source mount of the embodiment in FIG. 5 with two optical fibres 125 non-mechanically fastened to the light source 150. In particular, FIG. 6 shows two optical fibres held in place in the channel 190 in the tenon tongue 185.

FIG. 7 shows a schematic drawing of an embodiment modified from the previous embodiment of FIG. 2. The embodiment in FIG. 7 shows the adjustment module 105 having separate non-mechanical coupling or fastening mechanism between the adjustment module 105 (and hence the illuminator 100) to the objective lens 10 and a focal tube 35 of the microscope. The focal tube 35 may be connected to a viewer and/or image capture device at the opposite end of the connection to the adjustment module 105. An example of a non-mechanical fastening mechanism is a magnetic fastening mechanism. In FIG. 7, the mount 107 has a (first) mount mating member 130 on one side which mates with a complementary objective lens mating member on the objective lens or a portion of the housing 15. On the opposite side, the mount

107 may have another (i.e. a second) mating member 135 which mates with a focal tube mating member on the focal tube 35. The mating members 130, 135 may be magnets, for example, permanent magnets or temporary magnets that may be controlled by a switch. Advantageously, the use of a non-mechanical coupling to connect the objective lens 10 and mount 107 allows for switching of different objective lens 10 quickly, i.e. hot swapping. As each objective lens 10 has a unique illumination configuration of angle and position of the optical fibres 125, the quick and easy swapping of different objective lens 10 allow the image to be viewed differently, quickly and easily. The non-mechanical coupling of the mount 107 to the focal tube 35 also allows the focal tube 35 and viewer to be easily and quickly changed if needed.

FIG. 8 shows a schematic drawing of the light source 150 decoupled with the optical fibres 125, and the focal tube 35 decoupled with the mount 107. The mount 107 may further include a filter slot 145 configured to receive an optical filter 140. The bidirectional arrows 80 indicate that the components may be inserted or withdrawn to attach or detach the components. The optical filter 140 may be used to exclude or block certain fluorescence emission wavelengths from reaching the viewer and/or image capture device, and may improve the image observed. The optical filter 140 may be placed in a filter holder that may be received in the filter slot 145. The filter slot 145 may be configured with tracks or other similar configuration to allow for the optical filter 140 (and filter holder) to be easily inserted and withdrawn from the filter slot 145. FIG. 9 shows the embodiment in FIG. 8 with the optical filter 140 removed from the filter slot 145. Hence, the optical filter 140 may be considered in a sense to be non-mechanically coupled to or integrated into the optical path of the microscope. Advantageously, this enables the insertion, removal, and swapping of optical filters 140 while the system is in use, which is advantageous for excluding certain fluorescence emission wavelengths.

FIG. 10 shows a picture of an embodiment of the illumination device 100 holding the objective lens 10 and connected to the focal tube 35, with a partially inserted optical filter 140 (about the middle of the photo) along the optical signal detection path. The embodiment in FIG. 10 has four optical fibres 125 arranged approximately equidistant radially from the centre of the objective lens 10. Thus, each optical fibre

125 is arranged between two adjacent optical fibres 125, that is, one of the two adjacent optical fibres 125 being at approximately 90° to the left and the other one of the two adjacent optical fibres being at approximately 90° to the right, and opposite one optical fibre 125. FIG. 11 shows a top-down schematic view of the embodiment in FIG. 10 where the arrangement of the four optical fibres 125 (more specifically, 125a, 125b, 125c, 125d) from the top may be observed.

In some embodiments, illumination of the sample 5 may come from a subset of the optical fibres 125 at a given time. For example, when there are two or more optical fibres 125, the sample 5 may be illuminated by a subset first, and then the remaining optical fibres, the sequence may be repeated with the same or different subsets. In an example, there are two optical fibres 125, and the sample 5 is illuminated alternately by the two optical fibres 125. In another example, there are four optical fibres 125 as the embodiment in FIG. 10 and FIG. 11. The sample 5 may be illuminated by each optical fibre 125 sequentially. As viewed in FIG. 11, the sample 5 may be illuminated by each optical fibre 125 in a clockwise direction (e.g. from 125a to 125b to 125c to 125d), an anticlockwise direction, or any other sequence and/or combination of the four optical fibres. Thus, the sample 5 may be illuminated in any specific sequence or direction. The sequential illumination of the sample 5 by a combination and sequence of optical fibres 125 allows multiple images with each unique illumination direction to be obtained from the single imaged field of view. The unique two-dimensional images obtained from the sequential illumination may be processed or analysed to extract additional information such as, but not limited to, depth and surface topography which contribute to three-dimensional visualisation of the sample 5.

FIG. 12 shows the pictures of the same sample 5 (region) of a thick slice of rat heart illuminated in four directions sequentially with the embodiment in FIG. 11. Panels A, B, C and D of FIG. 12 show the illumination from the optical fibre 125a providing illumination from the back, the optical fibre 125b providing illumination from the right, the optical fibre 125c providing illumination from the front, and the optical fibre 125d providing illumination from the left in FIG. 11, respectively. The illumination directions are relative to each other and based with respect to the sample 5.

FIG. 13 shows a picture of a 3D rendered model of the thick slice of rat heart providing depth information, obtained from the images shown in the four panels A to D of FIG. 12.

5 Fluorescence microscopy on a sample may be performed by using the illuminator 100. The illuminator 100 may be used as an add-on to convert a basic widefield microscope into a fluorescence microscope with no modification to the original optical design or path. The illuminator 100 enables exceptionally low-cost deep UV
10 samples 5 which cannot be backilluminated because the illumination will be obstructed by the sample 5. Alternatively, the illuminator 100 may be used with a standalone objective lens 10 and coupled to a viewer or camera to capture the image. In an embodiment, a microscope may be set up having the illuminator 100 as described and a detection module having an objective lens 10 to receive light
15 from the sample 5 via the detection path to observe the sample 5. The fluorescence microscopy may be performed by setting a working distance 20 of the objective lens 10 to view a sample 5, wherein the working distance 20 is 2 mm and less; Illuminating the sample 5 with the illuminator 100; and optionally viewing the sample 5 and/or imaging the sample 5. The detection module may have any combination of
20 a viewer, an image detector, and a camera to image and/or view the sample 5.

FIG. 3A shows a photograph of a perspective view of an embodiment of the illuminator 100 attached to a housing 15. FIG. 3B shows an expanded view of the housing 15 with the adjustment module 105 attached to the housing 15.

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FIG. 4A and 4B respectively show captured images of a thick slice of rat brain and the inner wall of a rat stomach. In both figures, it may be seen that the illuminator 100 magnifies and resolves the features of the viewed samples 5.

30 The illuminator 100 described provides a method of delivering and directing sufficient illumination to a sample 5 using at least one optical fibre 125 via an illumination path separate from the optical detection path via a very small working distance 20 between the objective lens 10 and sample 5 for fluorescence microscopy. The illuminator 100 provides a method of achieving uniform illumination

or multiple wavelengths of illumination of the sample 5 via at least one optical fibre 125. An adjustment mechanism enables optimisation of the illumination angle by allowing each of the optical fibre 125 to move in at least 3 axes of freedom. The non-mechanical fastening of the optical fibre 125 to the light source mount 160, for example by using magnets, allows efficient optical coupling of optical fibre 125 to multiple light sources 150 and rapid swapping of light sources 150. The set of light sources 150 may have very close centre wavelengths, for example separated by at most 50 nanometres or have a tuneable wavelength range of up to 50 nanometres to generate images of specific molecular contrast.

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The illuminator 100 as described above may be used in a microscopy method. The method may include delivering and directing light from a light source 150 along an illumination path to a sample 5 via one or more optical fibres 125, adjusting independently each of the at least one optical fibre 125 spatially or angularly relative to the objective lens 10 such that the illumination path is separate from the detection path of the objective lens 10, adjusting the objective lens 10 if needed to its working distance, the objective lens 10 having a working distance of 2mm or less, and viewing and/or imaging the sample 5 via the detection path.

20 In an embodiment, the illumination path may be arranged with respect to the detection path to facilitate observation of the sample 5 in a reflective mode. In an embodiment, each of the optical fibres 125 may be independently adjusted in a manner to illuminate the sample 5 at an acute angle measured from a central axis of each optical fibre and a horizon (or a central axis of the objective lens 10).

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The use of multiple adjustable optical fibres 125 to illuminate outside of the objective lens optical detection path allows a small working distance 20 (2mm and less) to be used for the objective lens 10. The use of magnets achieves efficient and easily swapped optical coupling of multiple light sources with a centre wavelength separated by at most 50 nanometres.

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The illuminator 100 may be used in histopathology (i.e. preparation of tissue samples for medical diagnosis) as an add-on to an existing standard microscope with small space requirements to provide a very low cost and compact microscope

that may be switched between a standard microscope and a fluorescence microscope. Labs in the fields on tissue-based omics and 3D cell culture (e.g. cultured meat) may also utilise the illuminator 100 and fluorescence microscope described herein.

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Claims

1. An external illuminator for a microscope, the external illuminator comprising:
 - a light source;
 - at least one optical fibre optically coupled to the light source, the at
 - 5 least one optical fibre configured to deliver and direct light from the light
 - source along an illumination path to a sample being observed through an
 - objective lens of the microscope, the objective lens having a working
 - distance of 2mm or less; and
 - an adjustment module couplable to a portion of the microscope and
 - 10 arranged to support the at least one optical fibre,
 - wherein the adjustment module is configured to adjust the at
 - least one optical fibre spatially and/or angularly relative to the
 - objective lens in a manner such that the illumination path is separate
 - from a detection path of the objective lens, and the objective lens is
 - 15 movable to the working distance of the objective lens, and
 - the adjustment module comprises:
 - a mount configured to releasably couple to the portion
 - of the microscope;
 - at least one pillar extending from the mount;
 - 20 at least one tubular holder holding the at least one
 - optical fibre; and
 - a hinge connector assembly arranged between the at
 - least one pillar and the at least one tubular holder, the at least
 - one pillar and the at least one tubular holder being pivotally
 - 25 coupled to each other via the hinge connector assembly.
2. The external illuminator as claimed in claim 1,
 - wherein the at least one pillar comprises a distal end attached to the
 - mount, and a proximal end opposite to the distal end; and
 - 30 wherein the hinge connector assembly is disposed at the proximal
 - end.

3. The external illuminator as claimed in claim 1 or 2, wherein the mount comprises a ring mount configured to releasably couple around a housing of the objective lens.
- 5 4. The external illuminator as claimed in claim 1 or 2, wherein the mount configured to releasably couple to the portion of the microscope comprises a non-mechanical coupling of the mount to the portion of the microscope.
- 10 5. The external illuminator as claimed in claim 4, wherein the mount includes a first mount mating member to mate with an objective lens mating member of a housing of the objective lens.
- 15 6. The external illuminator as claimed in claim 5, wherein the mount includes a second mating member to mate with a focal tube mating member of the microscope.
- 20 7. The external illuminator as claimed in any one of claims 1 to 6,
wherein the at least one tubular holder comprises a first end, a second end opposite the first end, and a body extending between the first end and the second end; and
wherein the hinge connector assembly is disposed along a part of the body.
- 25 8. The external illuminator as claimed in any one of claims 1 to 7, wherein the mount includes a slot for receiving a removable optical filter.
- 30 9. The external illuminator as claimed in any one of claims 1 to 8, wherein the illumination path is arrangeable with respect to the detection path in a manner to facilitate observation of the sample in a reflective mode.
10. The external illuminator as claimed in any one of claims 1 to 9, wherein the light source has a wavelength of 300 nm or less.

11. The external illuminator as claimed in claim 10, wherein the light source is configured to emit light of another wavelength, and wherein a difference between the wavelength and the other wavelength is 50 nm or less.
- 5 12. The external illuminator as claimed in in any one of claims 1 to 11, further comprising a subsequent light source configured to optically couple to the at least one optical fibre, wherein the subsequent light source has a predetermined wavelength, wherein a difference between the predetermined wavelength of the subsequent light source and the wavelength of the light source is 50 nm or less.
- 10 13. The external illuminator as claimed in claim 12, wherein the subsequent light source is a light emitting diode or a laser.
- 15 14. The external illuminator as claimed in claim 12 or 13, further comprising a non-mechanically fastened mechanism operable to switch between the light source and the subsequent light source to allow the light source and the subsequent light source to each be coupled independently to the at least one optical fibre for illuminating the sample.
- 20 15. The external illuminator as claimed in claim 14, wherein the non-mechanically fastened mechanism is a magnetic fastened mechanism.
- 25 16. The external illuminator as claimed in claim 14 or 15, wherein the at least one optical fibre comprises a light-entry end configured to receive the light from the light source or the subsequent light source; and a light-emitting end opposite to the light-entry end, the light-emitting end configured to deliver the light to the sample,
- 30 wherein the non-mechanically fastened mechanism comprises a mating member coupled towards the light-entry end of the at least one optical fibre; and a complementary mating member configured to couple to the light source for a first period of time and to the subsequent light source for a second period of time, and

wherein the mating member and the complementary mating member are configured to mate each other to provide optical coupling between the at least one optical fibre and the light source for the first period of time, and between the at least one optical fibre and the subsequent light source for the second period of time.

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17. The external illuminator as claimed in any one of claims 1 to 16, wherein there are at least two optical fibres coupled to the light source which are arranged in at least one of the following conditions: in contact side by side, in a three-sided arrangement, and in a polygonal arrangement.

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18. The external illuminator as claimed in claim 17, wherein the adjustment module is configured to independently adjust the at least two optical fibres spatially and/or angularly relative to the objective lens such that the illumination path is separate from a detection path of the objective lens.

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19. The external illuminator as claimed in claim 17 or 18, wherein the at least two optical fibres are configurable to allow each optical fibre to illuminate the sample independently.

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20. The external illuminator as claimed in any one of claims 1 to 19, wherein the light source is a light emitting diode or a laser.

21. The external illuminator as claimed in any one of claims 1 to 20, wherein the adjustment module is configured to adjust the at least one optical fibre spatially and/or angularly relative to the objective lens to deliver the light onto the sample such that delivery of the light falls outside of an acceptance cone of the objective lens.

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22. The external illuminator as claimed in claim 21, wherein the adjustment module is configured to adjust the at least one optical fibre angularly relative to the objective lens at an acute angle measured from a central axis of each optical fibre and a horizontal plane of a sample holder for holding the sample

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when being observed through the objective lens of the microscope, the acute angle being greater than 0° but less than or equal to 30° .

23. A microscope comprising:

an external illuminator as claimed in any one of claims 1 to 22, the external illuminator configured to provide light via an illumination path to illuminate a sample being observed by the microscope; and

a detection module comprising an objective lens configured to receive the light from the sample via a detection path to observe the sample,

wherein the illumination path is separate from the detection path, and the objective lens of the detection module is movable to the working distance of the objective lens.

24. The microscope as claimed in claim 23, wherein the detection module further comprises at least one of the following: a viewer, an image detector, and a camera.

25. A microscopy method comprising:

(a) delivering and directing light from a light source, via at least one optical fibre, along an illumination path to a sample;

(b) adjusting, by an adjustment module, the at least one optical fibre spatially and/or angularly relative to an objective lens in a manner such that the illumination path is separate from a detection path of the objective lens;

(c) adjusting the objective lens if needed to its working distance, the objective lens having a working distance of 2mm or less; and

(d) viewing and/or imaging the sample via the detection path,

wherein the adjustment module comprises:

a mount releasably coupled to the portion of a microscope performing the microscopy method;

at least one pillar extending from the mount;

at least one tubular holder holding the at least one optical fibre;

and

a hinge connector assembly arranged between the at least one pillar and the at least one tubular holder, the at least one pillar and the at least one tubular holder being pivotally coupled to each other via the hinge connector assembly.

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26. The microscopy method as claimed in claim 25, wherein delivering and directing the light further comprises switching, via a non-mechanically fastened mechanism, the light source and a subsequent light source to deliver and direct the light.

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27. The microscopy method as claimed in claim 26, wherein each of the light source and the subsequent light source has a wavelength of 300 nm or less.

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28. The microscopy method as claimed in claim 27, wherein a difference between the wavelength of the light source and the wavelength of the subsequent light source is 50 nm or less.

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29. The microscopy method as claimed in any one of claims 25 to 28, wherein the illumination path is arrangeable with respect to the detection path in a manner to facilitate observation of the sample in a reflective mode.

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30. The microscopy method as claimed in any one of claims 25 to 29, wherein adjusting the at least one optical fibre comprises adjusting the at least one optical fibre in a manner to deliver the light onto the sample such that delivery of the light falls outside of an acceptance cone of the objective lens.

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31. The microscopy method as claimed in claim 30, wherein adjusting the at least one optical fibre comprises adjusting the at least one optical fibre at an acute angle measured from a central axis of each optical fibre and a horizontal plane of a sample holder for holding the sample when being observed through the objective lens, the acute angle being greater than 0° but less than or equal to 30°.

32. The microscopy method as claimed in any one of claims 25 to 31, wherein there are two or more optical fibres grouped into two or more sets of optical fibres,
wherein delivering and directing light from a light source comprises delivering and directing light sequentially from each set in the two or more sets of optical fibres.
33. An adjustment module couplable to a portion of a microscope and arranged to support at least one optical fibre for delivering and directing light via an illumination path to illuminate a sample observed by the microscope, the adjustment module comprising:
a mount configured to releasably couple to the portion of the microscope;
at least one pillar extending from the mount;
at least one tubular holder, each configured to hold the at least one optical fibre; and
a hinge connector assembly arranged between the at least one pillar and the at least one tubular holder, wherein the at least one pillar and the at least one tubular holder are pivotally coupled to each other via the hinge connector assembly,
wherein the adjustment module is configured to adjust the at least one optical fibres at least spatially or angularly relative to an objective lens of the microscope in a manner such that the illumination path is separate from the detection path, and wherein the objective lens has a working distance of 2 mm or less.
34. The adjustment module as claimed in claim 33,
wherein the at least one pillar comprises a distal end attached to the mount, and a proximal end opposite to the distal end; and
wherein the hinge connector assembly is disposed at the proximal end.

35. The adjustment module as claimed in claim 33 or 34, wherein the mount comprises a ring mount configured to releasably couple around a housing of the objective lens.
- 5 36. The adjustment module as claimed in claim 33 or 34, wherein the mount is non-mechanically couplable to the portion of the microscope.
37. The adjustment module as claimed in claim 36, wherein the mount includes a first mount mating member to mate with an objective lens mating member
10 of a housing of the objective lens.
38. The adjustment module as claimed in claim 37, wherein the mount includes a second mating member to mate with a focal tube mating member.
- 15 39. The adjustment module as claimed in any one of claims 33 to 38, wherein the at least one tubular holder comprises a first end, a second end opposite the first end, and a body extending between the first end and the second end; and
wherein the hinge connector assembly is disposed along a part of the
20 body.
40. The adjustment module as claimed in any one of claims 33 to 39, wherein the mount includes a slot for receiving a removable optical filter.