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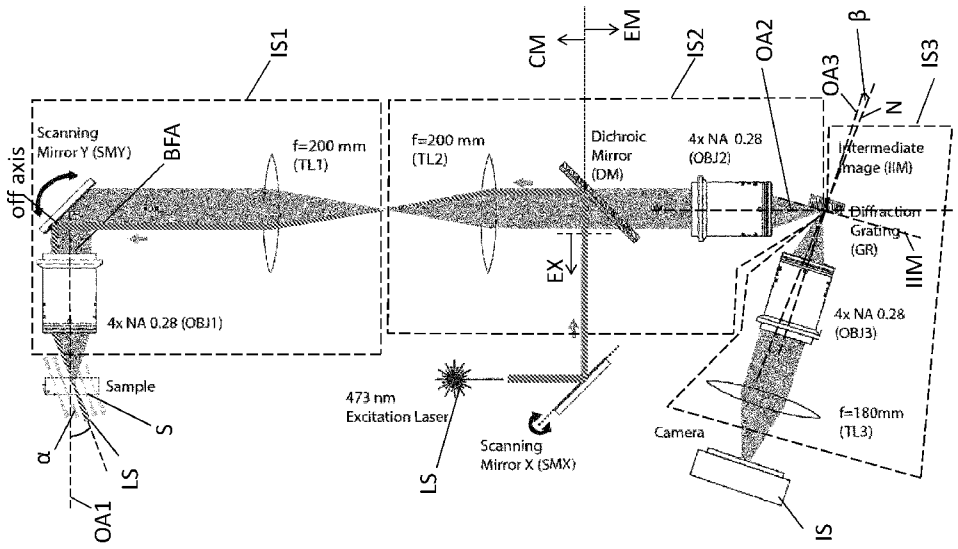

Fig. 2

(57) **Abstract:** The invention relates to a microscopy system particularly for oblique plane microscopy, comprising at least the following components: - A first imaging system (IS1) comprising a first objective lens (OBJ1) configured and arranged to provide excitation light to a sample space (S) and to collect light stemming from a sample in the sample space, - a second imaging system (IS2) comprising a second objective lens (OBJ2) configured and arranged such at the microscopy system that an intermediate image of the light collected by the first objective lens (OBJ1) is formed at an intermediate image plane (IIM), - an optical element (FA, GR) arranged at the intermediate image plane (IIM), wherein the optical element (FA, GR) is arranged at an oblique angle (α) with respect an optical axis (OA2) of the second object lens (OBJ2), particularly wherein the optical element (FA, GR) is configured to transfer and/or reproduce the intermediate image plane under a different orientation with respect to an optical axis (OA3) of an imaging device, such as a lens, particularly such that

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the transferred and/or reproduced image plane can be imaged with the imaging device (OBJ3) having its optical axis (OA3) essentially oriented perpendicular to said transferred and/or reproduced image plane, - a third imaging system (IS3) comprising a third objective lens (OBJ3) arranged such that a surface of the optical element (FA, GR) is imaged onto an image sensor (IS).

Microscopy system for oblique plane microscopy

Specification

The invention relates to a microscopy system for oblique plane microscopy.

In oblique plane microscopy a light sheet is scanned across a particularly fluorescently labelled sample, wherein the light sheet encloses an oblique angle with the optical axis of the imaging objective lens. In contrast to other light sheet microscopy techniques where a light sheet is oriented essentially perpendicular to the imaging objective lens, such as selective plane illumination microscopy, oblique plane microscopy, uses only a single objective lens for illumination and collection of emitted light from the sample allowing a more compact microscopy geometry at the sample space and also circumvents sample specific constraints that prohibit illumination or light collection from vastly different directions.

In oblique plane microscopy the light sheet therefore propagates at oblique angles smaller than 90° with respect to the optical axis of the imaging objective lens. The maximum oblique angle for excitation is predetermined by the numerical aperture (NA) of said imaging, or first objective lens.

However, as excitation is provided to a portion in the sample space that extends non-perpendicular to the optical axis, downstream imaging components have to compensate for this unusual excitation geometry.

For this purpose, a second objective lens essentially images the light emitted stemming from the oblique excitation plane or portion to an intermediate image plane, particularly without an effective magnification. This second objective lens is typically comprised in a second imaging system.

A third objective lens comprised in a tertiary or third imaging system is then arranged coplanar to the intermediate image plane, i.e. the optical axis of the third objective lens is arranged perpendicular to the intermediate image plane allowing to image the intermediate image plane on a planar image sensor, such as a camera.

This geometry however does not allow using objective lenses with arbitrary numerical apertures NA as too small objective apertures will lead to a complete loss of the emitted light by the third objective lens (this is illustrated in Fig. 1b).

Particularly, it is not possible to perform oblique microscopy for numerical apertures smaller than 0.5 of the second objective lens.

In order to minimize the introduction of optical aberrations by the first and the second imaging system, the first and the second objective lens have the same numerical aperture and magnification.

The numerical aperture of the first objective lens in turn defines the size of the field of view in the sample space; the larger the numerical aperture the smaller the field of view.

In many applications this is an unwanted limitation.

An object of the present invention is to provide a microscopy system for oblique plane microscopy that overcomes this limitation. The object is achieved by the microscopy system having the features of claim 1.

Advantageous embodiments are described in the subclaims.

According to claim 1, a microscopy system for oblique plane microscopy comprises at least the following components:

- A first imaging system (IS1) comprising a first objective lens (OBJ1) configured and arranged to provide excitation light to a sample space (S) and to collect light stemming from a sample in the sample space,
- A second imaging system (IS2) comprising a second objective lens (OBJ2) configured and arranged such at the microscopy system that an intermediate image of the light collected by the first objective lens (OBJ1) is formed at an intermediate image plane (IIM) ,
- An optical element (FA, GR) arranged at the intermediate image plane (IIM), wherein the optical element is arranged at an oblique angle (α) with respect an optical axis (OA2) of the second object lens (OBJ2), particularly wherein the optical element (Fa, GR) is configured to reproduce the intermediate image plane (IIM) at a different orientation with respect to an optical axis of an imaging element, such as a lens for collecting photons

from said reproduced intermediate image plane, and/or particularly wherein the optical element (Fa, GR) is configured to transfer the intermediate image plane (IIM) to a different orientation with respect to an optical axis of an imaging element, such as a lens for collecting photons from said transferred intermediate image plane,

- A third imaging system (IS3) comprising a third objective lens (OBJ3) arranged such that a surface of the optical element is imaged by the third OBJ3 onto an image sensor (IS).

The term “objective lens” particularly refers to a lens or a lens assembly with a plurality of lenses being arranged as a microscope objective.

The light stemming from the sample is particularly caused by some kind of photon interaction between a sample and the excitation light in the sample space, such that the emitted light is particularly confined to a portion in the sample space that is exposed to the excitation light.

The first objective lens is configured and arranged to provide excitation to the sample and collect the emitted light from the sample. The emitted light might have its origin in a scattering or fluorescent process triggered by the excitation light.

The second objective lens has the purpose of “reimaging” the emitted light from the sample to the intermediate image plane.

In oblique plane microscopy the intermediate image plane encloses an angle with the optical axis of the second objective lens that particularly corresponds to an angle enclosed by the oblique excitation light sheet or light beam. Light propagation at the image plane however is particularly along the optical axis of the second objective lens and particularly not perpendicular to the image plane.

The optical element is particularly configured to adjust the photon propagation direction such that light at the intermediate image plane can be collected by the third objective lens particularly also for low numerical apertures of the first and/or second imaging system objective lenses.

The optical element is designed such that the intermediate image plane or its information content can be imaged to or from an image plane that is oriented parallel to the intermediate image plane, i.e. perpendicular to an optical axis of the imaging system, particularly the third imaging system, particularly such that for a planar image sensor, the image plane is in focus over the whole image plane.

For this purpose, the optical element has at least one surface that is arranged co-planar with the intermediate image plane in the intermediate image plane. Another surface of the optical element can be arranged along a different orientation.

The oblique angle at which the optical element is arranged with respect to the optical axis of the second objective lens is particularly identical or corresponds to the oblique angle of the light sheet or the light beam in the sample space.

According to another embodiment of the invention, the microscopy system comprises a light source, wherein the light source is configured and arranged such that light the light from the light source forms a light sheet (LS) and/or a light beam in the sample space (S) that propagates at the oblique angle (α) with respect to an optical axis (OA1) of the first objective lens.

By scanning the light beam in the sample space laterally, a light sheet can be generated. Thus, the term “light sheet” particularly refers to a portion in the sample space that is exposed to excitation light either sequentially or simultaneously.

In case a light sheet is generated by scanning a light beam, the scanning rate of the light beam is particularly larger than a frame rate of the image senor of the microscopy system.

Alternatively the excitation light can be shaped such that a light sheet is generated even without scanning the excitation light.

A volumetric image of a sample is particularly achieved by scanning the light sheet over the sample.

The oblique angle is particularly larger than 0°, particularly larger than 20° and smaller than an angle under which total internal reflection happens.

According to another embodiment of the invention, the optical element is a diffraction grating.

The diffraction grating is particularly arranged coplanar with the intermediate image plane at the intermediate image plane with one surface.

Incident light on the diffraction grating is diffracted according to the physical laws applicable to the diffraction grating. Particularly, the diffraction grating will give rise diffraction angles along which diffracted light propagates “preferentially” due to interference conditions. Said diffraction angles, inter alia depend on the wavelength of the incident light, an angle under which the incident light impinges on the diffraction grating normal, and a spacing of the grating structure.

Particularly by arranging the third objective lens such that it images the diffraction grating surface particularly under one of the diffraction angles or perpendicular to the diffraction grating surface, the intermediate image can be imaged with an optical axis that is optically perpendicular to the intermediate image plane.

According to another embodiment of the invention the diffraction grating (GR) is a reflective diffraction grating, particularly wherein the diffraction grating is a blazed reflective diffraction grating. The surface arranged in the intermediate image plane corresponds to the reflective surface of the diffraction grating. This surface is also the surface to be imaged by the third objective lens.

The blazed diffraction grating allows accumulating a large portion of the incident light along a single diffraction angle.

According to an alternative embodiment of the invention, the diffraction grating (GR) is a transmission diffraction grating, particularly wherein the diffraction grating is a blazed transmission diffraction grating.

According to another embodiment of the invention, an optical axis (OA3) of the third objective lens (OBJ3), particularly the optical axis of the third imaging system (IS3), is arranged at an angle (β) with respect to a surface normal (N) of the diffraction grating, wherein said angle (β) corresponds to a diffraction angle of the diffraction grating (GR), particularly wherein the diffraction angle corresponds to the diffraction

angle along which the largest amount of light incident from the second objective lens (OBJ2) is diffracted.

The term “diffraction angle” has been elaborated above in an exemplary way.

This arrangement of the third objective lens allows a particularly efficient collection of the emitted light.

Diffraction angles can be calculated according to the well-known relation

$$m\lambda = d(\sin(90 - \alpha) - \sin\beta)\,;$$

Wherein λ corresponds to the wavelength of the emitted, i.e. incident light, d corresponds to the spacing of the grating structures, m assumes integer numbers and corresponds to the diffraction order, α corresponds to the oblique angle of the light sheet or light beam, wherein 90-α corresponds to the incident angle of the emitted light onto the surface of the diffraction grating with respect to its surface normal and β corresponds to the diffraction angle.

According to another embodiment of the invention, the optical element (FA) is a fibre array (FA), wherein the fibre array comprises a plurality of optical fibres (F), wherein the fibres are arranged adjacent to each other and wherein each fibre has a first and a second end face, particularly wherein said end faces extend perpendicular to the optical axis of the fibre, wherein the first end faces form a particularly planar input surface (IN) and the second end faces form a particularly planar output surface (OUT), wherein the input surface (IN) is arranged in and oriented along the intermediate image plane (IIM) and wherein the output surface (OUT) is imaged by the third objective (OBJ3), particularly wherein the output surface (OUT) of the fibre array (FA) is oriented perpendicular to the optical axis (OA3) of the third objective lens (OBJ3), such that the output surface (OUT) is imaged by the third objective (OBJ3).

According to another embodiment of the invention, the fibres of the fibre array are arranged in coherent bundles, particularly such that image transmission from the input surface to the output surface of the fibre array is possible.

The fibre end faces particularly form pixels for receiving incident light at the intermediate image plane, in case the end faces are comprised by the input surface or for outputting light, in case the end faces are comprised by the output surface.

According to another embodiment of the invention, the end faces of the fibres of the input and/or the output surface are particularly planar and enclose and angle other than 90° with the optical fibre, particularly wherein said angle is enclosed with an outer surface along the direction of extension of the fibre, particularly with a main light propagation axis of the fibre.

The fibres are particularly arranged such that all end faces of the input surface and/or the output surface of the fibre array extend parallel to each other.

As each fibre end face at the output surface can be understood as a point light source, the third objective can collect the light at the output surface, particularly wherein the third objective lens is arranged perpendicular to the end faces of the output surface.

According to another embodiment of the invention, the fibre array is a fibre optic face plate, particularly a fibre optic taper.

The optical fibres of the fibre array are particular multi-mode fibres.

According to another embodiment of the invention, a numerical aperture of the first and/or the second and/or the third objective lens (OBJ1, OBJ2, OBJ3) is smaller than 0.5, particularly smaller than 0.3.

Oblique microscopy systems exhibiting such low numerical apertures without the optical element such as the diffractive grating or the fibre array loose the complete emission signal and are thus inoperable.

According to one embodiment of the invention, the first objective lens has a numerical aperture smaller than 0.5, particularly smaller than 0.3.

According to one embodiment of the invention, the second objective lens has a numerical aperture smaller than 0.5, particularly smaller than 0.3.

According to one embodiment of the invention, the first and the second objective lens both have a numerical aperture smaller than 0.5, particularly smaller than 0.3, wherein particularly the numerical apertures of the first and the second objective lens are identical.

According to one embodiment of the invention, the first, the second and the third objective lens all have a numerical aperture smaller than 0.5, particularly smaller than 0.3, wherein particularly the numerical apertures of the first and the second objective lens are identical.

According to one embodiment of the invention, the first and the second objective lens have the same magnification.

According to one embodiment of the invention, the first and the second objective lens are identical, i.e. have the same optical specifications.

According to another embodiment of the invention, the microscopy system is configured to position the excitation light off-axis with respect to an optical axis (OA1) of the first objective lens (OBJ1) at a back focal aperture (BFA) of the objective lens (OBJ1), particularly such that the light beam or a light sheet (LS) propagating at an oblique angle (α) is generated in the sample space.

In order to positon the excitation light off-axis the microscopy system particularly comprises optical components such as lenses and mirrors, particularly wherein said optical components are adjustable for alignment reasons.

The position of the excitation light is particularly defined and determined by a positon of an intensity maximum of the excitation light perpendicular to the propagation direction.

For example in case the exaction light is a collimated light beam, the centre in terms of intensity of the collimated light beam is arranged off-axis.

For example in case the exaction light is an elongated or oval light beam, the elongated intensity profile is arranged off-axis with along its particularly elongated intensity maximum.

The back focal aperture of the first objective lens is particularly located at the first objective lens and particularly coincides with the back focal plane of the first objective lens.

According to another embodiment of the invention, the microscopy system comprises a light shaping device configured to generate an elongated light intensity profile of the excitation light from the light source, wherein the elongated intensity profile extends along a first axis [transverse to the propagation direction of the light] at a back focal aperture (BFA) of the first objective lens, particularly wherein said elongated intensity profile is arranged off-axis with respect to an optical axis (OA1) of the first objective lens (OBJ1).

The light shaping device can achieve its capability to generate the elongated light intensity profile at the back focal aperture of the first objective lens by means of other optical components, such as appropriately placed mirrors, and lenses, that are particularly configured to guide the light propagation accordingly towards the back focal aperture of the first objective lens.

According to another embodiment of the invention, the light shaping device is a first scanner (SMX) configured to deflect an incident, particularly essentially circular and particularly collimated light beam from the light source around a first scan axis, such that the light beam from the light source is scanned particularly back and forth along the first axis at the back focal aperture (BDA) of the first objective lens (OBJ1), generating the elongated light intensity profile in a time-averaged fashion, particularly wherein a frame rate of the image sensor is lower than the scan rate of the scanner.

This embodiment allows for a precise alignment of the excitation light and a precise shaping of the elongate intensity profile particularly having a uniform intensity distribution along the first axis.

According to another embodiment of the invention, the light shaping device is an optical element, such as a lens, particularly a cylindrical lens, or convex mirror, configured to generate the elongated intensity profile at the back focal aperture of the first objective lens along the first axis.

This embodiment omits moving parts for generating the light sheet. Furthermore, the light sheet excites the sample simultaneously and not in a sequential manner.

According to another embodiment of the invention, the microscopy system comprises a beam splitter, particularly a wavelength-dependent beam splitter, such as a dichroic mirror, for separating excitation light from emitted light of the sample by means of at least one particularly optical property, particularly by its wavelength, wherein the beam splitter optically separates the microscopy system in an excitation path along which only excitation light propagates towards the beam splitter, a common light path along which both the excitation light and the emitted light propagate, and an emission light path along which only emission light propagates after, particularly optically downstream of, the beam splitter.

According to another embodiment of the invention, the light shaping device is arranged in the excitation path.

This embodiment allows for a non-de-scanned provision of the emitted light from the light sheet to the intermediate image plane.

According to another embodiment of the invention, the microscopy system comprises a second scanner (SMY) configured to deflect an incident light beam around a second scan axis, particularly optically perpendicular to the first scan axis, particularly wherein said second scanner is arranged close to or at a back focal aperture (BFA) of the first objective lens (OBJ1), particularly such that the elongated intensity profile is deflected around the second scan axis, wherein said second scan axis particularly extends along the first axis.

This embodiment allows for scanning the light sheet through the sample, such that volumetric image data can be acquired by the image sensor by scanning the light sheet over the sample.

In order to optimize excitation performance, the second scanner is arranged at or at least close to the back focal aperture of the first optical lens such that scanning induced “beam wandering” at the back focal aperture of the first objective lens is avoided or minimized.

The second scanner particularly scans along he so-called slow axis.

The second scanner is particularly arranged such that the emitted light from the sample is de-scanned.

According to another embodiment of the invention, the first imaging system (IS1) comprises a first tube lens (TL1) and wherein the second imaging system (IS2) comprises a second tube lens (TL2), wherein the first and the second tube lens (TI1, TI2) form a telecentric lens system with respect to the light shaping device (SMX), particularly the first scanner, and the second scanner (SMY), particularly wherein the focal lengths of the first and the second tube lens (TL1, TL2) are identical.

The telecentric system allows for the provision of the non-de-scanned and de-scanned scan portions.

In case the first and the second tube lens have the same focal length, the second scanner and particularly the back focal aperture are arranged at an optical distance of one focal length of the first tube lens, wherein the second tube lens is arranged twice the focal distance from the firs tube lens, and particularly wherein the first scanner is arranged at one focal distance away from the tube lens in the excitation path. Particularly, in between the first scanner and the second tube lens the beam splitter is arranged. This configuration particularly is referred to as a telecentric system.

As for practical reasons either the second scanner or the back focal aperture can be placed at the focal distance from the first tube lens, the second scanner can be arranged closer to the first tube lens (than the focal distance) and the back focal aperture can be arranged further away from the first tube lens, such that the focal plane of the furs tube lens lies in between the second scanner and the back focal aperture of the first objective lens.

This embodiment allows also for a combined magnification of one in case the first and the second objective lens have the same optical specification.

According to another embodiment of the invention, an optical magnification, particularly a lateral magnification, of the first and the second imaging system are

identical with regard to the lateral magnification, such that the optical, particularly lateral magnification of the combined first and second imaging system is one.

According to this embodiment of the invention, for example the optical specification of the first and the second tube lens are identical and the optical specification of the first and the second objective lens are identical.

According to another embodiment of the invention, an optical magnification, particularly a lateral magnification, of the third imaging system is identical to the first and/or second imaging system.

According to another embodiment of the invention, the oblique angle a maximum angle achievable for the first objective lens.

Particularly, exemplary embodiments are described below in conjunction with the Figures. The Figures are appended to the claims and are accompanied by text explaining individual features of the shown embodiments and aspects of the present invention. Each individual feature shown in the Figures and/or mentioned in said text of the Figures may be incorporated (also in an isolated fashion) into a claim relating to the device according to the present invention.

Fig. 1a shows a schematic cross-section of an oblique light sheet LS or light beam generated by the first objective lens OBJ1, wherein the z-axis is oriented along the optical axis OA1 of the first objective lens OBJ1. The arrows pointing along the y-axis indicate the scanning of the light sheet LS through the sample by means of the second scanner SMY.

The light sheet LS encloses an oblique angle α with the optical axis OA1 of the first objective lens OBJ1 that is smaller than 90° and particularly limited by the numerical aperture NA of the first objective lens OBJ1.

Emitted light is collected by the first objective lens OBJ1 and guided to the second objective lens OBJ2 that images the emitted light on the intermediate image plane IIM as shown in Figs. 1b and 1c.

The intermediate image plane IIM encloses an angle α with the second objective lens OBJ2 that is particularly identical to the oblique angle in the sample space S. In conventional oblique plane microscopes, a third objective lens OBJ3 is arranged perpendicular to the intermediate image plane IIM. However, as the light propagates

essentially along the optical axis OA2 of the second objective lens OBJ2 a certain light portion is missed out by the third objective lens (Fig. 1b left panel, hatched light cone). In case of a small aperture, the third objective lens OBJ3 does not collect any of the emitted light.

In Fig. 1c an embodiment according to the invention is shown, where the diffractive grating GR is arranged at the intermediate image plane IIM and oriented along the intermediate image plane IIM, i.e. its surface normal N is perpendicular to the intermediate image plane IIM. Diffracted light propagates essentially along the diffraction angles β of the diffraction grating GR. The third objective lens OBJ3 is arranged such that it images the intermediate image plane IIM particularly under an diffraction angle β, preferably under the diffraction angle β under which the largest portion of light is diffracted.

The use of the diffraction grating GR allows for low numerical aperture objectives to be used and thus for a larger field of view to be imaged.

In Fig. 2 a schematic layout of one embodiment of the microscopy system according to the invention is shown.

The system comprises a light source in from of a laser LS that emits essentially collimated excitation light. The excitation light is guided over a first scanner SMX that deflects the incident light around a first scan axis.

Downstream, i.e. along the propagation direction of the excitation light, of the first scanner SMX, a dichroic mirror DM is arranged deflecting the excitation light through a second tube lens TL 2 of the second imaging system IS2 and then through the first tube lens TL1 of the first imaging system IS1.

Close to the back focal aperture BFA of the first objective lens OBJ1, the second scanner SMY is arranged. At the second scanner SMY, particularly its reflective surface, the essentially collimated excitation light is located off-axis with respect to the optical axis OA 1 of the first objective lens OBJ1 such that the light sheet LS is generated in the sample space S. The emitted light is collected by the first objective lens OBJ1 and guided over the second scanner SMY, i.e. de-scanned with respect to the second scan axis and propagates through the telecentric lens system consisting of the first and the second tube lens TL1 and TL2. As the dichroic mirror DM is transparent for the emitted light, the light passes through the dichroic mirror DM and is imaged by the second objective lens OBJ2 onto the intermediate image plane IIM. The diffraction grating GR is arranged at the intermediate image plane IIM and

oriented essentially along the intermediate image plane IIM, and diffracts the incident light along its diffraction angles β. The third objective lens OBJ3 is arranged such that it images the surface of the diffraction grating GR under the diffraction angle β that comprises the largest portion of diffracted light.

A third tube lens TL3 images the light collected by the third objective lens OBJ3 onto an images sensor IS such as a camera. The system according to the invention allows for the use of low numerical objectives and imaging of large filed of views or large volumes in oblique plane microscopy.

Fig. 3 shows an alternative embodiment for the optical element, wherein the optical element is a coherent fibre array FA that is arranged with its input surface IN at the intermediate image plane IIM and with its output surface OUT at a different plane. The optical axis of the third objective lens OBJ3 is arranged perpendicular to the output surface OUT and positioned such that the output surface is imaged to the image sensor (not shown).

The input surface and the output surface enclose an angle and are not parallel to each other, this allows for oblique plane microscopy using small aperture objectives.

Claims

1. A microscopy system particularly for oblique plane microscopy, comprising at least the following components:
   - A first imaging system (IS1) comprising a first objective lens (OBJ1) configured and arranged to provide excitation light to a sample space (S) and to collect light stemming from a sample in the sample space,
   - a second imaging system (IS2) comprising a second objective lens (OBJ2) configured and arranged such at the microscopy system that an intermediate image of the light collected by the first objective lens (OBJ1) is formed at an intermediate image plane (IIM),
   - an optical element (FA, GR) arranged at the intermediate image plane (IIM), wherein the optical element (FA, GR) is arranged at an oblique angle (α) with respect an optical axis (OA2) of the second object lens (OBJ2), particularly wherein the optical element (FA, GR) is configured to transfer and/or reproduce the intermediate image plane under a different orientation with respect to an optical axis (OA3) of an imaging device (OBJ3), such as a lens, particularly such that the transferred and/or reproduced image plane can be imaged with the imaging device (OBJ3) having its optical axis (OA3) essentially oriented perpendicular to said transferred and/or reproduced image plane,
   - a third imaging system (IS3) comprising a third objective lens (OBJ3) arranged such that a surface of the optical element (FA, GR) is imaged onto an image sensor (IS).

2. The microscopy system according to claim 1, wherein the microscopy system comprises a light source, wherein the light source is configured and arranged such that light the light from the light source forms a light sheet (LS) and/or a light beam in the sample space (S) that propagates at the oblique angle (α) with respect to an optical axis (OA1) of the first objective lens.

3. The microscopy system according to claim 1 or 2, wherein the optical element (FA) is a fibre array (FA), wherein the fibre array comprises a plurality of optical fibres (F), wherein the fibres are arranged adjacent to each other and wherein each fibre has a first and a second end face formed by the ends of the fibre, wherein the first end faces form a particularly planar input surface (IN) and the

second end faces form a particularly planar output surface (OUT), wherein the input surface (IN) is arranged in and oriented along the intermediate image plane (IIM) and wherein the output surface (OUT) is imaged by the third objective (OBJ3), particularly wherein the output surface (OUT) of the fibre array (FA) is oriented perpendicular to the optical axis (OA3) of the third objective lens (OBJ3), such that the output surface (OUT) is imaged by the third objective (OBJ3).

4. The microscopy system according to claim 3, wherein the fibre array (FA) is a fibre optic face plate, particularly a fibre optic taper.

5. The microscopy system according to claim 1 or 2, wherein the optical element (GR) is a reflective diffraction grating (GR), particularly wherein the diffraction grating is a blazed reflective diffraction grating, or wherein the optical element (GR) is a transmission diffraction grating (GR), particularly wherein the diffraction grating is a blazed transmission diffraction grating.

6. The microscopy system according to any of the claims 1 or 2 and 5, wherein an optical axis (OA3) of the third objective lens (OBJ3) is arranged at an angle (β) with respect to a surface normal (N) of the diffraction grating (GR) , wherein said angle (β) corresponds to a diffraction angle of the diffraction grating (GR), particularly wherein the diffraction angle corresponds to the diffraction angle along which the largest amount of light incident from the second objective lens (OBJ2) is diffracted.

7. The microscopy system according to any of the preceding claims, wherein a numerical aperture of the first and/or the second and/or the third objective lens (OBJ1, OBJ2, OBJ3) is smaller than 0.5, particularly smaller than 0.3.

8. The microscopy system according to any of the preceding claims, wherein the microscopy system is configured to position the excitation light off-axis with respect to an optical axis (OA1) of the first objective lens (OBJ1) at a back focal aperture (BFA) of the objective lens (OBJ1), particularly such that the light beam or light sheet (LS) propagating at an oblique angle (α) is generated in the sample space.

9. The microscopy system according to any of the preceding claims, wherein the microscopy system comprises a light shaping device configured to generate an elongated light intensity profile of the excitation light from the light source, wherein the elongated intensity profile extends along a first axis at a back focal aperture (BFA) of the first objective lens, particularly wherein said elongated intensity profile is arranged off-axis with respect to an optical axis (OA1) of the first objective lens (OBJ1).

10. The microscopy system according to claim 9, wherein the light shaping device is a first scanner (SMX) configured to deflect an incident light beam from the light source around a first scan axis, such that a light beam from the light source is scanned along the first axis at the back focal aperture (BFA) of the first objective lens (OBJ1), generating the elongated light intensity profile in a time-averaged fashion.

11. The microscopy system according to claim 9, wherein the light shaping device (SMX) is an optical element, such as a lens, particularly a cylindrical lens, or convex mirror, configured to generate the elongated intensity profile at the back focal aperture of the first objective lens (OBJ1) along the first axis.

12. The microscopy system according to any one of the preceding claims, wherein the microscopy system comprises a beam splitter (DM), particularly a wavelength-dependent beam splitter for separating excitation light from emitted light of the sample, wherein the beam splitter optically separates the microscopy system in an excitation path (EX) along which only excitation light propagates towards the beam splitter, a common light path (CM) along which both the excitation light and the emitted light propagate, and an emission light path (EM) along which only emission light propagates after the beam splitter (DM).

13. The microscopy system according to any one of the claims 9 to 12, wherein the light shaping device (SMX) is arranged in the excitation path (EX).

14. The microscopy system according to any of the preceding claims, wherein the microscopy system comprises a second scanner (SMY) configured to deflect an incident light beam around a second scan axis, particularly optically

perpendicular to the first scan axis, particularly wherein said second scanner is arranged at a back focal aperture (BFA) of the first objective lens (OBJ1), particularly such that the elongated intensity profile is deflected around the scan second axis, wherein said second scan axis particularly extends along the first axis.

15. The microscopy system according to any of the preceding claims, wherein the first imaging system (IS1) comprises a first tube lens (TL1) and wherein the second imaging system (IS2) comprises a second tube lens (TL2), wherein the first and the second tube lens (TL1, TL2) form a telecentric lens system with respect to the light shaping device (SMX), particularly the first scanner, and the second scanner (SMY), particularly wherein the focal lengths of the first and the second tube lens (TL1, TL2) are identical.

Fig. 1

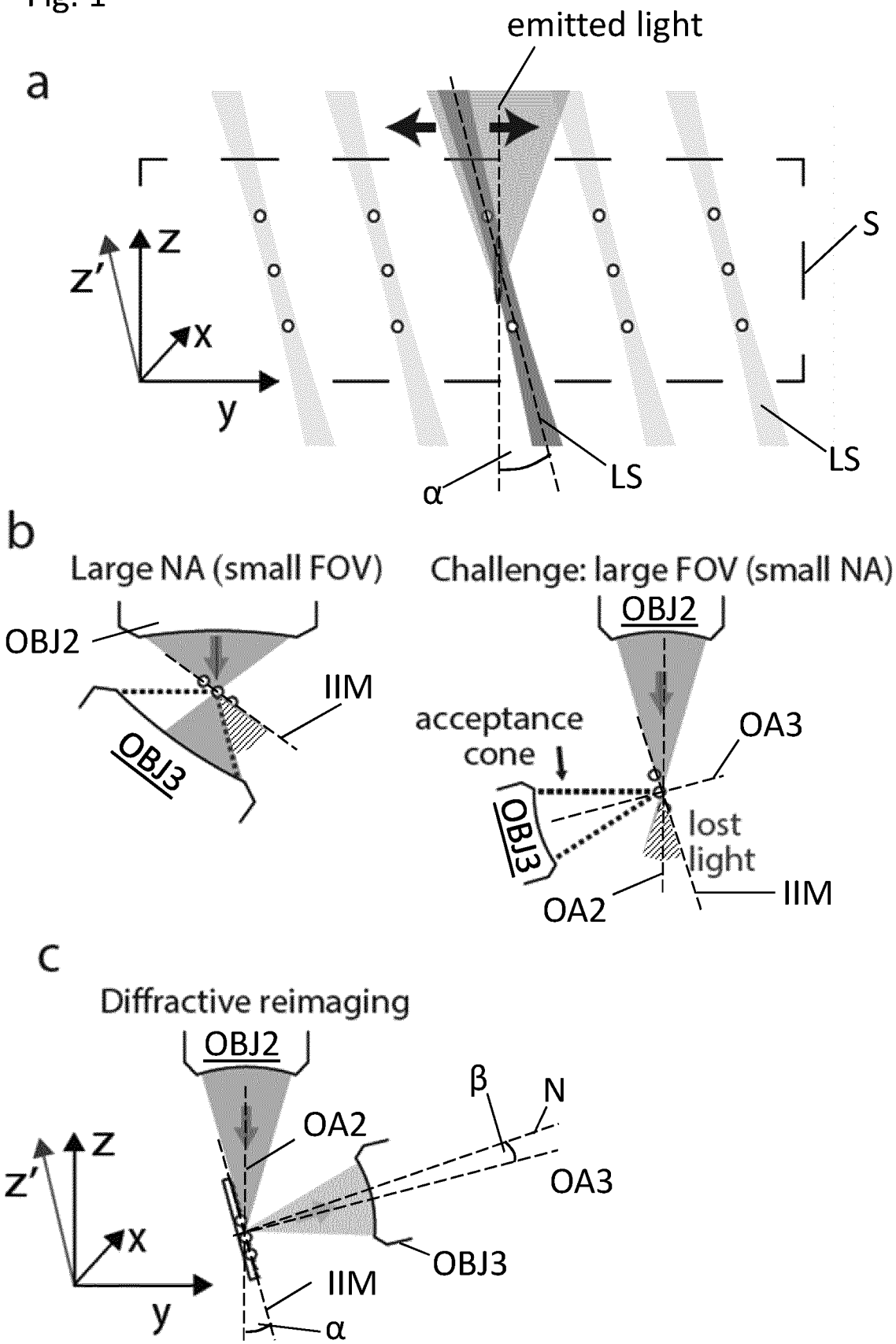

a
emitted light
z
z'
x
y
S
LS
LS
α
b
Large NA (small FOV)
OBJ2
IIM
OBJ3
Challenge: large FOV (small NA)
OBJ2
acceptance cone
OA3
OBJ3
lost light
OA2
IIM
c
Diffractive reimaging
OBJ2
β
N
OA2
z
z'
x
y
OA3
OBJ3
IIM
α

Fig. 2

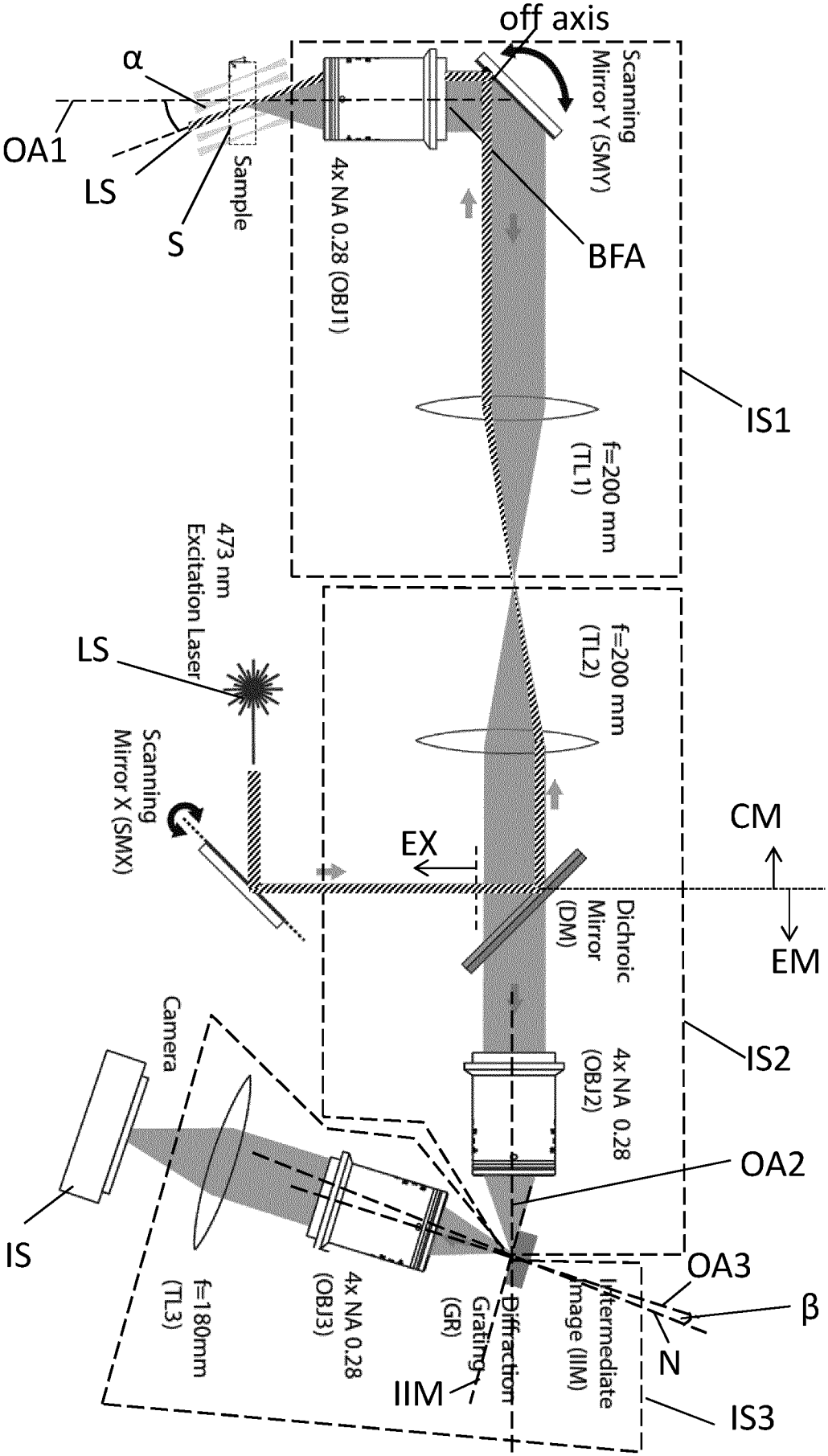
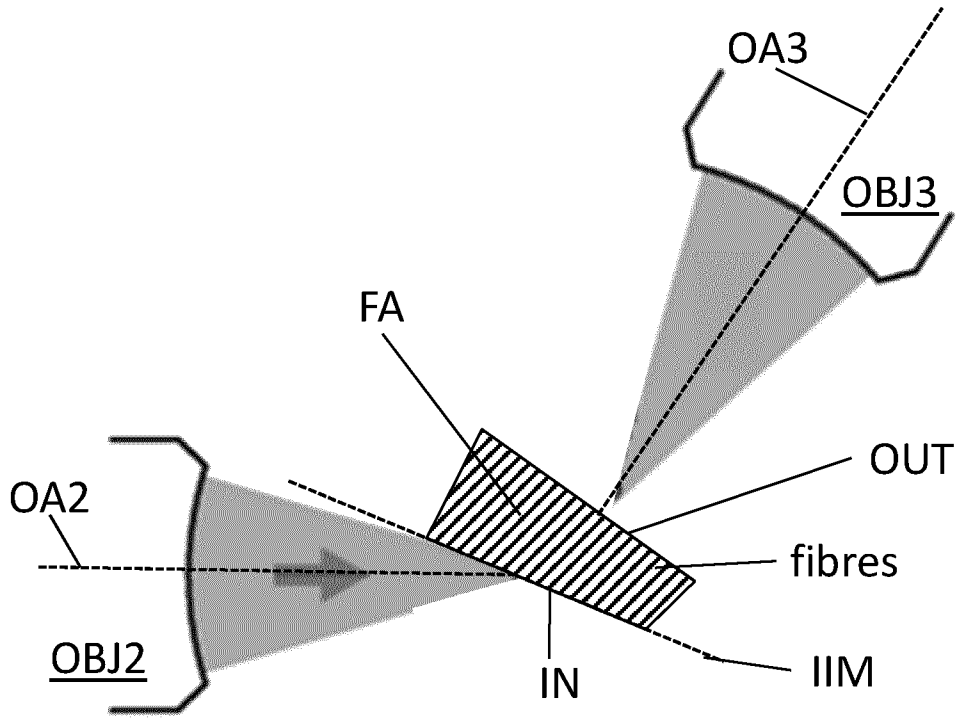

off axis
α
OA1
LS
S
Sample
4x NA 0.28 (OBJ1)
Scanning Mirror Y (SMY)
BFA
IS1
f=200 mm (TL1)
473 nm Excitation Laser
LS
f=200 mm (TL2)
Scanning Mirror X (SMX)
EX
CM
EM
Dichroic Mirror (DM)
IS2
4x NA 0.28 (OBJ2)
OA2
Camera
IS
f=180mm (TL3)
4x NA 0.28 (OBJ3)
Grating (GR)
Diffraction
Intermediate Image (IIM)
OA3
β
N
IIM
IS3

Fig. 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/057569

A. CLASSIFICATION OF SUBJECT MATTER
INV. G02B21/00 G02B21/36
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G02B

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)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/327779 A1 (HILLMAN ELIZABETH [US]) 10 November 2016 (2016-11-10)	1-4,7-15
Y	abstract; figures 1-6,15-20 paragraph [0267] - paragraph [0280] paragraph [0210] - paragraph [0226]	5,6
Y	US 2003/058432 A1 (DRAKE STEPHEN JOHN [GB]) 27 March 2003 (2003-03-27) the whole document	5,6
X	WO 2017/015077 A1 (UNIV COLUMBIA [US]) 26 January 2017 (2017-01-26) page 50, line 22 - page 51, line 34; figure 20B	1

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 June 2020	19/06/2020

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2020/057569

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