(12) UK Patent Application (19) GB (11) 2628556 (13) A

(43) Date of A Publication 02.10.2024

(21) Application No: 2304502.4

(22) Date of Filing: 28.03.2023

(71) Applicant(s):
SMI Drug Discovery Limited
23 Longmeadow, Lode, Cambridge, Cambridgeshire, CB25 9HA, United Kingdom

(72) Inventor(s):
James Alan Brozik
Michael Joe Martinez
Evan Lewis Taylor
Andrew James Thompson
Mark Peter Rogers

(74) Agent and/or Address for Service:
Reddie & Grose LLP
The White Chapel Building,
10 Whitechapel High Street, London, E1 8QS,
United Kingdom

(51) INT CL:
G02B 21/00 (2006.01) ***G01N 21/64*** (2006.01)
G02B 21/06 (2006.01) ***G02B 27/09*** (2006.01)

(56) Documents Cited:
CA 003017188 A1 **CN 115291381 A**
CN 113625439 A

(58) Field of Search:
INT CL **G01N, G02B**

(54) Title of the Invention: **An optical microscope for wide-field microscopies**
Abstract Title: **A wide-field optical microscope with beam shaping element for producing a flat-top beam**

(57) An optical microscope 100 for wide-field (epifluorescent) microscopies is disclosed. The optical microscope comprises an optical train and a microscope objective 115. The optical train comprises: an illumination pathway comprising: a beam shaping element (203) configured to convert a Gaussian excitation beam into a flat-top, or top-hat, beam; and an optical element (301) configured to focus the flat-top beam into the microscope objective 115 in a wide area of uniform illumination; and an optical collection pathway comprising an imaging means 107 configured to provide direct and simultaneous imaging of the wide area of uniform illumination. The imaging means could comprise a sCMOS sensor, and the collection pathway could include a tube lens 303. The invention could find application in single molecule imaging for example in the development of pharmaceuticals.

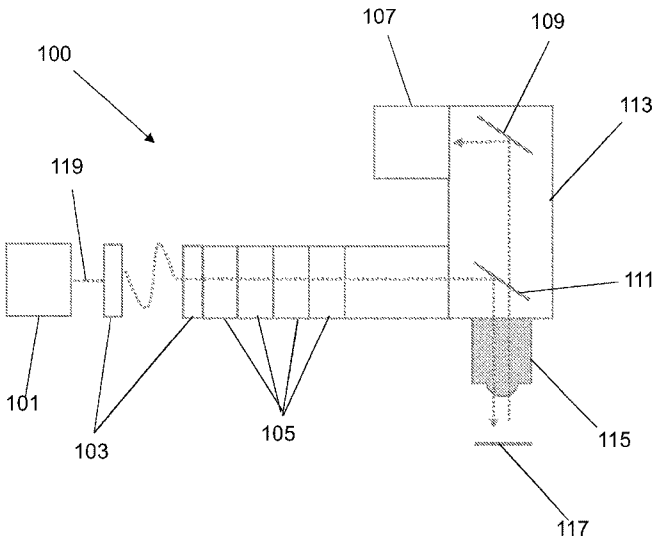

Figure 1

GB 2628556 A

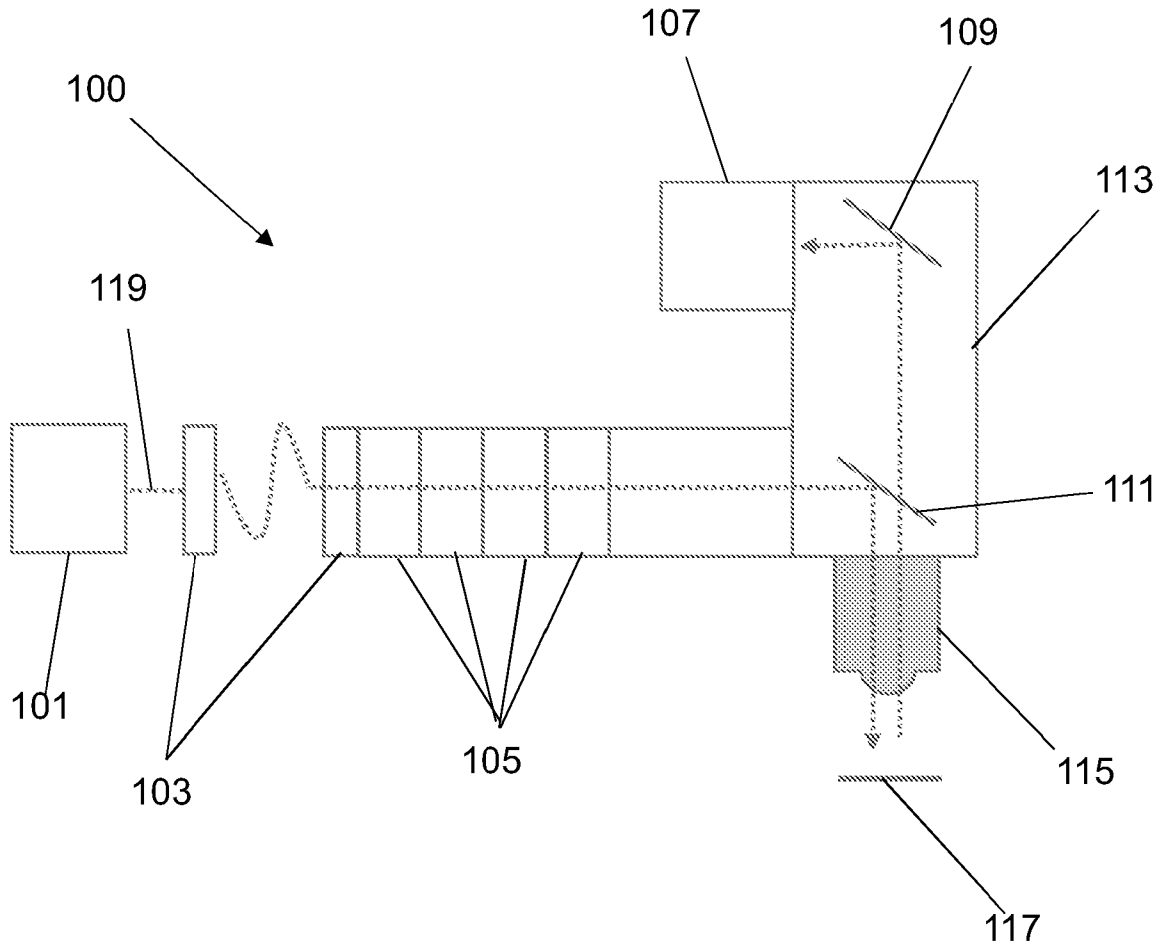

Figure 1

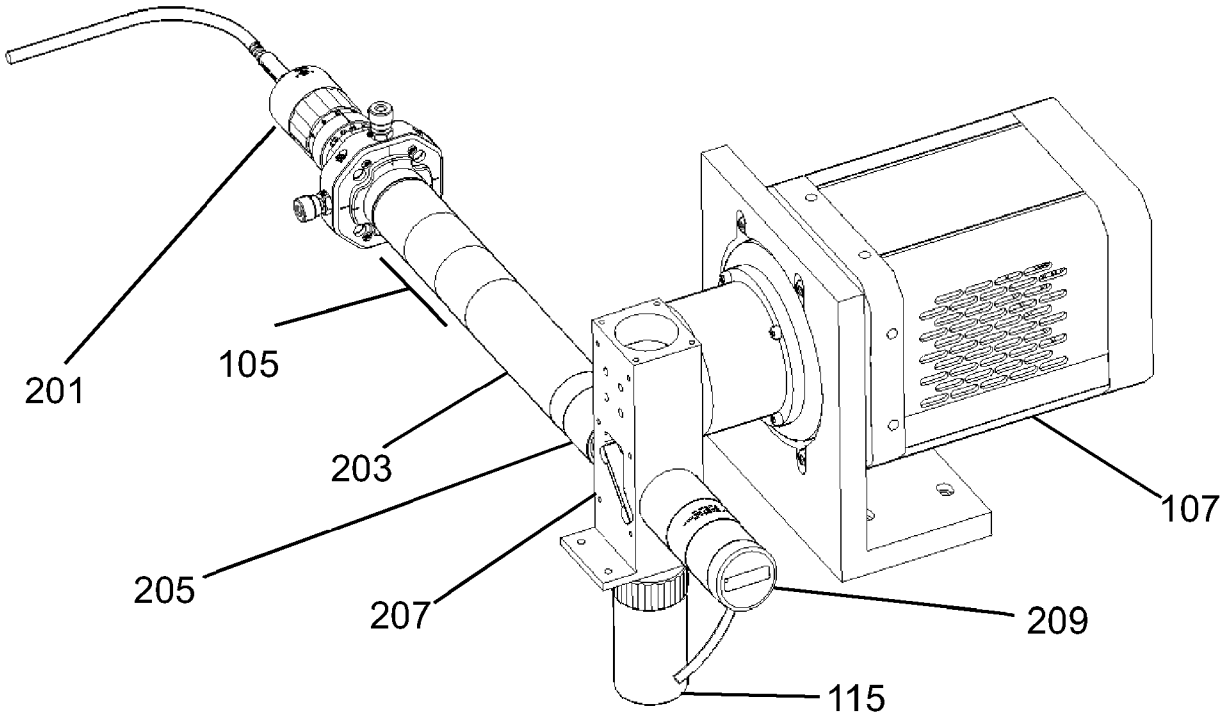

Figure 2

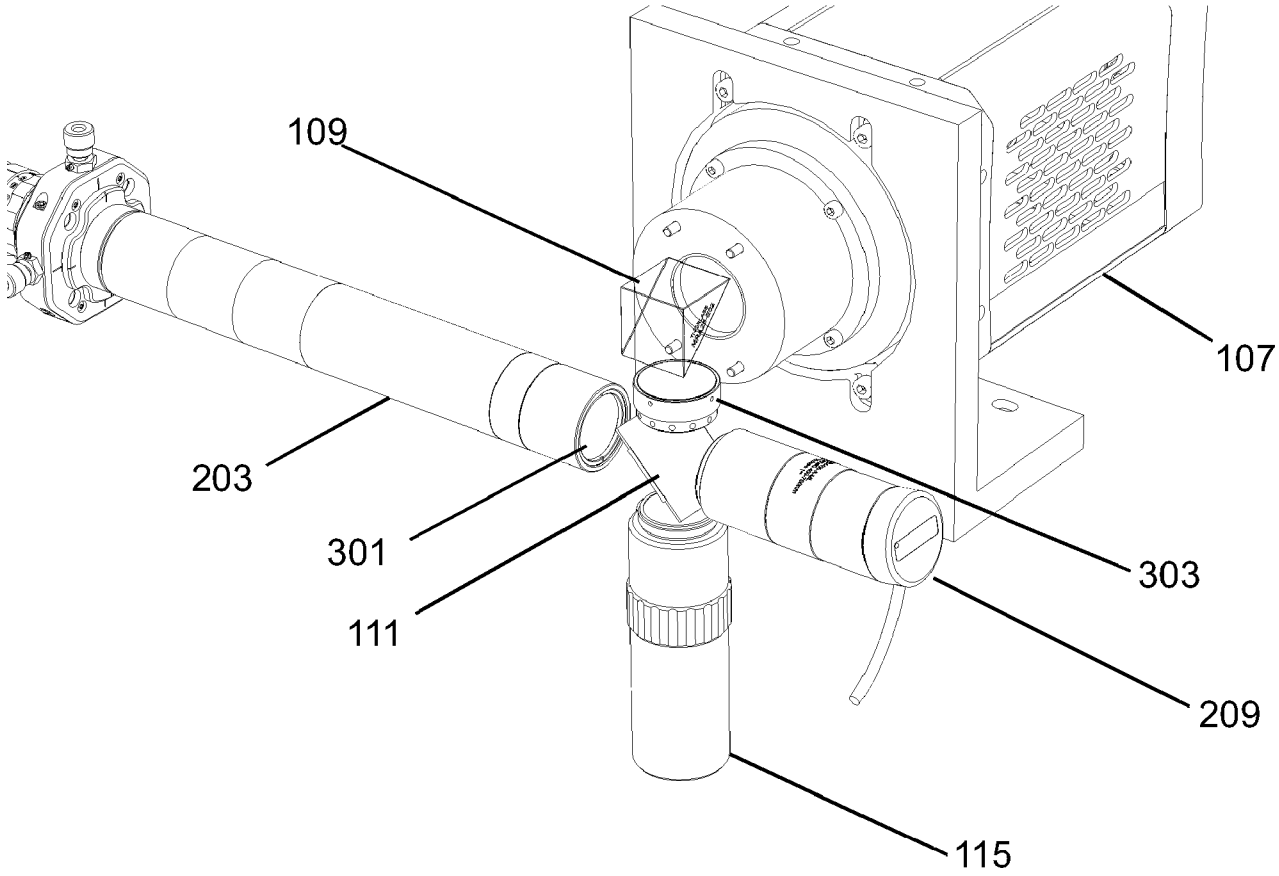

Figure 3

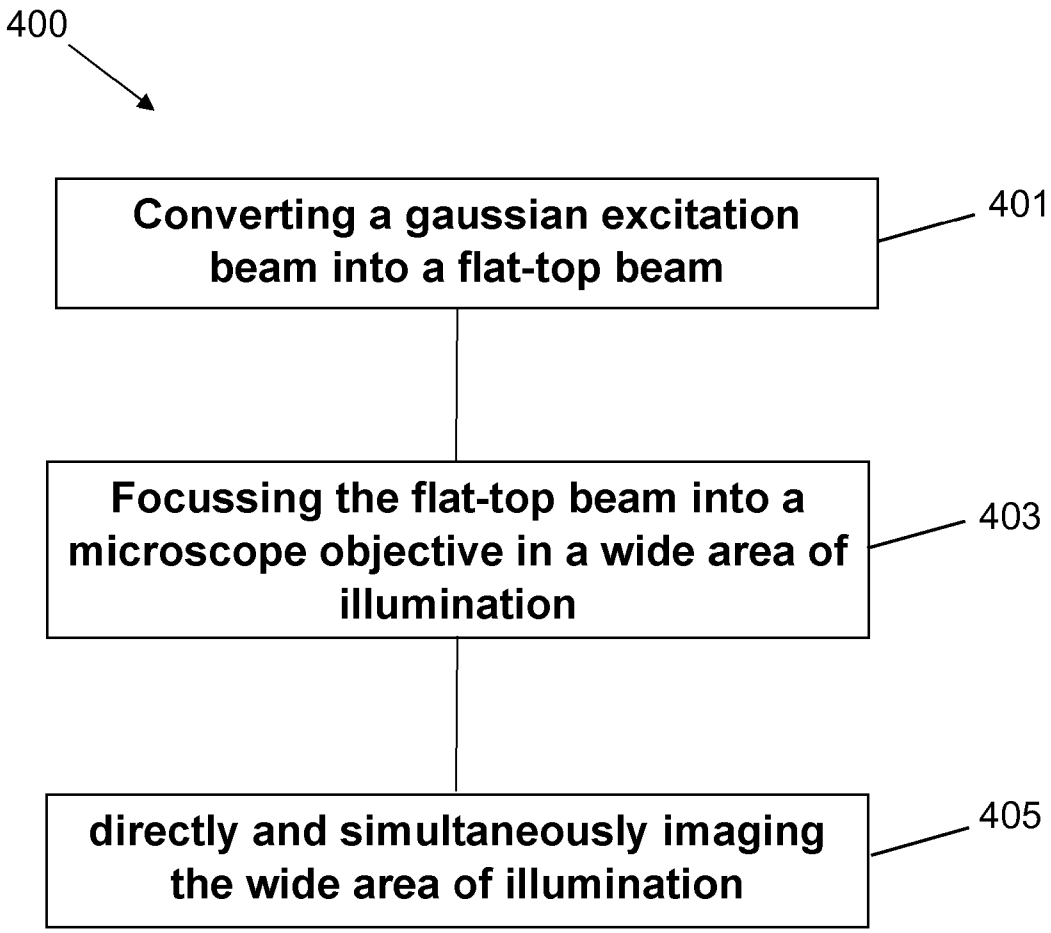
400
Converting a gaussian excitation beam into a flat-top beam
401
Focussing the flat-top beam into a microscope objective in a wide area of illumination
403
directly and simultaneously imaging the wide area of illumination
405

Figure 4

AN OPTICAL MICROSCOPE FOR WIDE-FIELD MICROSCOPIES

FIELD OF INVENTION

The present invention relates to an optical microscope for wide-field microscopies. In particular, the present invention relates to an optical microscope for wide-field microscopies that produces a wide-area of uniform illumination. The present invention also relates to a method for wide-field microscopy with an optical microscope.

BACKGROUND OF THE INVENTION

Microscopes are instruments that allow things to be seen that are too small to be seen by the human eye. Optical microscopy is a microscopy technique that uses light to produce an image. Optical microscopes are available that operate in different modes. That is to say; while they all use light to capture magnified images of small objects, the underlying methodology differs. For example, basic types of optical microscopes are simple microscopes that use a single lens for magnification and compound microscopes that use a plurality of lenses. A compound microscope uses a so-called objective lens close to the object being viewed to collect light. This provides the viewer with an enlarged inverted virtual image of the object. This focuses a real image of the object being imaged inside the microscope. This image is then magnified by a second lens or group of lenses in an eyepiece of the microscope. Other modes of optical microscopy include confocal microscopy and total internal fluorescence (TIRF) microscopy.

Optical microscopy is a microscopy technique that can be used to image single molecules. An optical microscope for single-molecule imaging is disclosed in an international patent application with publication No. WO 2020/245579 with the same applicant as the present patent application. Single-molecule imaging can be defined as the visualisation of individual molecules with a resolution measured in nanometres. Broadly, the optical microscope of international patent application with publication No. WO 2020/245579 comprises, in a single housing, a first optical microscope in the form of a confocal microscope and a second optical microscope in the form of a total internal reflection fluorescence microscope. Significantly, to provide this very high resolution, the second optical microscope is used to correct drift from the first optical microscope.

Single molecules can range in size from on the order of picometres to nanometres, a nanometre being one-billionth of a meter. By comparison, a human hair has a diameter of

80,000 to 100,000 nanometres; a single gold atom is about a third of a nanometre in diameter; a single water molecule is about 1.5 nanometres; and a strand of human DNA is 2.5 nanometres in diameter.

The use of optical microscopy to observe biological processes, in particular, has undergone huge advances in the last decade. There have been significant advances in resolution, including high resolution, often called, super-resolution microscopy, with resolutions as low as 100nm or less.

High-resolution microscopy with resolutions as low as 15 to 20nm or even lower, such as 1.5 nm resolution, is desirable, particularly where it is valuable to observe single molecules.

Understanding how drugs bind to their target(s) is a fundamental purpose of pharmacological research that enables the effective development of novel therapeutics. Monitoring kinetic properties is critical but is often based on limited macroscopic measurements that yield results that are ensemble averages of thousands, millions, or more individual molecules.

Current pharmacokinetic models for drug metabolism are based entirely on macroscopic kinetics experiments and crude deterministic kinetic models, devoid of the actual stochastic molecular detail of the enzymes, receptors, or other samples, resulting in numerous promising drugs never reaching clinical trials. There is a need to provide a tool for researchers, healthcare institutions, governmental drug regulatory bodies, and the pharmaceutical industry to monitor the underlying molecular scale properties such as drug-protein, antibody-epitope, and protein-protein interactions by providing raw data for realistic stochastic kinetic modeling. Incorporating these concepts and measurements could result in greater drug safety and increased life-saving pharmaceuticals reaching clinical trials.

SUMMARY OF THE INVENTION

The invention in its various aspects is defined in the independent claims below to which reference should now be made. Optional features are set forth in the dependent claims.

Broadly, the inventors have appreciated that single molecules may be imaged in-focus over a large area using optical microscopy. Arrangements are described in more detail below and take the form of an optical microscope for epi-illumination wide-field microscopy, and in

particular microscopy for imaging single molecules. The inventors have particularly appreciated that in order to provide optical microscopy of single molecules over a large area, modifications to existing optical illumination and collection pathways are required. In particular, at least three aspects have been identified in order to provide such advantageous microscopy. Described in greater detail below, these aspects include providing laser excitation over a wide area while maintaining high magnification and optical resolution, providing flat or uniform illumination to the illumination area, and providing the ability to directly and simultaneously image over the wide area of uniform illumination. In combination, these aspects provide an advantageous optical microscopy system allowing for in-focus imaging of single fluorescent molecules over a wide area.

Arrangements of the optical microscope described herein are widely applicable and provide, for example, a direct optical microscopy method of monitoring drug binding that provides single molecule imaging. Further applications may include but are not limited to, therapeutics research, disease diagnosis, or the detection of sample contaminants.

According to one aspect of the present invention, there is provided an optical microscope for wide-field microscopies, the optical microscope comprising: an optical train and a microscope objective, the optical train having: an optical illumination pathway comprising: a beam shaping element configured to convert a gaussian excitation beam into a flat-top beam; and an optical element configured to focus the flat-top beam into the microscope objective in a wide area of uniform illumination; and an optical collection pathway comprising an imaging means configured to provide direct and simultaneous imaging of the wide area of uniform illumination.

Thus, an optical microscope is provided enabling a flat-top beam, a wide area of uniform illumination, and direct and simultaneous imaging of the wide area of uniform illumination.

Optical microscopes according to embodiments described herein produce a large flat-field laser illumination area that is suitable for wide-field microscopies requiring a uniform laser excitation field using a combination of high numerical aperture and beam shaping optics. This combination of properties is particularly advantageous for microscopies involving the detection of single molecules.

Wide-field microscopy may also be referred to as epifluorescence microscopy. In epifluorescence microscopy, a parallel beam of light is passed through a microscope objective and directly through the sample, maximizing the amount of illumination. In

epifluorescence microscopes, a high-intensity light source is used and both the illuminated and emitted light travel through the same objective lens.

In one example, the optical element comprises an achromatic lens having an unconventional focal distance to focus the flat-top beam into the wide area of uniform illumination.

It will be understood that a wide area of uniform illumination comprises a wide area relative to illumination areas provided by existing arrangements. For comparison, the typical area of illumination for single molecule fluorescence assays using conventional focal distances creates a gaussian field approximately 100μm to 250μm in diameter. In one example, the wide area of uniform illumination comprises a diameter of at least 0.5 mm. For example, the wide area of uniform illumination may comprise a diameter of between 1 mm and 2.5 mm. It will be appreciated that the diameter may be equal to 1 mm or 2.5 mm.

In one example, the imaging means comprises a scientific complementary metal oxide semiconductor (sCMOS) sensor configured to provide the direct and simultaneous imaging of the wide area of illumination. In one example, the sCMOS sensor comprises a sensor chip area of at least $5cm^2$. Such a sensor is particularly advantageous in order to detect fluorescence emitted from a single molecule.

In general, charge-coupled device (CCD)-based cameras offer high sensitivity but slow sampling speeds. Conventional CMOS cameras provide fast frame rates but compromise dynamic range. sCMOS image sensors also provide fast frame rates, and have extremely low noise, wide dynamic range, high quantum efficiency, high resolution, and provide a large field of view simultaneously in one image. sCMOS are therefore particularly suitable for high fidelity, quantitative scientific measurement and low-light-level conditions.

In one example, the optical train comprises a plurality of components, one or more of the plurality of components being in-line directly coupled. In one example all of the plurality of components are in-line directly coupled. The plurality of components may comprise the optical element, and any other components or optical elements described herein in relation to the optical illumination or collection pathways. Directly coupling one or more components of the optical train gives rise to a number of advantages. Firstly, direct coupling components greatly stabilizes the relative positions of each component within the optical train, misalignments of which can cause a deterioration of the uniformity and size of the excitation beam and quality of the image produced by the emitted signal. This therefore

provides inherently stabilized excitation and emission light paths resulting from the direct coupling of components. Secondly, direct coupling prevents the ingress of dust or other potential contaminants into the illumination path. Thirdly, directly coupling components ultimately reduces the number of components needed in the optical train. A reduced number of components results in less attenuation of the excitation and emission intensity, improving efficiency of the microscope. Fourthly, the resultant design has the most compact footprint possible for the optical path, making the optical microscope arrangement suitable for applications, for example, in a wide range of bench-top instruments.

In one example, the optical collection pathway is configured to provide the wide area of uniform illumination to illuminate a sample surface for imaging a sample. The sample may comprise a single molecule, or a plurality of single molecules.

In one example, the microscope objective is configured to collect fluorescence emitted from single molecules. In one example, the optical collection pathway further comprises a second optical element having an unconventional focal distance configured to focus the fluorescence emitted from the single molecules. The second optical element may be configured to focus the fluorescence emitted from the single molecules into an image detectable by the imaging means.

In one example, the second optical element is configured to be arranged at an unconventional proximity to the microscope objective. In one example, the second optical element comprises a tube lens.

In one example, the imaging of the wide area of uniform illumination comprises a resolution of at least 2048x2048 pixels. That is, the imaging means may be configured to provide an imaging resolution of at least 2048x2048 pixels.

In one example, the optical microscope further comprises a beam source, or light source, configured to provide the gaussian excitation beam to the optical illumination pathway. The gaussian excitation beam may be configured to provide an optimal signal-to-noise ratio for a sample to be imaged by the imaging means. For example, the light source may provide a gaussian excitation beam having a photon flux I_e of 1×10^{22} to 2×10^{22} photons cm^{-2} sec^{-1} for a typical organic fluorophore in water. In other words, the properties of the excitation beam such as the photon flux may be adjusted based on the sample to be imaged such that the beam provides an optimal signal-to-noise ratio. The properties of the gaussian excitation beam may also be represented by a laser intensity I_o in kJ cm-2 sec-1. This

depends on the wavelength of the light beam and the absorption wavelength which differs depending on the molecule(s) of the sample being imaged. For a wavelength of 640nm, the laser intensity I_o may be approximately 4.26 KW sec-1 with a photon flux I_e of 1.36 x 10^{22} photons cm^{-2} sec^{-1}, providing the optimal signal-to-noise ratio for a sample. In one example, the imaging means is configured to image single molecules.

According to another aspect of the present invention, there is provided a method for wide-field microscopy with an optical microscope, the method comprising: converting a gaussian excitation beam into a flat-top beam; focussing the flat-top beam into a microscope objective in a wide area of uniform illumination; and directly and simultaneously imaging the wide area of uniform illumination.

Examples of the method may include use of the optical microscope according to any embodiment described herein.

In one example, the focussing the flat-top beam into a microscope objective in a wide area of uniform illumination comprises focussing the flat-top beam using an achromatic lens having an unconventional focal distance.

In one example, the wide area of uniform illumination comprises a diameter of at least 0.5 mm. In one example, the wide area of uniform illumination comprises between 1 mm and 2.5 mm. It will again be appreciated that this diameter may be equal to 1 mm or 2.5 mm.

In one example, the method further comprises illuminating a sample surface with the wide area of uniform illumination to image a sample.

In one example, the method further comprises collecting, at a microscope objective, fluorescence emitted from single molecules.

In one example, the method further comprises focussing the fluorescence emitted from single molecules using an optical element having an unconventional focal distance. In one example, the optical element is a tube lens.

In one example, the method further comprises imaging the wide area of uniform illumination at a resolution of at least 2048x2048 pixels.

Arrangements of the present disclose may include one or more of the following particularly advantageous features: direct coupling of components into a single, in-line physically

connected optical train; extremely large beam-diameter, flat-field illumination that provides an equal or uniform level of excitation across a large area using a combination of high numerical aperture and beam shaping optics; an extremely large field of view with single molecule resolution throughout; inherently stabilised excitation and emission light paths resulting from the direct coupling of components; reduced numbers of components compared to conventional microscopes dramatically reducing the attenuation of the excitation and emission intensity; and inherently compact design.

Methods described herein also enhance the usefulness of other well-known techniques such as laser-induced fluorescence microscopy, fluorescence lifetime microscopy, Raman microscopy, fluorescence cross-correlation microscopies, and two-photon laser microscopies, among others.

BRIEF DESCRIPTION OF DRAWINGS

The invention will be described in more detail by way of examples with reference to the accompanying drawings, in which:

Figure 1 is a schematic diagram of showing an optical microscope according to aspects of the present disclosure;

Figure 2 is a schematic diagram of showing an optical microscope according to aspects of the present disclosure;

Figure 3 is a schematic diagram showing of a mirror mount block assembly of an optical microscope according to aspects of the present disclosure; and

Figure 4 is a flow diagram showing the method for wide-field microscopy according to aspects of the present disclosure.

Like features are denoted by like reference numerals.

DETAILED DESCRIPTION

An example optical microscope 100 for wide-field microscopies will be described with reference to Figures 1 to 3.

The optical microscope 100 in this example is contained in a single housing. The optical microscope 100 comprises an optical train having an optical illumination path and an

optical collection path. These may also be termed optical excitation and optical emission paths respectively. The light path is denoted by the dashed lines of Figure 1. Initially, a gaussian excitation beam is provided by a light source 101 at the beginning of the optical illumination path. The optical illumination path also comprises a fibre coupler 103 and one or more beam expanders 105. The gaussian excitation beam is provided to the fibre coupler 103 allowing the transmission of the beam to the one or more beam expanders 105. The beam expanders 105 are configured to expand the diameter of the gaussian excitation beam provided by the light source 101.

As will be described in more detail below with respect to Figure 2, significantly, the optical illumination path comprises a beam shaping element 203 configured to convert the gaussian excitation beam into a flat-top beam.

With respect to Figure 3, the optical illumination path then provides the flat-top beam to a dichroic mirror 111. In this example, the dichroic mirror 111 is at an angle of 45° to the optical illumination path from originating at the light source 101. A dichroic mirror is a mirror with significantly different reflection or transmission properties at two different wavelengths. In this way, the dichroic mirror 111 reflects light from the optical illumination path towards a microscope objective 115 while allowing light reflected from a sample on a sample surface 117 being imaged to not be reflected and to be transmitted through the dichroic mirror 111 along the optical collection path.

The objective lens or microscope objective 115 is located in the optical train after reflection from the dichroic mirror 111. The microscope objective 115 makes a magnified real image of the object or sample being imaged. However, the image that would be observed would be a virtual image that cannot be detected by an electronic detector such as an sCMOS. Thus, as explained further below in relation to Figure 3, the optical collection path comprises a tube lens 303 configured to create an image that an electronic detector such as an sCMOS 107 can observe or detect. The optical collection path also comprises a dielectric mirror 109 arranged at an angle of 45° to the optical collection path from the tube lens 303. The dielectric mirror is designed to allow transmission of particular wavelengths of light.

A sample surface 117 is located underneath the objective lens 115. The sample surface 117 is configured to hold, or retain, samples comprising single molecules. In this example, the sample surface 117 forms part of an assay plate which is placed into the assay plate

holder. In this example the surface 117 forms part of a 96-well assay plate. However, in other examples the surface 117 could form part of any surface configured to hold samples comprising single molecules. Examples include, but are not limited to, a glass microscope slide, a glass surface etched with microfluidic channels, a metal plate, an engraved glass surface, or a glass coverslip. Surface 117 could also, in other examples, form part of an assay plate with a different number of wells. For example, 6, 24, 384 or 1536-well assay plates. The sample surface does not form part of the microscope as such. That is, it is not located in the same housing as the optical microscope.

The optical microscope 100 comprises a plurality of components which includes those components or elements described herein. Significantly, one or more of these components are in-line directly coupled. As described, direct coupling: provides improved stabilization of the relative positions of each component within the optical train; prevents the ingress of dust or other potential contaminants into the illumination path; reduces the number of components needed in the optical train; and provides the most compact footprint possible for the optical path, making the optical microscope arrangement suitable for applications, for example, in a wide range of bench-top instruments. In some examples, all components of the optical microscope 100 are in-line directly coupled.

The optical microscope 100, and particularly the optical illumination path, will now be described in more detail with respect to Figure 2.

The gaussian excitation beam provided by the light source 101 is configured to provide an optimum signal-to-noise ratio for a sample being imaged. In this example, the sample comprises a typical organic fluorophore in water, and the excitation beam comprises a photon flux I_e of 1.36×10^{22} photons cm^{-2} sec^{-1}, providing the optimal signal-to-noise ratio for this sample. Before being provided to beam expanders 105, the excitation beam is passed through a fibre coupler 103, which in this example is a fibre collimator 201 such as a zoom fibre collimator. The fibre collimator 201 converts the light from the optical fibre to a free-space collimated beam.

This collimated beam is subsequently provided to beam expanders 105 which expand the diameter of the gaussian excitation beam along the optical illumination path.

Significantly, the gaussian excitation beam having an expanded beam diameter is subsequently passed through a beam shaping element configured to convert the gaussian

excitation beam into a flat-top beam. In this example, the beam shaping element comprises a top-hat beam shaping technology such as an Aspericon a|-TopShape beam shaper. Such a beam shaping element is configured to convert collimated gaussian beams into collimated flat-top/top-hat beams with uniform intensity distribution. The beam shaping element may also slightly enlarge (M ≈ 1,5) the beam.

By providing such a beam shaping element and converting the beam into a flat-top beam profile, advantageously, illumination of equal intensity is provided across the whole field of view illuminated by the beam.

The optical illumination pathway further comprises an optical element configured to focus the flat-top beam into the microscope objective 115 in a wide area of illumination. In this example, the optical element is an achromatic lens 301 having an unconventional focal distance, and the achromatic lens 301 is housed within a collar 205. By focussing the beam with the achromatic lens 301, illumination of a wide area is achieved. In combination with the flat-top beam, equal intensity illumination is advantageously provided across the entirety of the wide area of illumination. This area may comprise a diameter of at least 1mm, and in this example, the beam area comprises a diameter of between 1mm and 2.5mm.

The wide area of illumination illuminates a sample on the sample surface 117. Light reflected from the sample passes through the dichroic mirror 111 through a mirror mount block assembly 207 along the optical collection path. The mirror mount block assembly 207, together with the optical collection path, is described in more detail with reference to Figure 3 below.

The optical collection path ultimately comprises an imaging means 107 for imaging the sample. In this example, the imaging means comprises an imaging sensor such as a scientific complementary metal oxide semiconductor (sCMOS) sensor configured to provide the direct and simultaneous imaging of the wide area of illumination. This, in combination with the provision of equal intensity illumination and illumination itself being over a wide area provides a particularly advantageous arrangement. That is, the three-fold modification to existing arrangements enables in-focus observation of single molecules over a large area.

The mirror mount block assembly 207 also comprises a laser power sensor 209 configured to monitor the power of the light illuminating the sample.

Figure 3 illustrates the mirror mount block assembly 207 and optical collection path in more detail.

As has been described, the flat-top beam converted by the beam shaping element 203 passes through the achromatic lens 301 and is provided to the microscope objective 115 through reflection from the dichroic mirror 111 for illuminating a sample on sample surface 117.

The sample on the sample surface 117 comprises single molecules. That is, the microscope objective 115 is configured to collect fluorescence emitted from single molecules, and a fluorescent molecule of an image sample will experience sufficient excitation flux to create a fluorescent signal. Detection of the photons arising from this fluorescence demands particular requirements from the imaging means. In this example, the imaging means or sCMOS sensor comprises a chip area of at least 5 cm^2 and provides a resolution of at least 2048x2048 pixels in order to detect photons arising from fluorescence of single molecules.

Light from the sample on the sample surface 117 is reflected and transmitted through the dichroic mirror 111 and passed through the mirror mount block assembly 207. The objective lens 115 makes a magnified real image of the object or sample being imaged. To allow observation by the imaging means 107, the light from the sample is passed through a tube lens 303 in the optical collection path. Similarly, to the achromatic lens 301, the tube lens 303 has an unconventional focal distance. The tube lens 303 also has an unconventional proximity to the microscope objective 115.

The light transmitted through the tube lens 303 is reflected by the dielectric mirror 109 angled at 45° to the optical collection path, and the tube lens 303 creates a detectable image of the sample at the imaging means 107 for imaging of the sample.

Figure 4 is a flow diagram illustrating a method 400 for wide-field microscopy using an optical microscope according to embodiments described herein. At step 401, a gaussian excitation beam is converted into a flat-top beam. At step 403, the flat-top beam is

focussed into a microscope objective in a wide area of illumination. At step 405, the wide area of illumination is directly and simultaneously imaged.

The method described may be applied to the optical microscope according to any embodiment described herein. In this specific example, the flat-top beam is focussed using an achromatic lens having an unconventional focal distance to provide a wide area of illumination having a diameter of between 1 mm and 2.5 mm.

To produce an image of the sample, the method comprises illuminating a sample surface with the wide area of illumination to image a sample. The sample comprises one or more single molecules, and the microscope objective collects fluorescence emitted from single molecules excited by the flat-top excitation beam. The fluorescence emitted from the single molecules is focussed by an optical element, in this example a tube lens, having an unconventional focal distance.

In summary, the optical microscope and corresponding method described provide in-focus epi-illumination wide-field microscopy capable of imaging single molecules. the inventors have appreciated that this is possible particularly through the combination of providing a flat-top beam, a wide area of uniform illumination, and direct and simultaneous imaging of the wide area of uniform illumination.

Embodiments of the present invention have been described with particular reference to the examples illustrated. However, it will be appreciated that variations and modifications may be made to the examples described within the scope of the present invention.

CLAIMS

1. An optical microscope for wide-field microscopies, the optical microscope comprising:

an optical train and a microscope objective, the optical train having:

an optical illumination pathway comprising: a beam shaping element configured to convert a gaussian excitation beam into a flat-top beam; and an optical element configured to focus the flat-top beam into the microscope objective in a wide area of uniform illumination; and

an optical collection pathway comprising an imaging means configured to provide direct and simultaneous imaging of the wide area of uniform illumination.

2. The optical microscope of claim 1 wherein the optical element comprises an achromatic lens having an unconventional focal distance to focus the flat-top beam into the wide area of uniform illumination.

3. The optical microscope of claim 1 or claim 2 wherein the wide area of uniform illumination comprises a diameter of at least 0.5 mm.

4. The optical microscope of any preceding claim wherein the wide area of uniform illumination comprises a diameter of between 1 mm and 2.5 mm.

5. The optical microscope of any preceding claim wherein the imaging means comprises a scientific complementary metal oxide semiconductor, sCMOS, sensor configured to provide the direct and simultaneous imaging of the whole wide area of uniform illumination.

6. The optical microscope of claim 5 wherein the sCMOS sensor comprises a sensor chip area of at least 5 cm^2.

7. The optical microscope of any preceding claim wherein the optical train comprises a plurality of components, one or more of the plurality of components being in-line directly coupled.

8. The optical microscope of any preceding claim wherein all of the plurality of components are in-line directly coupled.

9. The optical microscope of any preceding claim wherein the optical collection pathway is configured to provide the wide area of uniform illumination to illuminate a sample surface for imaging a sample.

10. The optical microscope of any preceding claim wherein the microscope objective is configured to collect fluorescence emitted from single molecules.

11. The optical microscope of claim 10 wherein the optical collection pathway further comprises a second optical element having an unconventional focal distance configured to focus the fluorescence emitted from the single molecules.

12. The optical microscope of claim 11 wherein the second optical element is configured to be arranged at an unconventional proximity to the microscope objective.

13. The optical microscope of claim 11 or claim 12 wherein the second optical element comprises a tube lens.

14. The optical microscope of any preceding claim wherein the imaging of the wide area of uniform illumination comprises a resolution of at least 2048x2048 pixels.

15. The optical microscope of any preceding claim further comprising a beam source configured to provide the gaussian excitation beam to the optical illumination pathway, the gaussian excitation beam being configured to provide an optimal signal-to-noise ratio for a sample to be imaged by the imaging means.

16. The optical microscope of claim 15 wherein the gaussian excitation beam comprises a photon flux of 1×10^{22} to 2×10^{22} photons cm^{-2} sec^{-1}.

17. The optical microscope of any preceding claim wherein the imaging means is configured to image single molecules.

18. A method for wide-field microscopy with an optical microscope, the method comprising:

converting a gaussian excitation beam into a flat-top beam;

focussing the flat-top beam into a microscope objective in a wide area of uniform illumination; and

directly and simultaneously imaging the wide area of uniform illumination.

19. The method of claim 18 wherein the focussing the flat-top beam into a microscope objective in a wide area of uniform illumination comprises focussing the flat-top beam using an achromatic lens having an unconventional focal distance.

20. The method of claim 18 or claim 19 wherein the wide area of uniform illumination comprises a diameter of at least 0.5 mm.

21. The method of any of claims 18 to 20 wherein the wide area of uniform illumination comprises a diameter of between 1 mm and 2.5 mm.

22. The method of any of claims 18 to 21 further comprising illuminating a sample surface with the wide area of uniform illumination to image a sample.

23. The method of any of claims 18 to 22 further comprising collecting, at a microscope objective, fluorescence emitted from single molecules.

24. The method of claim 23 further comprising focussing the fluorescence emitted from single molecules using an optical element having an unconventional focal distance.

25. The method of any of claims 18 to 24 further comprising imaging the wide area of uniform illumination at a resolution of at least 2048x2048 pixels.

Intellectual Property Office

Application No:	GB2304502.4	**Examiner:**	Alan Phipps
Claims searched:	1-25	**Date of search:**	28 September 2023

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1-25	CN 113625439 A YU BIN et al., see whole document
A	-	CN115291381 A LANG SONG et al., see sCMOS camera 148
A	-	CA 3017188 A1 VOKHMIN, see Summary section

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

G01N; G02B

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC

International Classification:

Subclass	Subgroup	Valid From
G02B	0021/00	01/01/2006
G01N	0021/64	01/01/2006
G02B	0021/06	01/01/2006
G02B	0027/09	01/01/2006