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(72) Inventor; and
(71) Applicant : **HARRIS, Martin Russell** [AU/AU]; 196 Southern Road, Heidelberg West, Victoria 3081 (AU).

(74) Agent: **GRIFFITH HACK**; GPO Box 1285, Melbourne, Victoria 3001 (AU).

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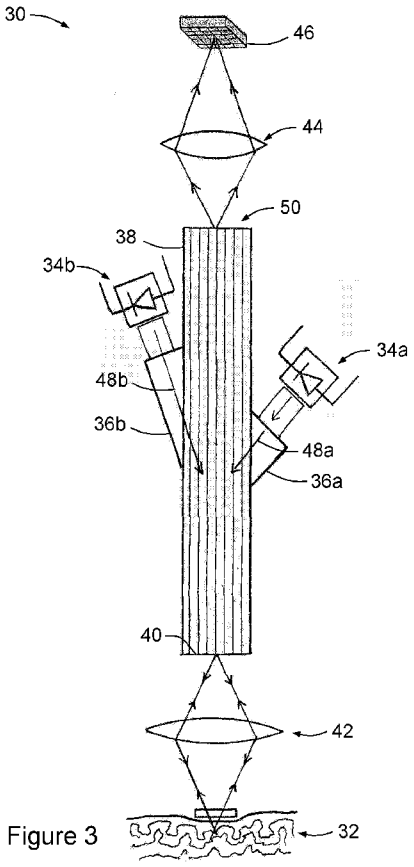


Figure 3

(57) Abstract: A microscope, endoscope or endomicroscope, comprising: a first light source for illuminating a specimen at a first wavelength; a second light source for illuminating the specimen at a second wavelength; a photodetector for collecting light from the specimen when under illumination; and an image differentiator; wherein the microscope, endoscope or endomicroscope is configured to form a first image of the specimen using the first light source and a second image of the specimen using the second light source, and the image differentiator is configured to form a differential image comprising a difference between the first and second images.

WO 2014/005193 A1

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ENDOSCOPY OR MICROSCOPY APPARATUS AND METHOD

Field of the Invention

The present invention relates to an apparatus and method for performing phase gradient and structured illumination microscopy (SIM), of particular but by no means exclusive application in observing intensity patterns within a specimen formed by light emitted from the tip of an optic fibre bundle or in conducting oblique illumination phase contrast microscopy or endoscopy, and to an apparatus and method in microscopy, endoscopy and endomicroscopy, of particular application in performing phase gradient microscopy with, optionally, structured illumination fluorescence microscopy.

Background of the Invention

Structured illumination microscopy (SIM) illuminates a specimen with structured or patterned light, which excites fluorescence in the specimen according to that pattern. A plurality of images are collected with the illumination shifted between each image collection. Analysis of a plurality of such images is used generate a super-resolution image.

Jerome Mertz (Dept. Biomedical Engineering, Boston University, MA) has described a method for phase contrast endoscopic imaging in thick biological tissues, termed Oblique Illumination Phase Contrast Endoscopy. This involves modifying an endoscope, such as that made by Muana Kea Technologies (trade mark) by adding two 1-mm wide LED illumination fibres to the central 2.8 mm optical fibre core detector. This configuration allowed Mertz to use Monte Carlo modelling to reveal a virtual oblique light source deep within the illuminated tissue. Subtraction of the left and right images generates a phase image. This technique was tested using 45-μm beads in agarose, and is said to have revealed good phase images at a depth of about 150 μm. Mertz also reports examining chick embryos and detecting red blood cells flowing in capillaries.

Figure 1 is a view of a system described in *Nature Methods*, **9** (2012) 1195 (Ford, Chu and Mertz). In use, light emitting diode 1 emits a very brief high power pulse of light which travels along multimode plastic optical fibre 2 and is emitted from the tip of the fibre 3 into the tissue 4. A photograph is taken with camera at 5 during this very brief interval. The image is conveyed from the tissue through lenses 6 and coherent image transfer bundle 7. After a very short period of time a second brief high power pulse is emitted, this time from light emitting diode 8. This pulse is of the same wavelength as that from laser diode 1. This pulse from light emitting diode 8 passes along multimode plastic optical fibre 9, is emitted from the tip 10 and illuminates the tissue around the tip. A second image is acquired from camera 5. A specialised camera allows sequential images to be obtained with a less than 10 μs interval between exposures. Image processing involving smoothing and subtraction of one image from the other greatly enhances the contrast of phase objects in the field of view.

Summary of the Invention

According to a first broad aspect, the present invention provides a microscope, endoscope or endomicroscope, comprising:

a first light source for illuminating a specimen at a first wavelength;

a second light source for illuminating the specimen at a second wavelength;

a photodetector for collecting light from the specimen when under illumination; and

an image differentiator;

wherein the microscope, endoscope or endomicroscope is configured to form a first image of the specimen using the first light source and a second image of the specimen using the second light source, and the image differentiator is configured to form a differential image comprising a difference between the first and second images.

In one embodiment, the first wavelength and the second wavelength are different, and the microscope, endoscope or endomicroscope is arranged to perform phase gradient microscopy of the specimen. The first and second light sources may be configured or operable to illuminate the specimen simultaneouly.

The first and second wavelengths may correspond to red light and green light.

The microscope, endoscope or endomicroscope may comprise at least one additional light source (and typically two) for illuminating a specimen at a third wavelength (though the third wavelength may be identical to either the first or third wavelength);

wherein the microscope, endoscope or endomicroscope is arranged to perform structured illumination fluorescence microscopy of the specimen by successive excitation of spots in the specimen and portions surrounding the spots, and form a differential image comprising a difference between the first and second images.

In another embodiment, the first wavelength and the second wavelength are substantially equal, the first and second light sources are configured or operable to illuminate the specimen at different times, and the microscope, endoscope or endomicroscope is arranged to perform structured illumination fluorescence microscopy of the specimen by successive excitation of spots in the specimen and portions surrounding the spots, and form a differential image comprising a difference between the first and second images.

The image differentiator may subtract one of the first and second images from the other of the first and second images.

The microscope, endoscope or endomicroscope may be configured to illuminate the specimen with the first and second light sources and collect the light from the specimen on a single side of the specimen.

The microscope, endoscope or endomicroscope may be arranged to provide oblique back illumination.

The microscope, endoscope or endomicroscope may comprise first and second light transmitters arranged to transmit light from the first and second light sources respectively to the specimen, wherein the first and second light transmitters have distal tips close to the specimen.

The microscope, endoscope or endomicroscope may include a lens for collecting light from the specimen and the distal tips are located on opposite sides of the lens.

According to a first broad aspect, the present invention provides a microscopy or endoscopy method, comprising:

illuminating a specimen at a first wavelength;

illuminating the specimen at a second wavelength;

collecting light from the specimen when under illumination;

forming a first image of the specimen using the first light source and a second image of the specimen using the second light source; and

forming a differential image comprising a difference between the first and second images.

The first wavelength and the second wavelength may be different, and the method include performing phase gradient microscopy of the specimen.

The method may include illuminating the specimen at the first and second wavelengths simultaneouly.

The first and second wavelengths may correspond to red light and green light.

The method may include illuminating the specimen at at least one additional wavelength, performing structured illumination fluorescence microscopy of the specimen by successive excitation of spots in the specimen and portions surrounding the spots, and forming a differential image comprising a difference between the first and second images.

In one embodiment, the first wavelength and the second wavelength are substantially equal, and the method includes illuminating the specimen at the first and second wavelengths at different times, performing structured illumination fluorescence microscopy of the specimen by successive excitation of spots in the specimen and portions surrounding the spots, and forming a differential image comprising a difference between the first and second images.

The image differentiator may subtract one of the first and second images from the other of the first and second images.

The method may include illuminating the specimen with the first and second light sources and collecting the light from the specimen on a single side of the specimen.

The method may include illuminating the specimen with oblique back illumination.

The method may include transmitting illuminating light to the specimen with first and second light transmitters having distal tips close to the specimen. The method may include collecting light from the specimen with a lens and locating the distal tips on opposite sides of the lens.

According to another broad aspect, the present invention provides a structured illumination microscopy apparatus and method, in which the successive images that are required to isolate the focal plane (using known structured illumination principles) are generated by moving the specimen, the bundle tip or an optical component near the specimen by a small distance (in X, Y or Z directions) between exposures.

According to a further broad aspect, the present invention provides an apparatus and method in which the two (or more) images are produced by generating two different patterns of light in a specimen by separate illumination pulses delivered through different channels within an optic fibre bundle (e.g. core and cladding modes, respectively).

According to a still further broad aspect, there is provided a microscope or endoscope for conducting oblique illumination phase contrast microscopy or endoscopy, and a method of conducting oblique illumination phase contrast microscopy or endoscopy.

It should be noted that these various aspects may be employed in combination.

There are several channels that can readily be accessed to carry light within a single fused bundle. Any pair of these might be illuminated in succession to generate the pattern shift in the focal plane and to generate the two or more images for SIM imaging.

Among these channels are:

- Fundamental mode of the few moded cores (or single mode cores)
- Higher order modes of "few-moded" cores. Radially symmetrical, elliptical or other geometries
- Low order cladding modes of entire bundle matrix
- Cladding modes of entire bundle that matching the NA of the cores
- Higher order cladding modes of entire bundle matrix
- Orthogonal polarisation modes of fundamental mode within each core.

The light from the specimen that returns to form the image may travel within the cores of the same bundle. Alternatively the light may return via a different bundle. In this case it would be desirable that both bundle faces came from a single cleave of one bundle in order for the cores to match; bundles with such matching faces are referred to herein as 'conjugate tip bundles'. The tips are mirror images of one another, which is a requirement if a beamsplitter cube is employed.

Alternatively, light may be focussed to a photodetector (such as a CCD), without passing through the cores of a bundle.

The present invention is applicable to a range of techniques, such as GSD microscopy and STED microscopy, in which subtraction can be performed optically rather than in a computer processor. Using this invention super-resolution could also be obtained in fluorescence mode if light intensity is sufficient to saturate the fluorophore (Mats Gustafsson). It is also possible that the invention could be used to facilitate and increase the speed of STORM/PALM imaging by reducing out of focus or near neighbour fluorescent interference.

The invention is applicable to the areas of microscopy and endoscopy.

It is clear that with several available channels within one single fibre bundle to carry light to the specimen and the number of optical configurations of the components that it will not be possible to enumerate all cases.

In general it is preferable to hold the proximal bundle tip stationary. Computation should be easier when the light from each core is always projected onto the same collection of pixel elements of the photodetector array.

Scanning the distal bundle tip or other distal component is used to change the projected pattern in the specimen if the same light delivery channel is used for both exposures. Scanning the distal tip is also used to "fill in" the space between the cores and give proper Nyquist interval sampling. The same scanning mechanism can be used to fulfil both functions.

A number of scanning and positional feedback mechanisms could be employed, including for example the hydraulic actuator of U.S. Patent Application No. 12/065,203 which has the advantage of compactness.

The shift in the pattern position between successive image frame acquisitions is desirably a fraction of the core centre spacing. The difference between successive pairs of images would be transferred to an accumulating frame store. As the scanning would typically be continuous and in one direction (simultaneously fulfilling Nyquist sampling criteria), compensation for the continuing lateral shift must be made before the addition to the cumulative image.

The amount of shift required in the frame store could be computed from the outputs of positional feedback mechanisms linked to the bundle tip. Alternatively autocorrelation could be done on the features of successive images, possibly aided by bright reference objects previously applied to the tissue.

For some embodiments it is desirable to scan the light pattern within the specimen (such as by scanning the distal tip of a delivery fibre bundle) and to simultaneously and synchronously scan another component at the proximal end so as to produce a real image with a fixed position on the EMCCD photo-array. The frame acquisition can then be continuous, with no downtime required for CCD readout. A single exposure thus integrates the image.

A number of embodiments are suitable for use in GSD and STED. These can be implemented by software manipulation of the image or by directly viewable techniques.

It should be noted that any of the various features of each of the above aspects of the invention, and of the various features of the embodiments described below, can be combined as suitable and desired.

It should also be noted that the optical arrangements of the embodiments described herein, implemented variously as microscopes or photolithographic apparatuses, may in certain applications be implemented to provide, for example, microscopes, endoscopes, endomicroscopes, photolithographic apparatuses, light sources, apparatuses for trapping Bose-Einstein condensates, and apparatuses for photopolymerisation.

Also, many components of the described embodiments are omitted for clarity, but the characteristics of such omitted components will be readily understood or determined by those skilled in the art, who similarly and be varied will readily understand how these components should be varied for other applications.

Brief Description of the Drawing

In order that the invention may be more clearly ascertained, embodiments will now be described, by way of example, with reference to the accompanying drawing, in which:

Figure 1 is a schematic view of a microscope of the background art;

Figure 2 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 3 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 4 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 5 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 6 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 7 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 8 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 9 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 10 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 11 is a schematic view of a photolithography apparatus according to an embodiment of the present invention;

Figure 12A is a schematic view of a photolithography apparatus according to an embodiment of the present invention;

Figure 12B is a schematic view of a structure for directing de-activating light into the beamsplitter cube of the photolithography apparatus of figure 12A according to an embodiment of the present invention;

Figure 13A is a schematic view of a photolithography apparatus according to an embodiment of the present invention;

Figures 13B to 13D are schematic representation of various modes in which activating light is transmitted in the cores of the fibre bundle of the photolithography apparatus of figure 13A;

Figure 13E is a schematic representation of the halo effect produced by simultaneously coupling two de-activating laser light sources into the cores of the fibre bundle of the photolithography apparatus of figure 13A;

Figure 14A is a schematic view of a photolithography apparatus according to an embodiment of the present invention;

Figures 14B is a schematic representation of a mode that is not supported by the elliptical cores of the fibre bundle of the photolithography apparatus of figure 14A;

Figures 14C is a schematic representation of the fundamental mode of the fibre bundle of the photolithography apparatus of figure 14A;

Figure 15 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 16 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 17 is a schematic illustration of a technique for manufacturing elliptical core bundles according to an embodiment of the present invention;

Figure 18 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 19 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 20 is a schematic view of a rotating fused fibre bundle system according to an embodiment of the present invention;

Figure 21A to 21C are schematic views of various components of the rotating fused fibre bundle system of figure 20;

Figure 22 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 23 is a schematic view of the prism stage of the microscope of figure 22;

Figure 24 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 25 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 26 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 27 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 28 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 29 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 30 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 31 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 32 is a cross sectional view of the microscope of figure 31;

Figure 33 is a schematic view of a disposable microscope according to an embodiment of the present invention;

Figure 34 is a schematic view of a microscope according to an embodiment of the present invention;

Figures 35A and 35B are structural diagrams of possible compounds for use with the microscope of figure 34;

Figure 36 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 37 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 38 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 39 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 40 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 41 is a schematic view of a microscope according to an embodiment of the present invention;

Figure 42 is a schematic view of a microscope according to an embodiment of the present invention; and

Figure 43 is a schematic view of a microscope according to an embodiment of the present invention.

Detailed Description

Figure 2 is a schematic view of a microscope 10 according to an embodiment of the present invention, shown with a specimen in the form of a tissue sample 12. Microscope 10 includes a light source (not shown), a prism coupler 14, a fused optic fibre bundle 16 (with a distal tip 18), a focussing lens 20 and a photodetector in the form of a CCD chip 22.

Referring to figure 2, in use a light beam 24 from the light source is coupled through prism coupler 14 into the higher order cladding modes of fused optic fibre bundle 16. The light leaves the bundle at distal tip 18 and passes into the tissue sample 12. The distal tip 18 can slide against the tissue sample 12, so—to effect scanning—distal tip 18 is moved relative to tissue sample 12 (by movement of distal tip 18, tissue sample 12 or both) such that distal tip 18 moves in direction 26 relative to tissue sample 12. This movement may be, for example, linear or rotational. Light from within the tissue (being backscatter light, fluorescence or a combination thereof) enters the cores of the bundle 16 and passes to the other end 28 of bundle 16, from which it is emitted. It is then focussed by focussing lens 20

to form an image on CCD chip 22. Two separate exposures are taken, the distal tip 18 being moved relative to the tissue sample 12 therebetween preferably by a fraction of the core centre spacing of the bundle 16.

A differential image is then formed from the two separate exposures. This differential image contains mostly high frequency information, low frequency information being largely removed in the formation (such as by subtraction) of the differential image, and thus provides a greater contrast for structures that are very close to the distal tip 18 of bundle 16.

Figure 3 is a schematic view of a microscope 30 according to another embodiment of the present invention, shown with a specimen in the form of a tissue sample 32. Microscope 30 includes two light sources 34a, 34b, respective prism couplers 36a, 36b, a fibre bundle 38 with a polished distal tip 40, a first focussing lens 42, a second focussing lens 44 and a photodetector in the form of a CCD chip 46.

In use, light 48a, 48b from respective light sources 34a, 34b enters the cladding of the bundle 38 as high and low order modes respectively. The light from each source leaves the polished distal tip 40 and is focused by first lens 42 into the tissue sample 32. The focus structure of the two light sources 34a, 34b differs, as the NA of the light from the 38 bundle is not the same. Light from the tissue sample 32 returns to the bundle 38; some of it enters the cores of bundle 38 and emerges from the other end 50 of the bundle 38, and is focused by second lens 44 onto CCD 46 to form an image. Light sources 34a and 34b are flashed alternately to provide the change in the projected light structure so that a differential image can again be formed. Relative movement of distal tip 40 and tissue sample 32 is not required in this embodiment for focal plane isolation but is effected to satisfy Nyquist sampling criteria. With this embodiment the differential image signal may be enhanced by the use of a lens having slight chromatic or spherical aberration.

Figure 4 is a schematic view of a microscope 60 according to another embodiment of the present invention, shown with a specimen in the form of a tissue sample 62. Microscope 60 includes a large area source 64, a collimating lens 66, a beamsplitter in the form of a beamsplitter cube 68, a focussing lens 70, a fibre bundle 72 (with a proximal tip 74 and distal tip 76), a lens train 78, a focussing lens 80 and a photodetector in the form of a CCD array 82.

In use, light from large area source 64 is collimated by lens 66 and (partially) reflected by beamsplitter cube 68 towards the cores at the proximal tip 74 of fibre bundle 72. The light is emitted from the cores at the distal tip 76 and focussed by lens train 78 into the tissue sample 62. Fluorescence and/or other return light from the tissue sample passes in the opposite direction through the cores of fibre bundle 72 and is emitted from the proximal tip 74, passes through beamsplitter cube 68 and is focused by focussing lens 80 onto CCD array 82. Differential images are generated from pairs of exposures, including effecting relative movement of distal tip 76 and the tissue sample 62 (in the illustrated example, by moving distal tip 76 in the direction shown by arrow 84). This movement provides the desired shift in the structured light. Alternatively, the lens train 78, or lens train 78 and fibre

bundle 72, or tissue sample 62, may be moved to the same effect between exposures. The movement can also function to provide proper sampling intervals.

Figure 5 is a schematic view of a microscope 90 according to another embodiment of the present invention, shown with a specimen in the form of a tissue sample 92. Microscope 90 employs two fibre bundles, one to deliver illuminating light and a second 'conjugate' bundle to collect return light. These bundles are oriented so that all the cores are in mirror-image correspondence and advantageously are affixed to the sides of a beamsplitter cube with optical cement, as discussed below.

Referring to figure 5, microscope 90 includes a light source 94, a focussing lens 96, a first fibre bundle 98 and a beamsplitter cube 100. The distal tip 102 of first fibre bundle 98 is affixed to one side of beamsplitter cube 100 with optical cement. Microscope 90 also includes a second light source 104 and a coupling prism 106 for coupling light from second light source 104 into the cladding modes of the first fibre bundle 98. Microscope 90 includes a collimating lens 108 optically downstream of beamsplitter cube 100, an objective lens 110 for focussing light to within tissue sample 92, a second 'conjugate' bundle 112 with a proximal tip 114 affixed to another side of beamsplitter cube 100 with optical cement, a converging lens 116 and a photodetector in the form of a CCD 118.

In use, a first light beam from light source 94 is coupled by focussing lens 96 into the cores of first fibre bundle 98. A second light beam, from second light source 104, is coupled by prism 106 into the cladding modes of first fibre bundle 98. Both light beams emerge from the distal tip 102 of first fibre bundle 98 and pass into the glass of beamsplitter cube 100. The light beams pass through beamsplitter cube 100 and, upon emerging, are collimated by collimating lens 108, and pass to objective lens 110 which brings them to a focus within tissue sample 92. Return light from tissue sample 92 is collected by objective lens 110 focussed by collimating lens 108 towards beamsplitter cube 100, and partially reflected by beamsplitter cube 100 to a focus at the proximal tip 114 of second bundle 112. The light that enters the cores of second bundle 112 passes to the other end 120 of second bundle 112, from which it emerges and is converged by converging lens 116 to a focus on CCD 118. A differential image is formed by flashing first light source 94 and then second light source 104, and thereby collecting a pair of exposures. The pixel values from the exposure from second light source 104 are subtracted from the pixel values from the exposure taken with first light source 94. No relative movement—such as by translation or rotation of tissue sample 92—is required between exposures, but translational relative movement is employed to give proper sampling intervals (as discussed above).

Figure 6 is a schematic view of a microscope 130 according to another embodiment of the present invention, which has a number of novel features and is shown with a specimen in the form of a tissue sample 132. Microscope 130 includes an excitation source 134, a delivery optic fibre 136 (which is single moded, or may be multimoded for low coherence length light) with a distal tip 138, a collimating lens 140 and a fibre bundle 142.

Microscope 130 also includes sources of de-excitation light; a first de-excitation light source 144 is arranged in a manner comparable to that of excitation source 134, with an optic fibre 146 for delivering light from first de-excitation light source 144 to collimating lens 140. The distal tip 148 of optic fibre 146 is positioned in the Fourier space away from the optic axis of collimating lens 140 and fibre bundle 142, so that the beam 150 of de-excitation light emerging from it is collimated (by collimating lens 140) and enters fibre bundle 142 at an angle that causes the beam to enter the cores of fibre bundle 142 as higher order modes.

Microscope 130 also includes second, third and fourth de-excitation sources 152a, 152b, 152c, and respective multimode optic fibres 154a, 154b, 154c, mode conditioners 156a, 156b, 156c and prism couplers 158a, 158b, 158c, to couple de-excitation light from second, third and fourth de-excitation sources 152a, 152b, 152c into the cladding of fibre bundle 142 as low order, medium order and high order cladding modes respectively.

The de-excitation pulses from de-excitation sources 144, 152a, 152b, 152c may operate by GSD/STED mechanisms, or de-excitation sources 144, 152a, 152b, 152c may act as the illumination source that is used to produce the second frame that is subtracted from the frame produced by the excitation light pulse from excitation source 134.

Downstream of fibre bundle 142, microscope 130 includes collimating lens 160 and an objective lens 162, the last arranged to focus light into tissue sample 132.

Microscope 130 also includes a converging lens 164 and a CCD 166, the converging lens 164 being arranged to direct return light emitted by fibre bundle 142 and collimating lens 140 to a focus on CCD 166. In addition, microscope 130 includes two low coherence light sources 168a, 168b, and respective multimode optic fibres 170a, 170b connected thereto with distal tips for emitting light from low coherence light sources 168a, 168b positioned on either side of the optic axis at a distance of approximately 2f away from objective lens 162; these emit light alternately (by alternate illumination of low coherence light sources 168a, 168b) to carry out Oblique Illumination Phase Contrast Endoscopy in conjunction with the fluorescence mode imaging described above.

In use, light from excitation source 134 is transmitted along optic fibre 136, and is emitted from distal tip 138 as a diverging beam 172 that is collimated by collimating lens 140 and passes into the proximal tip 174 of fibre bundle 142 as the fundamental mode of the cores of fibre bundle 142.

Meanwhile, de-excitation light is provided either by first de-excitation light source 144 or by second, third and fourth de-excitation sources 152a, 152b, 152c. Light from de-excitation light source 144 is delivered by optic fibre 146 as described above, entering the cores of fibre bundle 142 as higher order modes. De-excitation light from second, third and fourth de-excitation sources 152a, 152b, 152c is transmitted by respective multimode optic fibres 154a, 154b, 154c to mode conditioners 156a, 156b, 156c; light passing through the mode conditioners 156a, 156b, 156c passes through respective prism couplers 158a, 158b, 158c, which couple the light into the cladding of fibre bundle 142 as low order, medium order and high order cladding modes respectively.

The light and de-excitation light passes out through distal tip 176 of fibre bundle 142, passes through collimating lens 160 and is focussed by objective lens 162 to a focus in tissue sample 132. Light from the tissue returns through objective lens 162 and collimating lens 160, and the cores of the fibre bundle 142, and is focused by converging lens 164 to form a focus on CCD 166.

In some applications it may be desirable that the fibre bundle 142 be made from depressed cladding, W profile core fibres.

GSD/STED Imaging

The embodiments described above by reference to figures in 4, 5 and 6 can be used for conducting ground state depletion (GSD) microscopy/stimulated emission depletion (STED) microscopy, in which case each would additionally include an image processor (such as in the form of image processing software) for finding the centroid and the intensity sum for each core spot of all the EMCCD pixel elements that receive light from every individual spot. The image processor then maps every centroid as a reduced spot in the cumulative frame store for that image and then continues this to give proper high resolution sampling.

However, in an alternative embodiment, a micro-lens plate is used to avoid this complexity. Figure 7 is a schematic view of a microscope 180 according to another embodiment of the present invention (shown with a user's eye 182), for ground state depletion GSD microscopy or stimulated emission depletion STED microscopy in which an entire image may be integrated in one single exposure on an EMCCD. This allows one to avoid readout downtime of the CCD, a drawback of multiple exposure systems. For bright objects it would be possible for it to be viewed directly by eye.

Referring to figure 7, microscope 180 includes a light source 184, a pair of lenses 186, 188 and an optic fibre bundle 190. Microscope 180 also includes de-activation light sources 192 and 194 and respective prisms 196 and 198 for coupling the de-excitation light from de-activation light sources 192 and 194 into the cladding of fibre bundle 190. Microscope 180 includes a beamsplitter in the form of a beamsplitter cube 200, a collimating lens 202 and an objective lens 204 for brings light to a focus at a plane 206 inside a specimen (not shown).

Microscope 180 also includes, for processing return light in the manner described below, a collimating lens 208, filters 210a, 210b, a focussing lens 212, a microlens array plate 214 and an ocular lens 216.

In use, excitation light from light source 184 is focused by lenses 186, 188 into the cores of fibre bundle 190. Light from the de-activation sources 192, 194 is coupled by prisms 196, 198 into the cladding of fibre bundle 190. Light from both de-activation sources 192, 194 is emitted from the distal end 218 of fibre bundle 190, passes through beamsplitter cube 200 and is collimated by collimating lens 202. The light then passes to objective lens 204, which brings it to a focus at plane 206 inside the specimen.

Light emitted from, for example, sub-resolution fluorescent objects A and B in the specimen in focal plane 206 returns through objective lens 204 and collimating lens 202, and is brought to a focus as separate diffraction limited spots at A' and B' at an exit face of beamsplitter cube 200. Light emitted by that face is collimated by collimating lens 208 forming respective beams 220 and 222, and passes through filters 210a, 201b. The beams 220 and 222 then pass through focussing lens 212, which focuses them to respective Gaussian waists / Airy discs 224 and 226. The now parallel light beams (each emitted from a sub-resolution area in the specimen) then encounter microlens array plate 214; the optical elements in the microlens array match the projected spot pattern in the image. Thus, microlens 228—for example—brings the light emitted from A to a focus at 230, while microlens 232 brings the light from B to a focus at 234. These focal points 230 and 234 are located in a plane 236. Ocular lens 216 then converges and collimates the light from plane 236 and brings the image to the retina 238 as focused spots A" and B" respectively.

Synchronised scanning between the optic fibre distal tip 218 and microlens array plate 214 creates a continuous and properly sampled super-resolution image.

Optically Obtained Super-Resolution Images, the Microlens Array Plate and Methods of Fabricating the Expanded Array Component

As described above, it should be possible to obtain a purely optical super-resolution raw image directly without the necessity for electronic transduction and translation to a screen. The Abbé limit could be exceeded optically without empty magnification.

In all cases where purely optical super-resolution is desired it is necessary to employ an array of optical elements at or close to the focus of an enlarged image of the core spots. These elements must be arranged in a pattern that accurately matches the spacing of the cores.

A number of methods of creating a lens array would be apparent to those skilled in the art. Some of these involve photo-activation of an optically transparent material. The light exposure and the resulting lenses may be formed by photopolymerisation or by photo-dissolution.

1. PHOTOFABRICATION
    a. Forming an expanded image of the bundle waveguide pattern in a layer of monomer or polymer on a glass sheet.
        i. Photopolymerisation of convex micro-lenses by light projected from the cores of the bundle.
        ii. Photopolymerisation of concave micro-lenses by light projected from the cladding of the bundle.
        iii. Photo-dissolution to form convex micro-lenses by light projected from the cladding of the bundle.

iv. Photo-dissolution to form concave micro-lenses by light projected from the cores of the bundle forming an expanded image on a glass sheet.

b. Formation of the lens array directly on the polished expanded end of an adiabatically tapered bundle.

i. Using photofabrication in a layer of the material on the expanded face. This may be done by any of the four techniques described above.

ii. Chemically etching the glass of the expanded polished end of the waveguide bundle. This can be done with alkaline or fluoride solutions.

1. Etching the cladding to give convex microlenses
2. Etching the cores to give concave microlenses.

2. Allowing glass balls (ballotin) to position themselves into the array of concave structures formed from any of the above. (Glass balls of precise dimensions used as spacers in LCD screens would be suitable.)

3. Employing an adiabatically tapered bundle made from fibres which have a sub-core at the centre of each of the cores. This sub-core has no effect on the light in the thin section but it can act as a single mode core of an axially symmetric coupler in the expanded section. If made correctly

4. Using a pick and place robot to position and glue the microlenses on the expanded plate. This would be done by the active control using the optical signal from the core as measured from the output of a quadrant photodiode. The robot would take each lens to its approximate position on the glass plate. The robot would move the lens, under active feedback control, until the outputs of the quadrant photodiodes were equal. Then a photopolymerisation pulse would affix each lens in position. Hemispherical planoconvex lenses would be easiest to manipulate.

Figure 8 is a schematic view of a microscope 240 according to another embodiment of the present invention, with a specimen in the form of a tissue sample 242. Microscope 240 is designed for use as a tissue contact endomicroscope through a flexible endoscope biopsy channel. It combines structured illumination fluorescence imaging with Oblique Illumination Phase Contrast Endoscopy.

Microscope 240 includes two light emitting diodes 244 and 246 and a Sumitomo (trade mark) or Furakawa (trade mark) fused optic fibre bundle 248 that has shallow grooves 250 and 252 in its sides close to its proximal end 254. Fibre bundle 248 has a polished frusto-conical distal tip 256 (though other distal tip shapes are envisaged, and it may be advantageous in some applications to have, for example, a polished convex distal tip).

Microscope 240 also includes, for collecting return light ultimately emitted by proximal tip 254 of fibre bundle 248, a collimating lens 258, a beamsplitter in the form of a beamsplitter cube 260, a focussing lens 262 and a CCD array 264.

Microscope 240 further includes a large area excitation wavelength light source 266, and a collimating lens 268 for collimating light from light source 266. Microscope 240 also includes an alternately illuminated light source 270, a pair of focussing lenses 272 and 274 for collecting and focussing light from alternately illuminated light source 270, a multimode optic fibre 276 arranged to receive light from focussing lenses 272 and 274, and a coupler prism 278 that is optically glued to fibre bundle 248 and couples light into the cladding modes of bundle 248.

Microscope 240 further includes cladding mode strippers 280 and 282 located towards the proximal end 254 of fibre bundle 248 and a long pass filter 284 to prevent stray light from source 266 from reaching CCD 264.

In use, light emitting diodes 244 and 246 couple light into the adjacent cores on the distal side of the grooves 250, and this light travels as rays 14 and 16 to the distal end of the bundle 18. The light enters the tissue sample 242 as rays 286 and 288; as these rays leave fibre bundle 248, they are bent towards the central axis of the bundle 248 by refraction at the angle polished sections 290 and 292 of the distal tip 256.

The light emitting diodes 244 and 246 are pulsed in alternation. Light from backscatter within the tissue sample 242 form "virtual oblique light sources" (at regions 294 and 296, at depths approximating the mean free path distance within the tissue sample 242) re-enters the bundle in the central area of the distal tip 256 and travels to the proximal end 254 of the bundle 248. The light is then emitted from the cores at the proximal tip 254 and collimated by collimating lens 258, then reflected from the internal, oblique reflecting surface 298 of beamsplitter cube 260 and focused by focussing lens 262 into CCD array 264. Images are derived by subtraction of one frame from the other. Phase objects close to the distal bundle face are enhanced while scatter by deeper objects is nulled out.

A similar two exposure differential image method is used for isolating the fluorescence from objects close to the distal tip 256 and rejecting fluorescence from more distant sources. Light from briefly illuminated large area excitation wavelength light source 266 is collimated by collimating lens 268, passes through beamsplitter cube 260 and is coupled into the cores of the proximal tip 254 of the bundle 248 via lens 258. The light emerges from the distal tip 256 and generates fluorescence in fluorophore labeled structures within the tissue sample 248. Some of this fluorescence returns through the cores of bundle 248, is reflected by the beamsplitter cube 260 and is focussed onto the CCD array 264 to form an image.

A second fluorescence image is generated by alternately illuminated source 270. The light from this source 270 is focussed by lenses 272 and 274, and is carried by multimode optic fibre 276 to coupler prism 278, which couples the light into the cladding modes of the bundle 248. This light is reflected from the opposite sides of the bundle 248 at

the interface between the glass and a fluoropolymer layer 300. This cladding mode light also leaves the distal tip 256 of the fibre bundle 248 and causes fluorescence. Very little of the light energy from this second pulse is detectable in the region directly in front of each of the cores; hence the differential image will strongly enhance the visibility of those features.

Thus, dual phase and fluorescence images from the contact surface of a bare bundle tip may be produced. All required hardware components can be located within 15 cm of the proximal tip 254. The main length of the fibre bundle 248 is available to pass along the biopsy channel to the flexible endoscope tip.

The following two embodiments provide microscope that each have a fibre bundle with a distal tip that is polished at an acute angle to the axis of the bundle. Light passing down the cores of the fibre bundle is totally internally reflected when it reaches the angled tip interface. The polished face may resemble a hypodermic needle or it may be formed into a conical tip.

By the principle of optical reciprocity, the light from the bulk of the tissue sample is unable to enter the cores at an angle that would allow them to be guided within them back to the proximal tip. Objects within the tissue that are within a very short distance of the core end surface, typically less than a wavelength, are able to evanescently couple scattered light or fluorescence into the cores. This light is guided to the proximal tip and acts to form the image. The cladding modes act as the source of the light in this embodiment. They can exceed the angle of TIR in their initial propagation mode or they can increase the angle as they undergo multiple reflections.

Thus, figure 9 is a schematic view of a microscope 310 according to another embodiment of the present invention, shown with a specimen in the form of a tissue sample 312. Microscope 310 includes a fibre bundle 314 with a polished distal tip 316 in the shape of a hypodermic needle (shown in isometric view as detail 318). Microscope 310 includes a pair of prism couplers 320 and 322 attached to bundle 314. Light rays 324 and 326 from respective light sources (not shown) enter bundle 314 via prism couplers 320 and 322, and propagate along the bundle 314 as rays that are reflected at the glass air interface of the bundle 314. At the distal tip 316 of bundle 314, these beams escape into the tissue sample 312 through the flat, angle polished tip surface of the distal tip 316. The distal portion of bundle 314 (apart from the angle polished tip surface of the distal tip 316) has a coating comprising a thin layer of silver 328, which increases the light output. Scatter or fluorescence from objects (such as at 330) return into the cores of bundle 314 and pass to the proximal tip 332 of fibre bundle 314, emerging as ray bundles 334 and 336. Microscope 310 includes lenses 338 and 340, and a CCD 342; ray bundles 334 and 336 are collected and focussed by lenses 338 and 340 to CCD 342, on which an image is formed. Microscope 310 also includes a cladding mode stripper 344, located near proximal tip 332 of bundle 314.

Figure 10 is a schematic view of a microscope 350 according to another embodiment, shown with a specimen in the form of a tissue sample 352; microscope is

comparable to microscope 310 of figure 9, and like numerals have been used to identify like elements. However, instead of a fibre bundle with a hypodermic needle-like distal tip, microscope 350 has a fibre bundle 354 with a tip 356 polished in the form of a cone (shown in isometric view as detail 358). As with microscope 350, light rays 324 and 326 from respective light sources (not shown) enter bundle 354 via prism couplers 320 and 322, and propagate along the bundle 354 as rays that are reflected at the glass air interface of the bundle 354. At the distal tip 356 of bundle 354, these beams escape into the tissue sample 352 through the surface of the conical polished distal tip surface 356. The bundle 354 is rotated during each exposure by any suitable mechanism, as shown by arrow 360. This improves the resolution of the image. Scatter or fluorescence from objects such as that at 362 return into the cores of bundle 314 and pass to the proximal tip 332 of fibre bundle 314, emerging as ray bundles 334 and 336 which are collected and focussed by lenses 338 and 340 to CCD 342, on which an image is formed

The conical tip 356 might be a desirable form to provide images of the os of the cervix. In solid tissue it might be desirable to pass a trochar through the tissue and to introduce a contrast medium and a reference object such as a thin fluorescent thread prior to the observation being made.

Cladding mode applications; Photolithography; photopolymerised nanofabrication

Figure 11 is a schematic view of a photolithography apparatus 370 according to another embodiment of the present invention, shown with a silicon wafer 372. Microscope 370 is adapted for high density photolithography applications, such as for the exposure of resist material on silicon wafers (for example) in the fabrication of semiconductor chip devices.

Photolithography apparatus 370 includes a TEMoo laser 374 with an output wavelength that can activate the photoresist material on silicon wafer 372, a first microlens array 376 (comprising microlenses or lenslets 378 on a transparent plate 380), a second microlens array 382 (also comprising microlenses or lenslets 384 on a transparent plate 386) and, between the first and second microlens arrays 376, 383, a mask 388 that consists of a pattern defining the features desired to be transferred to the wafer 372. Each microlens 378 of first microlens array 376 brings a portion of the incident light to a diffraction limited point focus, the array of such foci being in the plane as that of mask 388. The mask 388 of this embodiment comprises a thin layer of chromium metal on a silica glass sheet substrate. Light incident on first microlens array 376 but not subsequently blocked by mask 388 passes to second microlens array 382, whose microlenses 384 match the microlenses 378 of first microlens array 376. The two microlens arrays 376, 382 are separated by a distance such that the foci of the microlenses 378 of first microlens array 376 are co-incident with the foci of the microlenses 384 of second microlens array 382.

Photolithography apparatus 370 also includes, optically downstream of microlens arrays 376, 382, a first lens 390, a second lens 392 and a fused fibre optic bundle 394 (with

polished proximal tip 396 and distal tip 398, and comprising single moded cores 400). Downstream of fibre bundle 394, photolithography apparatus 370 includes a pair of lenses 402, 404 for focussing light to diffraction limited spots on the photoresist layer 406 of the silicon wafer 372.

Photolithography apparatus 370 also includes light sources 408, 410 for providing light with a wavelength that can de-activate the latent image in the photoresist (STED mechanism) or prevent the formation of the activated species in the photoresist (GSD mechanism), and respective coupling optics 412, 414 for coupling light from light sources 408, 410 into fibre bundle 394 so that the light passes as cladding modes along the bundle 394 to its distal tip 398.

In use, a collimated beam of light 416 of a wavelength that can activate the photoresist material is emitted from TEMoo laser 374 and encounters first microlens array 376. As explained above, each microlens 378 brings a portion of the incident light to a diffraction limited point focus in the plane of mask 388. The light that is not blocked by mask 388 passes to the set of matching microlenses 384 of second microlens array 382.

Consequently the light from microlenses 384 of second microlens array 382 emerges as narrow collimated beams 418 but, as these beams are narrow, they rapidly spread out as diverging cones of light 420. These cones of light 420 encounter first lens 390, which collimates the light from each cone to form collimated ray bundles 424. These collimated ray bundles 424 encounter second lens 392, which brings each beam to a focus at the polished proximal end of a respective, separate single moded core 400 at the proximal tip 396 of fibre bundle 394.

The light enters the cores 400 and passes along them to emerge from the distal tip 398. It then passes through the pair of lenses 402, 404 to form diffraction limited spots on the photoresist layer 406 of the semiconductor wafer 372.

Light from light sources 408, 410 is coupled into the cladding of the bundle 394 by coupling optics 412, 414. This light passes as cladding modes along the bundle 394 to the distal tip 398, and emerges from the distal tip 398 from between the cores 400 and hence is focused by the pair of lens 402, 404 as a reticulated pattern that surrounds the focused spots formed by the light from the cores 400.

Super-resolution photolithography is effected by moving the wafer in the direction shown by arrow 426. At the same time the mask 388 is moved in the direction shown by arrow 428 at a speed that allows the image of the mask 388 to maintain its position on the surface of wafer 372.

In this photolithography embodiment, the activating spot of light is surrounded by a ring of light of the reticulated cladding pattern or the doughnut mode. This shrinks/erodes the dimensions of the feature in both the X and the Y dimensions. Much of the 'real estate' on a silicon wafer chip, however, is taken up by linear tracks. Also, many of the devices fabricated in the silicon surface are made up of structures that are thin only in one direction (see, for example, an Ivy Bridge 22 nm tri-Gate Transistor.) Exposing the photoresist that

forms the tracks or other thin structures using a halo of light for de-activation is thus inefficient, as the de-activated state for GSD processes in the photoresist has a finite decay time (and STED may require a light intensity that is too high to write many spots), so if a line is the desired feature it would be more efficient to write it as sections of a line in a few exposures rather than to fabricate it by exposures as a large number of spots.

Figure 12A is a schematic view of a photolithography apparatus 430 according to another embodiment of the present invention, as might be applied to high density photolithography, shown with a silicon wafer 432. This embodiment uses an array of planar waveguide structures to produce a de-activating light source. Several features of photolithography apparatus 430 are identical with corresponding features of photolithography apparatus 370 of figure 11, and like reference numerals have been used to identify like features.

Thus, photolithography apparatus 430 includes a TEMoo laser 374 with an output wavelength that can activate the photoresist material on silicon wafer 432, a first microlens array 376, a second microlens array 382 and, between the first and second microlens arrays 376, 383, a mask 388 that consists of a pattern defining the features desired to be transferred to the wafer 432. Each microlens of first microlens array 376 brings a portion of the incident light to a diffraction limited point focus, the array of such foci being in the plane as that of mask 388, while light incident on first microlens array 376 but not subsequently blocked by mask 388 passes to second microlens array 382, whose microlenses match the microlenses of first microlens array 376, the two microlens arrays 376, 382 being separated by a distance such that the foci of the microlenses of first microlens array 376 are co-incident with the foci of the microlenses of second microlens array 382.

Photolithography apparatus 430 also includes, optically downstream of microlens arrays 376, 382, a first lens 390 and a second lens 392. However, downstream from first and second lenses 390, 392, photolithography apparatus 430 includes a beamsplitter in the form of a beamsplitter cube 434 and a pair of lenses 436, 438.

Photolithography apparatus 430 also includes a structure 440 (shown in greater detail in isometric view in figure 12B) for directing de-activating light into beamsplitter cube 434, comprising a plurality of parallel planar waveguide layers 442.

Photolithography apparatus 430 further includes light sources 446 optically coupled to structure 440 such that light coupled into structure 440 from light sources 446 is emitted by structure 440 into a side face 448 of beamsplitter cube 434.

In use, a collimated beam of light 416 of a wavelength that can activate the photoresist material is emitted from TEMoo laser 374 and encounters first microlens array 376. As explained above, each microlens brings a portion of the incident light to a diffraction limited point focus in the plane of mask 388. The light that is not blocked by mask 388 passes to the set of matching the microlenses of second microlens array 382. The light from the microlenses of second microlens array 382 emerges as narrow collimated beams 418 but, as these beams are narrow, they rapidly spread out as diverging cones of light 420.

These cones of light 420 encounter first lens 390, which collimates the light from each cone to form collimated ray bundles 424. These collimated ray bundles 424 encounter second lens 392, which brings each beam to a focus at a proximal face 450 of beamsplitter cube 434; the beams then pass through beamsplitter cube 434 followed by the pair of lenses 436, 438, which form the beams into diffraction limited spots A, B, and C in the photoresist layer 452 on the wafer 432.

Light from de-activating sources 446 enters structure 440 as described above, and exits structure 440 through side face 448 into beamsplitter cube 434, is (partially) reflected by the dichroic surface of beamsplitter cube 434 towards, and is focussed by, the pair of lenses 436, 438. D and E indicate two exemplary planar waveguide layers 442; D' and E' indicate the positions of the light from layers D and E once focussed onto wafer 432.

To effect The mask and the wafer are moved synchronously as shown by arrows 392 and 48.

Photolithography is effected by moving the wafer 432 in the direction shown by arrow 426. At the same time the mask 388 is moved synchronously in the direction shown by arrow 428, such that the image of the mask 388 maintains its position on the surface of wafer 432.

Figure 13A is a schematic view of a photolithography apparatus 460 according to still another embodiment of the present invention, as might be applied to high density photolithography, shown with a silicon wafer 462. Several features of photolithography apparatus 460 are identical with corresponding features of photolithography apparatus 370 of figure 11, and like reference numerals have been used to identify like features.

Thus, photolithography apparatus 460 includes a TEMoo laser 374 with an output wavelength that can activate the photoresist material on silicon wafer 462, a first microlens array 376, a second microlens array 382 and, between the first and second microlens arrays 376, 383, a mask 388 that consists of a pattern defining the features desired to be transferred to the wafer 432. Each microlens of first microlens array 376 brings a portion of the incident light to a diffraction limited point focus, the array of such foci being in the plane as that of mask 388, while light incident on first microlens array 376 but not subsequently blocked by mask 388 passes to second microlens array 382, whose microlenses match the microlenses of first microlens array 376, the two microlens arrays 376, 382 being separated by a distance such that the foci of the microlenses of first microlens array 376 are co-incident with the foci of the microlenses of second microlens array 382.

Photolithography apparatus 460 also includes, optically downstream of microlens arrays 376, 382, a first lens 390 and a second lens 392. However, downstream from first and second lenses 390, 392, photolithography apparatus 460 includes a fused fibre optic bundle 464 with a polished proximal tip 466 and a distal tip 468, followed optically by a pair of lenses 470 and 472 which bring the light to a focus at the surface of the wafer 462.

Photolithography apparatus 460 also includes a second light source 474 and a focussing lens 476 for focussing light from second light source 474 to a focus 478 at the

back focal plane of lens 476; the rays of light then impinge second lens 392 and fall on the polished proximal tip 466 of the bundle 464, to ultimately be brought to a focus at the surface of the wafer 462.

In use, a collimated beam of light 416 of a wavelength that can activate the photoresist material is emitted from TEMoo laser 374 and encounters first microlens array 376. As explained above, each microlens brings a portion of the incident light to a diffraction limited point focus in the plane of mask 388. The light that is not blocked by mask 388 passes to the set of matching the microlenses of second microlens array 382. The light from the microlenses of second microlens array 382 emerges as narrow collimated beams 418 that rapidly spread out as diverging cones of light 420. These cones of light 420 encounter first lens 390, which collimates the light from each cone to form collimated ray bundles 424. These collimated ray bundles 424 encounter second lens 392, which brings each beam to a focus at the polished end of a separate fibre core at the proximal tip 466 of fibre bundle 464. The activating light (from TEMoo laser 374) enters the cores as predominantly the fundamental mode of each core. This mode is shown in figure 13B.

The beam of light 480 from second light source 474 is brought to a focus 478, as described above, at the back focal plane of lens 476. The rays are collimated by this lens 476 and fall on the polished proximal tip 466 of the bundle 464. The rays are outside the cone of acceptance of the fundamental mode of the cores; they can, however, be coupled into the cores and guided as the **1,0** mode (see figure 13C). (The cores of the bundle have a slight degree of asymmetry, so they maintain the orientation of this mode in the cores through the length of the fibre.)

At the distal tip 468 of fibre bundle 464, the light from both sources 374, 474 emerges from each of the cores of bundle 464, each wavelength maintaining its modal pattern as it passes through lenses 470 and 472. The focused spots in the material of the photoresist 482, on the surface of the wafer 462, maintains these two separate modal patterns. Hence the de-activated "sharpening" is in one dimension only. (In this case the lines that are formed orthogonal to the plane of the figure are the ones that are sharpened.)

The mask 388 and the wafer 462 are moved synchronously, as described above.

Optionally, laser light from a further source (not shown, out of the plane of the figure and orthogonal to second light source 474) may be coupled into the cores as the **0,1** mode (see figure 13D), with the orientation of the modes maintained as the light passes through the cores. This would sharpen lines in the photoresist that are parallel to the plane of the figure (or in it). In another variation, both of the de-activating laser light sources (i.e. second light source 474 and the further source) are simultaneously coupled into the cores, producing effectively a halo (see figure 13E).

Figure 14A is a schematic view of a photolithography apparatus 490 according to still another embodiment of the present invention, as might be applied to high density photolithography, shown with a silicon wafer 492. Several features of photolithography

apparatus 490 are identical with corresponding features of photolithography apparatus 370 of figure 11, and like reference numerals have been used to identify like features.

Hence, photolithography apparatus 490 includes a TEMoo laser (not shown) with an output wavelength that can activate the photoresist material 494 on silicon wafer 492, a first microlens array 376, a second microlens array 382 and, between the first and second microlens arrays 376, 383, a mask 388 that consists of a pattern defining the features desired to be transferred to the wafer 432. Each microlens of first microlens array 376 brings a portion of the incident light to a diffraction limited point focus, the array of such foci being in the plane as that of mask 388, while light incident on first microlens array 376 but not subsequently blocked by mask 388 passes to second microlens array 382, whose microlenses match the microlenses of first microlens array 376, the two microlens arrays 376, 382 being separated by a distance such that the foci of the microlenses of first microlens array 376 are co-incident with the foci of the microlenses of second microlens array 382.

Photolithography apparatus 490 also includes, optically downstream of microlens arrays 376, 382, a first lens 390 and a second lens 392. However, downstream from first and second lenses 390, 392, photolithography apparatus 430 includes a tapered fused fibre optic bundle 496 with a polished proximal tip 498. Bundle 496 is single moded in proximal region 500 for the activating light, has an adiabatically tapered region 502 and an expanded (distal) region 504. The cores 506 are elliptical and can only support the **0,1** mode, not the **1,0** mode (see figure 14B).

Photolithography apparatus 490 also includes a cladding mode stripper 508 affixed to bundle 496 towards distal tip 510 thereof, a first de-activating light source 512 (light from which is admitted into proximal region 500 and can couple into the 01 mode in the tapered region 502), and a second de-activating light source 514 (light from which is admitted into expanded region 504 and can overlap the fundamental mode (see figure 14C) in the 01 direction in the expanded region 504, although not as one of the core modes).

The distal tip 510 of bundle 496 is provided with a microlens array 516 for focussing the activating light as a multitude of diffraction limited spots in the photoresist material 494. Each microlens of the microlens array 516 is aligned with a respective core 510 of bundle 496.

In use, a collimated beam of light 416 of a wavelength that can activate the photoresist material 494 is emitted from the TEMoo laser and encounters first microlens array 376. As explained above, each microlens brings a portion of the incident light to a diffraction limited point focus in the plane of mask 388. The light that is not blocked by mask 388 passes to the set of matching the microlenses of second microlens array 382. The light from the microlenses of second microlens array 382 emerges as narrow collimated beams 418 that rapidly spread out as diverging cones of light 420. These cones of light 420 encounter first lens 390, which collimates the light from each cone to form collimated ray bundles 424. These collimated ray bundles 424 encounter second lens 392, which brings

each beam to a focus at the polished end of a separate fibre core at the proximal tip 498 of fibre bundle 496. As mentioned above, the bundle 496 is single moded in proximal region 500 for the activating light, and cores 506 can support the **0,1** mode 40 but not the **1,0** mode.

Meanwhile, light from first de-activating light source 512 is coupled into the 01 mode in the tapered region 502 and excess light is removed by cladding mode stripper 508, while de-activating light from second de-activating light source 514 is coupled into expanded region 504 and overlap the fundamental mode in the 01 direction in the expanded region 504.

The activating light continues as the fundamental mode to the distal tip 510 of the bundle 496 and is focused as a multitude of diffraction limited spots in the photoresist material 494 by microlens array 516. The de-activating light surrounds these diffraction limited spots. As in embodiments described above, the mask 388 and the wafer 492 are moved synchronously.

The system could also be operated with the distal optics of the embodiment of figure 13A.

Microscopes according to still further embodiments of the present invention are depicted schematically in figures 15 and 16, generally at 520 and 530 respectively, and may broadly be compared in operation with microscope 180 of figure 7.

One way of producing polarisation maintaining bundles is to give the cores a small degree of ellipticity. Elliptical core bundles can be manufactured as shown generally at 540 in figure 17. Referring to figure 17, a glass fibre bundle preform or preferably a partly drawn bundle 542 is held in a clamp 544 and slowly fed (as indicated by arrow 546) into an electrically heated coil 548 of wire. The heat from the coil 548 softens the glass and the free end 550 of the bundle 542 is pulled away from the heated coil 548 as shown by arrow 552. The speed of the pulling is greater than the input feed rate. The pulling force direction is at an angle θ to the axis 554 of the bundle 542 at free end 550. This angle θ can be small and, in this figure, the angle is the same at the top and bottom of the figure.

Use of Light Emitted From Cladding and Cores of Fused Coherent Bundle in Stochastic Localization Microscopy (STORM, PALM, FPALM, etc)

The embodiment in figure 18 is configured to employ the cladding mode light from a fibre bundle to improve the performance of localization microscopy, stochastic optical reconstruction microscopy (STORM), photoactivated localization microscopy (PALM), fluorescence photoactivation localization microscopy (FPALM), etc.

Thus, figure 18 is a schematic view of a microscope 560 according to an embodiment of the present invention. Microscope 560 includes a first de-activation system A comprising a light source 562 of suitable wavelength and a converging lens 564. Microscope 560 also includes an optic fibre 566 with a proximal tip 568 and a distal tip 570,

an objective lens 572 and a moveable specimen stage 574. Light from light source 562 is thereby directed towards a specimen from the front of the specimen stage 574.

Microscope 560 further includes a second de-activation system B comprising a light source 576 and a converging lens 578. Light source 576 is located to direct light towards a specimen from the rear of the specimen stage 574. Both the light sources 562, 576 are of wavelength λ_1.

Microscope 560 also includes an activating light source 580 for producing activating wavelength pulses, a first collimating lens 582, a beamsplitter in the form of first beamsplitter cube 584, a coherent fused fibre bundle 586 with proximal tip 588 and distal tip 590, a beamsplitter in the form of second beamsplitter cube 592 and a second collimating lens 594.

Microscope 560 further includes a further light source 596 of de-activating light of wavelength λ_1, coupling optics 598 arranged to couple that light into the cladding modes of the bundle 586, a light source 600 of excitation light of wavelength λ_3 and a third collimating lens 602 for collimating excitation light 604 into a side face of first beamsplitter cube 584.

Finally, microscope 560 includes a converging lens 606 adjacent a side face of second beamsplitter cube 592 for receiving return light, and a pixellated photodetector 608 (such as a CCD or EMCCD) for detecting that return light.

In use, a specimen to be observed is located on specimen stage 574. The fluorophore in the specimen is de-activated by either de-activation system A or B (in which case the other may be omitted), or both de-activation systems A and B.

In the de-activation system A, light source 562 emits a pulse of light that is coupled into the proximal tip 568 of optic fibre 566. The pulse travels along the fibre 566 and is emitted as divergent rays 610 from the distal tip 570 of the fibre 566. This distal tip 570 is located at the back focal plane of objective lens 572. On passing through objective lens 572, the rays 610 are collimated and pass into the specimen as a uniform de-activating beam 612.

In de-activation system B, light source 576 is located to the rear of specimen stage 574 and, with lens 578, illuminates the specimen from the rear.

Light from activating light source 580, of wavelength λ_2, passes through first collimating lens 582 and first beamsplitter cube 584 and enters the cores of fibre bundle 586 at the proximal tip 588. The light exits the cores of the bundle 586 at the distal tip 590 and enters second beamsplitter cube 592. The portion of this light that is transmitted by second beamsplitter cube 592 is collimated by second collimating lens 594 and then passes to objective lens 572, which focuses it to an image of the core pattern at the focal plane 614 within the specimen.

A pulse of de-activating light from further light source 596 passes through coupling optics 598 into the cladding modes of the bundle 586 and is emitted from the distal tip 590 and focussed by the lens system (comprising collimating lens 594 and objective lens 572) as a reticulated pattern surrounding the positions of the previously activated spots. Wavelength λ_1 will also de-activate the molecules in the specimen. In this case the de-activation has

spatial selectivity defined by the projected 3D pattern of the cladding of fibre bundle 586. It will largely inactivate the fluorophores in the out of focus planes but will leave fluorescent entities in the centres of the hexagonal pattern units in the focal plane mostly unaffected.

The imaging process is carried out using excitation light generated by light source 600. Rays of light 604 from source 600 are collimated by third collimating lens 602 and reflected from the dichroic layer 616 of first beamsplitter cube 584 to enter the cores of the fibre bundle 586 at its proximal tip 588. This light exits the cores of the bundle 586 at the distal tip 590 and passes through the second beamsplitter cube 592 and the second collimating lens 594 to be focused by objective lens 572 as a pattern of spots at the focal plane of in the tissue. This light causes fluorescence of the isolated sparse fluorescent entities. Some of this fluorescent light passes through objective lens 572 and second collimating lens 594, and is reflected from the dichroic layer 618 of second beamsplitter cube 592 to a focal plane 620 between second beamsplitter cube 592 and converging lens 606. Converging lens 606 relays the image to photodetector 608. A conjugate bundle may optionally be positioned at focal plane 620 to convey the light to the photodetector 608, with converging lens 606 omitted, the cores of the conjugate bundle acting as further spatial filters and further sharpening the point spread functions (PSFs).

This image contains multiple spots each spot is produced by the light emitted from one single fluorescent entity. This allows the centroid of the PSF to be determined and thus the position of the individual fluorescent entity can be localised with a resolution far greater than that of the Abbé limit.

The fluorophores in the specimen are then de-activated by a further exposure pulse from de-activation system A or B, and the sequence reiterated, potentially several hundred times or more. Each iteration activates fresh fluorescent entities in the specimen.

A series of several hundred to several thousand exposures are made and the sample is moved to a new position. A further several hundred to several thousand exposures are made at this new position. The number of changes of position of the sample or the bundle tip required in this method has only to satisfy the Nyquist criterion in relation to core spacing and Airy disc size. It does not require the nano-stepping that must be done in implementing the STED and GSD embodiments.

Figure 19 is a schematic view of a microscope 622 according to another embodiment of the present invention. Most features of microscope 622 are identical with corresponding features of microscope 570 of figure 18, and like reference numerals have been used to identify like features.

This embodiment, however, uses only de-activation system B for de-activation by supplying a pulse of wavelength λ_1 to the specimen from the rear, while de-activation system A of microscope 570 of figure 18 is replaced, in microscope 622, with a light source 624 of excitation light of wavelength λ_3. and a focussing lens 626. The activating light pulse rays from light source 580 of wavelength λ_2 is collimated by lens 582 and coupled directly into the cores of the bundle 586 at the proximal tip 588 and travels through the optical system to

produce spatially defined areas in the specimen in which single fluorescent entities may be activated, as described above. A further deactivating pulse is supplied by further light source 596, which selectively de-activates fluorophores above and below the focal plane.

The excitation light produced by light source 624 are focused by lens 626 into the proximal tip 568 of optic fibre 566. The light emerges from the distal tip 570 as a divergent beam 610 that is collimated by objective lens 572 and uniformly illuminates the specimen. The fluorescence from the specimen travels to the photodetector 608 to form the image, as described in the previous embodiment.

Multiple images and Nyquist criterion shifting need to be acquired, as in the previous embodiments.

Some embodiments require that a fibre bundle be rotated (cf. figure 10). Figures 20 and 21A to 21C illustrate how this can be accomplished. Figure 20 is a schematic view of a rotating fused fibre bundle system 630 (including a detail of the distal portion 632 in the upper register and of the proximal portion 634 in the lower register), including a thin, long, flexible fused fiber bundle 636. Bundle 636 is sufficiently thin and long that it should be possible to use it through a biopsy channel. The optical and electronic components associated with the proximal portion 634 of the bundle 636 and those associated with the distal portion 632 of the bundle 636 are provided for illustrative purposes, and will differ according to application. As illustrated, however, these include a laser light source (not shown) that provides light of wavelength λ_2 injected at an injection location 646 near distal tip 648 of bundle 636, and a second light source 638 between the proximal tip 640 and the photodetector 642, optically after objective lens 644. Second light source 638 provides light at a wavelength λ_1 different from that of laser light λ_2.

Motor 650 causes rotation of gear 652 (directly coupled to motor 650), which engages and drives gear 654, which is coupled to bundle 636. Bundle 636 is located coaxially within gear 654 and is thus rotated. Close to the distal and proximal tips 648, 640 of the bundle 636 are lubricated sleeves or dashpots 656a and 656b, respectively, while the bundle is rotated within Teflon tubes 658a and 658b.

The construction of the distal and proximal dashpots 656a and 656b is shown in figures 21A and 21B, respectively. Referring to figure 21A, the distal rotary dashpot 656a comprises a tube 660 through which the distal portion of the bundle 636 passes and to which bundle 636 is glued. This tube 660 is fabricated to fit bundle 636, but is mounted to rotate freely within a second, larger tube 662. A further member (not shown) constrains axial movement in both directions. The space between the tubes 660, 662 is filled with Kilopoise grease 664.

Dashpot 656a has an aperture 666 through which light from an optical fibre 688 (or other device) is injected, via the Kilopoise grease, into the cladding modes of the bundle 636.

Figure 21B shows the proximal rotary dashpot 656b, which is identical in most regards (though without an aperture to admit light) to distal rotary dashpot 656a. A Peltier

device 668 is thermally connected to the outer tube 670, to maintain the temperature at the temperature of the distal end and hence to that of the specimen (such as 37°C when imaging the human body).

Figure 21C is a detail of figure 20 showing the motor 650 and gears 652, 654, for rotating the bundle 636.

Thus, the rotary motion of bundle 636 allows imaging to be carried out via a distal lens train 672 (as illustrated in figure 20). However, it should also be noted that imagining can alternatively be conducted without such distal optical elements but rather by direct contact of the distal tip 648 of the bundle 636 with the specimen.

Chromatic Shift Lateral

Figure 22 is a schematic view of a microscope 680 according to another embodiment of the present invention, shown with a specimen in the form of a tissue sample 682. In this embodiment, a prism is introduced in the Fourier space in the distal lens train. Thus, microscope 680 includes an excitation light source 684, a multi-moded optical fibre 686 with a proximal end 688 and a distal end 690, a coupling prism 692, a fused optic fibre bundle 694 with a distal tip 696, a collimating lens 698, a prism stage 700, a first converging lens 702 and a cover slip 704.

Microscope 680 also includes a second converging lens 706 located to receive return light emitted by the other tip 708 of fibre bundle 694, a long pass filter 710 and a photodetector in the form of a CCD 712.

In use, light from excitation light source 684 is introduced into the proximal end 688 of multi-moded optical fibre 686, transmitted to the distal end 690 of the fibre 686, and coupled into the fused optic fibre bundle 694 by coupling prism 692. The light is then emitted by distal tip 696 of fused optic fibre bundle 694, and diverges until collected by collimating lens 698, which collimates the light. The light then passes through prism stage 700 and is deviated slightly thereby, and continues as beam 714 which passes through converging lens 702 which produces an image of the cladding pattern in the focal plane 716 within the tissue sample 682.

A point object 718 within the tissue sample 682 that is excited by the light then emits fluorescent light of a longer wavelength. A portion of this light passes in the reverse direction through converging lens 702 until it reaches the prism stage 700. As the return light is Stokes shifted and of a longer wavelength, it is deviated to a slightly different extent (compared with the incident light) as it passes through the prism stage 700. It is then brought to a focus at distal tip 696, with a lateral displacement compared with the incident, excitation light, which causes the return light to enter and be guided by a core 720 of fused optic fibre bundle 694 to the other tip 708 of bundle 694. The light from the core 720 is emitted and passes as ray 722 through converging lens 706 and long pass filter 710 to a focus 724 on CCD 712, thus contributing to the formation of an image.

Figure 23 is an enlarged view of prism stage 700 and adjacent elements of the microscope 680 of figure 22. Prism stage 700 comprises a pair of glass plates 726, 728 between which is a compressible elastomeric "O" ring 730 which forms a seal against both glass plates 726, 728. The prism stage 700 also comprises a liquid 732 contained by "O" ring 730 and glass plates 726, 728. Pressure is maintained on the "O" ring 730 by two (or more) piezo transducer actuators 734, 736. During the operation of the microscope 680, the voltage applied to the actuators 734, 736 is first set so that the two glass plates 726, 728 are parallel. An initial image is collected and the voltages changed so that a slight prism angle is formed between the two glass plates 726, 728. A second image is then acquired. A pixel by pixel subtraction of one image from the other gives the difference and thus isolates the focal plane.

Further detail contributing to the formation of the image could be obtained by reversing the voltages applied to the actuators 734, 736 so that the prism angle is formed to produce deviation of the light in the opposite direction.

Optionally, a further pair of piezo transducer actuators (not shown) may be deployed to operate above and below the plane of the figure, to cause tilting in the orthogonal plane and would give further information that could contribute to the image.

It is also envisaged that the introduction of small amounts of aberration into the distal lens train could be used in a similar manner, e.g. normal chromatic aberration, astigmatism etc.

Figures 24 to 26 are schematic views of further embodiments, which may be used to conduct Oblique Illumination Phase Contrast Endoscopy.

Figure 27 is a schematic view of a microscope according to an embodiment of the present invention. A light source 1 of one specific primary colour, which in this description will be referred to as red, emits light into a large core high NA plastic optical fibre 2. The light passes to the other end of the fibre 3 from which it is emitted to pass into biological tissue 4. Backscatter light 5 returns through the tissue and enters the endoscope tip through lenses 6 and 7 to form an image on the distal tip 8 of optical fibre imaging bundle 9. The images transferred to the end 10 to be focus by lenses 11 and 12 onto colour CCD chip 13. A mode stripper 14 maintains the contrast of the image. Simultaneously, during the same exposure time another light source 15 of a different primary colour emits light. This light, which will be referred to as green in the following description, passes into a larger diameter core high NA optical fibre 16. At the other end 17 of bundle 16 it passes out into the tissue 4 and some of the light is backscattered as rays 18. Thus, a type of Rheinberg illumination is obtained and this can be processed to give a high contrast monochrome image. This differs from prior art of figure 1 in that a conventional colour CCD chip can be used and the illumination period can be extended over the full acquisition time of the CCD chip.

It should be noted that, in this and other embodiments, for convenience the two channels are referred to as "red" and "green." These colors are exemplary, and reference to them does not mean that other coding/colour schemes between the left and the right

channels are excluded. It should also be noted that a three chip colour CCD may be used in an alternative embodiment, and would give superior performance, but at the expense of increased size and cost.

Figure 28 is a schematic view of a microscope according to an embodiment of the present invention, in which the optical fibre bundle 1 is in direct contact with the surface 2 of the tissue 3. A red light source 4 (in this embodiment, a red LED) emits light into optical fibre 5. This is emitted into the tissue on one side of the optical fibre bundle tip 6. Light from a green light source 7 (in this embodiment, a green LED) passes along illumination fibre 8 and is emitted into the tissue on the other side of the optical fibre bundle. Backscattered light of both colours enters the optical fibre bundle and is emitted from tip 9 to be focused by lenses 10 and 11 as an image onto colour CCD chip 12. Phase objects / structures in the tissue that are in contact with tip 6 will cause colour ratio variations. These can be enhanced in monochrome through image processing.

Figure 29 is a schematic view of a microscope according to an embodiment of the present invention, comparable to that of figure 28 but with a colour CCD array 20 glued directly to the bundle 1 at exit tip 9. This allows the omission of lenses 10 and 11.

Figure 30 is a schematic view of a microscope according to an embodiment of the present invention, which does not use an optical fibre imaging bundle. The projected image falls directly on a colour chip CCD array. Such chips are now available in very small sizes and they have replaced optical fibre bundles in many endoscope applications. Light from red source 1 and the green source 2 pass along plastic optical fibres 3 and 4. They emit light at the distal tips 5 and 6 into the tissue that it is desired to observe 7. The light that is backscattered from the deeper tissue passes through the train of lenses 8 and falls as an image on colour CCD chip 9.

Figure 31 is a schematic view of a microscope according to an embodiment of the present invention, in which a cluster of gradient index lenses is employed to form the image. In figure 31, an object 1 emits rays of light 2. These enter a cluster of gradient index lenses 3 and the rays pass through the lenses and converge to focus as an erect image 4 on CCD array 5. Light is delivered to the specimen by multimode plastic optical fibres 6 and 7. The light may be temporally separated as with the system of Ford, Chu and Mertz illustrated in figure 1, or spectrally separated as with the red/green illuminated microscopes of the embodiments described above.

Figure 32 is a cross sectional view of the microscope of figure 31. An object 1 below the surface 2 of tissue 3 is illuminated by backscatter from regions 4 and 5 within the tissue. The light passes through a protective coverslip 6 and is focused by a cluster of gradient index lenses 7 to form an image 8 on CCD array 9.

Figure 33 is a schematic view of a disposable microscope according to an embodiment of the present invention, in which a CCD array chip 1 is held directly against the tissue to be observed 2. Light is delivered to the area to be observed 3 by multi-mode plastic optical fibres 4 and 5. Fibre 4 carries red light to the specimen. Fibre 5 carries green

light to the specimen. A thin layer waterproof material 6 protects the CCD chip and prevents degradation due to ingress of fluid. This could be formed by vapour deposition from Parylene. Chip connection leads are shown by 7 and 8. Colour contrast highlighting structure of objects close to the surface would be achieved.

Figure 34 is a schematic view of a microscope according to an embodiment of the present invention, in which light from sources 1 and 2 passes along optical fibre waveguides 3 and 4 to the tips 5 and 6 of the waveguides 3 and 4. The light is then reflected from respective mirrors 7 and 8, and is conveyed by total internal reflection within a glass plate 9. Both beams are directed towards one another and the plate 9 is in contact with the tissue specimen 10. The particular area under observation 11 of specimen 10 has previously had a drop of a particular compound applied to it. This compound mingles with the tissue fluid and increases the refractive index. This allows the totally internally reflected beams to pass out of the glass briefly and to follow the paths within the tissue shown by 12 and 13. Suitable compounds have been given approval as radio opaque liquids miscible with blood that can be used to visualise cardiac stenoses et cetera.

Figures 35A and 35B are structural diagrams of two examples of the suitable compounds for use in the embodiment in figure 34. That of figure 35A is diatrizoic acid, sodium salt, and that of figure 35B is Iopamidol. Both are derivatives of 1, 3, 5 tri iodinated benzene.

Figure 36 is a schematic view of a microscope according to an embodiment of the present invention, in which both the red and the green beams are directed by total internal reflection in a negative power lens to meet in the optic axis of the microscope system. Red light from delivery fibre 1 and green light from delivery fibre 2 pass into a cylinder of optic glass 3. They then pass into the plano concave lens 4 which has a surface 5 that reflects the beams towards the optical axis of the lenses. Beams 6 and 7 are totally internally reflected by the concave surface but are transmitted when they encounter the flat surface, emerging into the tissue 10 as beams 11 and 12. The use of a mode conditioner maximises the intensity of light in the desired observational area. Light that is scattered by phase objects in areas 13 passes through the train of lenses and focuses as an image on colour chip CCD 14.

Figure 37 is a schematic view of a microscope according to an embodiment of the present invention, which operates on essentially the same principle as that in figure 36. Light is transmitted by total internal reflection within the lens 1, and travels first as Whispering Gallery modes 2 and then is totally internally reflected from both surfaces 3 before escaping into the tissue as rays 4 and 5.

The present invention also provides embodiments that have the capability of producing fluorescence images as well is phase objects images from the same endoscope / microscope.

Figure 38 is a schematic view of a microscope according to an embodiment of the present invention, in which structured fluorescence mode imaging is added to a system

comparable to that of Ford, Chu and Mertz (*op. cit.*). Two single moded fibres 3 and 4 deliver excitation light at or around the back focal plane of the objective lens. The light from each fibre tip diverges until it meets the objective lens. The lenses collimate each beam and the two collimated beams intersect and give an interference pattern in the tissue. Any fluorescent structure 5 in this region will be brightly lit if it is in a region of constructive interference and dark if in destructive interference. The light from both fibres must come from the same long coherence laser source and the polarisation must be matched. A minimum of two separate images are acquired, with one fibre stretched between exposures to change the optical path length and thus shift the fringes. This embodiment can be readily implemented using polarisation maintaining fibre and an FBT coupler.

Figure 39 is a schematic view of a microscope according to an embodiment of the present invention. In this embodiment, red and green illuminating light is conducted to the specimen (in this example, tissue 18) by relatively stiff fibres 10 and 12 respectively. The forward or distal tips of fibres 10 and 12 are provided with or shaped into needle tips 14, 16, which facilitate the penetration of the fibres 10, 12 into the tissue 18.

The needle tips 14, 16 have angled rear faces (i.e. the outward faces in the figure), whose inner faces act as mirrors (and may optionally be provided with mirrored surfaces), so as to reflect illuminating light from fibres 10, 12 towards a common portion of the tissue 18. In the illustrated embodiment, this location is between and somewhat higher than tips 14, 16.

Light 20 from that common portion is scattered back into the microscope via glass cover 22, passes through lenses 24 and 26 to form an image on a colour CCD chip 28.

Figure 40 is a schematic view of a microscope according to an embodiment of the present invention in which red and green light sources enabling phase object imaging as described above. The following describes fluorescence imaging with this embodiment. In this embodiment a light source 1 is capable of exciting fluorescence from fluorophores in a specimen. The light source 1 emits light which is collimated by lens 2 and reflected by dichroic beam-splitter. A lens 4 focuses the light into the cores at the proximal end of optical fibre bundle 5. The light passes along the fibre and is emitted through the distal end 6 of the optical fibre bundle. The light then passes through lenses 7 and 8 and focuses as a series of spots the fluorescence generated within these spots passes back through lenses 8 and 7 into the cores of the optical fibre bundle within the tissue 9. Each core receives back the fluorescence from the focus spot generated by the excitation light which came from that core. The light passes back through the beam splitter and is focused by lens 10 to form an image on CCD 11. Light source 1 (having illuminated the specimen at, typically, a blue wavelength λ_1) is now turned off after an illumination period t_1 and a second excitation light source 12 (also of wavelength λ_1) is switched on for a time t_2. Light from light source 12 passes to a prism coupler 13 and mode conditioner (not shown) and is then is introduced into the fibre bundle 5 as cladding modes. The cladding modes pass to the distal tip of bundle 5 by successive total internal reflections and leave the bundle through the face 6 of bundle 5. The light from these modes passes through lenses 7 and 8 and focuses into the

specimen 9. At the focal plane the cladding light produces a reticular pattern surrounding each of the spots described above. This light also generates fluorescence within the specimen. Some of this fluorescence passes back through the lenses 8, 7 into the cores at the distal face 6 of bundle 5 and passes through to lens 10 which focuses it as an image onto CCD 11. A considerable improvement in the fluorescence image is obtained by subtracting this image from the image taken when the fluorescence was generated by light source 1. Thus, three exposures are employed: one for phase gradient microscopy using the red and green light sources, and second from light source 1 and a third from light source 12, the second and third being used to provide structured illumination fluorescence microscopy.

Figure 41 is a schematic view of a microscope according to an embodiment of the present invention, which is comparable to the microscope of figure 40 except that contact images are obtained (with the omission of lenses 7, 8 of the embodiment of figure 40). This embodiment also combines structured illumination fluorescence microscopy with phase gradient microscopy with oblique back illumination. The red and green light sources for the phase gradient microscopy are indicated by 20 and 22, respectively, with respective delivery fibres 24, 26; this phase gradient microscopy aspect of this embodiment operates as has been described above. Fluorescence is generated by excitation light from (typically blue) light source 1 within the tissue 9. Light from light source 12 passes to a prism coupler 13 and mode conditioner (not shown) and is then is introduced into the fibre bundle 5 as cladding modes. The cladding modes pass to the distal tip of bundle 5 by successive total internal reflections and, upon leaving the bundle, passes into the tissue 9. The cladding light produces a reticular pattern surrounding each of the spots created by the light from light source 1. This light also generates fluorescence within the specimen. Some of this fluorescence passes back into bundle 5 and passes through to lens 10, which focuses it as an image onto CCD 11. A considerable improvement in the fluorescence image is obtained by subtracting this image from the image taken when the fluorescence was generated by light source 1.

Figure 42 is a schematic view of a microscope according to an embodiment of the present invention, which is comparable to the microscope of figure 41 though with still further simplified optics. This embodiment also combines structured illumination fluorescence microscopy with phase gradient microscopy with oblique back illumination. The two light sources for the phase gradient microscopy are indicated by 10 and 12, with respective delivery fibres 14, 16 (as has been described above). Fluorescence is generated by excitation light from (typically blue) light source 18 within a specimen 20, having been coupled via a mode conditioner 22 into fibre bundle 24 as cladding modes. Light from light source 26, of the same wavelength as that of light source 18, is also coupled via a mode conditioner 28 into fibre bundle 24 as cladding modes. Structured illumination fluorescence microscopy can then be perfomred as described above, including the acquisition of two

images by CCD camera 30 and subsequent image processing, to give improved visual isolation of the focal plane in the specimen 20.

Figure 43 is a schematic view of a microscope according to an embodiment of the present invention, which is comparable to the microscope of figure 42 though with a pair of lenses 32, 34 between the distal tip 36 of fibre bundle 24 amd specimen 20.

Modifications within the scope of the invention may be readily effected by those skilled in the art. It is to be understood, therefore, that this invention is not limited to the particular embodiments described by way of example hereinabove.

For example, the red and green LEDs described above could be powered from the USB port of the computer that is acquiring the images. The voltage and total power deliverable from a USB are just right. Also the control of the light coud then be done directly by the computer.

Also, there could be two or more such light sources (such as LEDs or fibre tips delivering light) of the same colour on each side. If the brightness of each of these is adjustable independently, irregularities in tissue reflection can be compensated for.

In normal image processing software, smoothing operations are generally symmetrical about the pixel that is undergoing the correction transformation. For example Ford, Chu and Mertz (op. cit.) uses a radially symmetrical Gaussian filter. According to the present inevntion, however, such software may have an elongated bilateral symmetry (i.e. rectangular with the long axis directed along the line between the two light sources or parallel to it).

Experiments do not show any problem arising from the red and green light behaving differently in tissue specimens, but—if there was such a concern—the microscopes described above could be configured to generate firstly an image with Red Left/Green Right then a second image with Green Left/Red Right.

The third channel of the CCD/CMOS chip can be used for another purpose; for example, depending on filter characteristics, the blue channel is often sensitive to IR so an IR image could also be collected.

Modifications within the scope of the invention may be readily effected by those skilled in the art. For example, other uses of fused fibre optic bundles with light coupled into the cladding modes include:

- Rapidly changing uniform high intensity light sources, for microscopy, etc;
- Merging high intensity light sources;
- Independent control of angle of cone of light of each colour without light loss: a “mode conditioner”;
- Trapping Bose Einstein condensates (where the light source is separated to a spectrum and the spectrum is launched into the cladding as a spread of modes;
- Nanofabrication by photopolymerisation using projected cladding modes, providing a reticulated pattern.

It is to be understood, therefore, that this invention is not limited to the particular embodiments described by way of example hereinabove.

In the claims that follow and in the preceding description of the invention, except where the context requires otherwise owing to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, that is, to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

Further, any reference herein to prior art is not intended to imply that such prior art forms or formed a part of the common general knowledge in any country.

CLAIMS:

1. A microscope, endoscope or endomicroscope, comprising:

a first light source for illuminating a specimen at a first wavelength;

a second light source for illuminating the specimen at a second wavelength;

a photodetector for collecting light from the specimen when under illumination; and

an image differentiator;

wherein the microscope, endoscope or endomicroscope is configured to form a first image of the specimen using the first light source and a second image of the specimen using the second light source, and the image differentiator is configured to form a differential image comprising a difference between the first and second images.

2. A microscope, endoscope or endomicroscope as claimed in claim 1, wherein the first wavelength and the second wavelength are different, and the microscope, endoscope or endomicroscope is arranged to perform phase gradient microscopy of the specimen.

3. A microscope, endoscope or endomicroscope as claimed in claim 2, wherein the first and second light sources are configured or operable to illuminate the specimen simultaneouly.

4. A microscope, endoscope or endomicroscope as claimed in either claim 2 or 3, wherein the first and second wavelengths correspond to red light and green light.

5. A microscope, endoscope or endomicroscope as claimed in any one of claims 2 to 4, comprising at least one additional light source for illuminating a specimen at a third wavelength;

wherein the microscope, endoscope or endomicroscope is arranged to perform structured illumination fluorescence microscopy of the specimen by successive excitation of spots in the specimen and portions surrounding the spots, and form a differential image comprising a difference between the first and second images.

6. A microscope, endoscope or endomicroscope as claimed in claim 1, wherein the first wavelength and the second wavelength are substantially equal, the first and second light sources are configured or operable to illuminate the specimen at different times, and the microscope, endoscope or endomicroscope is arranged to perform structured illumination fluorescence microscopy of the specimen by successive excitation of spots in the specimen and portions surrounding the spots, and form a differential image comprising a difference between the first and second images.

7. A microscope, endoscope or endomicroscope as claimed in any one of claims 1 to 6, wherein the image differentiator subtracts one of the first and second images from the other of the first and second images.

8. A microscope, endoscope or endomicroscope as claimed in any one of claims 1 to 7, configured to illuminate the specimen with the first and second light sources and collect the light from the specimen on a single side of the specimen.

9. A microscope, endoscope or endomicroscope as claimed in any one of claims 1 to 7, wherein the first and second light sources are arranged to provide oblique back illumination.

10. A microscope, endoscope or endomicroscope as claimed in any one of claims 1 to 8, comprising first and second light transmitters arranged to transmit light from the first and second light sources respectively to the specimen, wherein the first and second light transmitters have distal tips close to the specimen.

11. A microscope, endoscope or endomicroscope as claimed in claim 10, wherein the microscope, endoscope or endomicroscope includes a lens for collecting light from the specimen and the distal tips are located on opposite sides of the lens.

12. A microscopy or endoscopy method, comprising:

illuminating a specimen at a first wavelength;

illuminating the specimen at a second wavelength;

collecting light from the specimen when under illumination;

forming a first image of the specimen using the first light source and a second image of the specimen using the second light source; and

forming a differential image comprising a difference between the first and second images.

13. A method as claimed in claim 12, wherein the first wavelength and the second wavelength are different, and the method includes performing phase gradient microscopy of the specimen.

14. A method as claimed in claim 13, including illuminating the specimen at the first and second wavelengths simultaneouly.

15. A method as claimed in either claim 13 or 14, wherein the first and second wavelengths correspond to red light and green light.

16. A method as claimed in any one of claims 13 to 15, including illuminating the specimen at at least one additional wavelength, performing structured illumination fluorescence microscopy of the specimen by successive excitation of spots in the specimen and portions surrounding the spots, and forming a differential image comprising a difference between the first and second images.

17. A method as claimed in claim 12, wherein the first wavelength and the second wavelength are substantially equal, the method including illuminating the specimen at the first and second wavelengths at different times, performing structured illumination

fluorescence microscopy of the specimen by successive excitation of spots in the specimen and portions surrounding the spots, and forming a differential image comprising a difference between the first and second images.

18. A method as claimed in any one of claims 12 to 17, wherein the image differentiator subtracts one of the first and second images from the other of the first and second images.

19. A method as claimed in any one of claims 12 to 18, including illuminating the specimen with the first and second light sources and collecting the light from the specimen on a single side of the specimen.

20. A method as claimed in any one of claims 12 to 18, including illuminating the specimen with oblique back illumination.

21. A method as claimed in any one of claims 12 to 19, including transmitting illuminating light to the specimen with first and second light transmitters having distal tips close to the specimen.

22. A method as claimed in claim 21, including collecting light from the specimen with a lens and locating the distal tips on opposite sides of the lens.

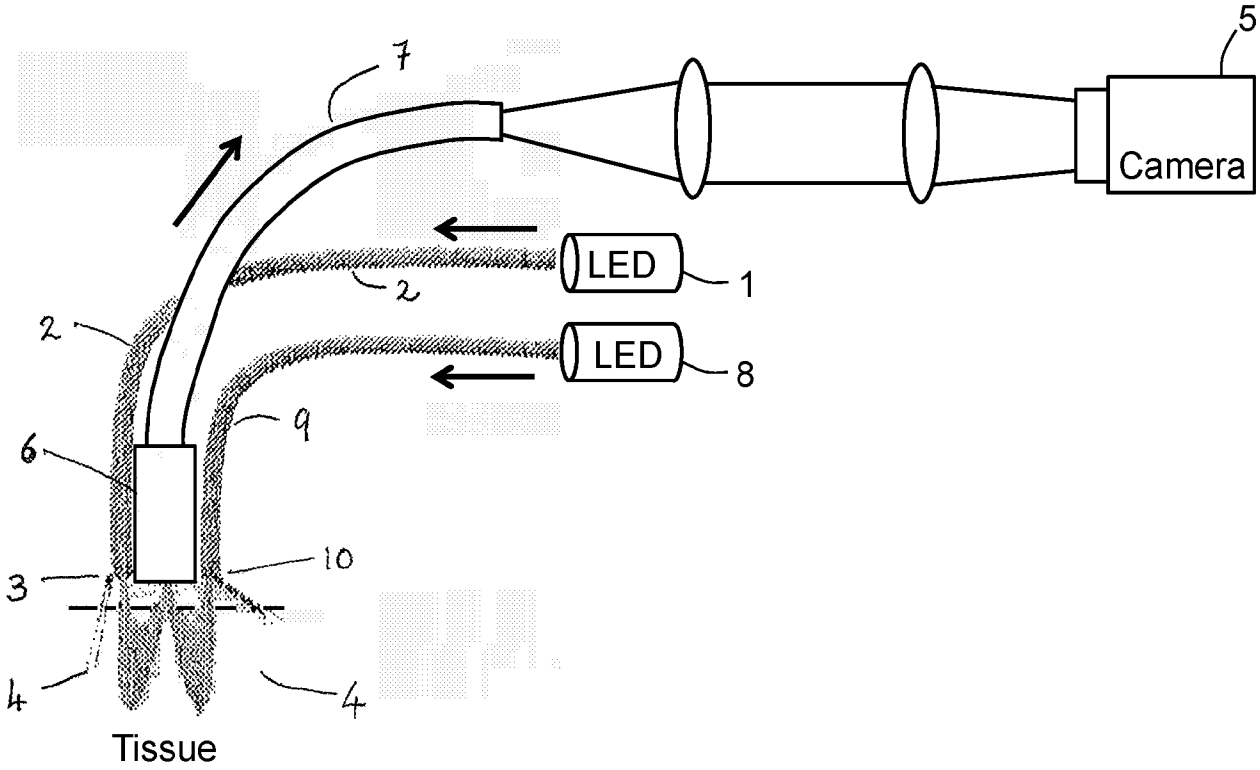


Figure 1
(*Background Art*)

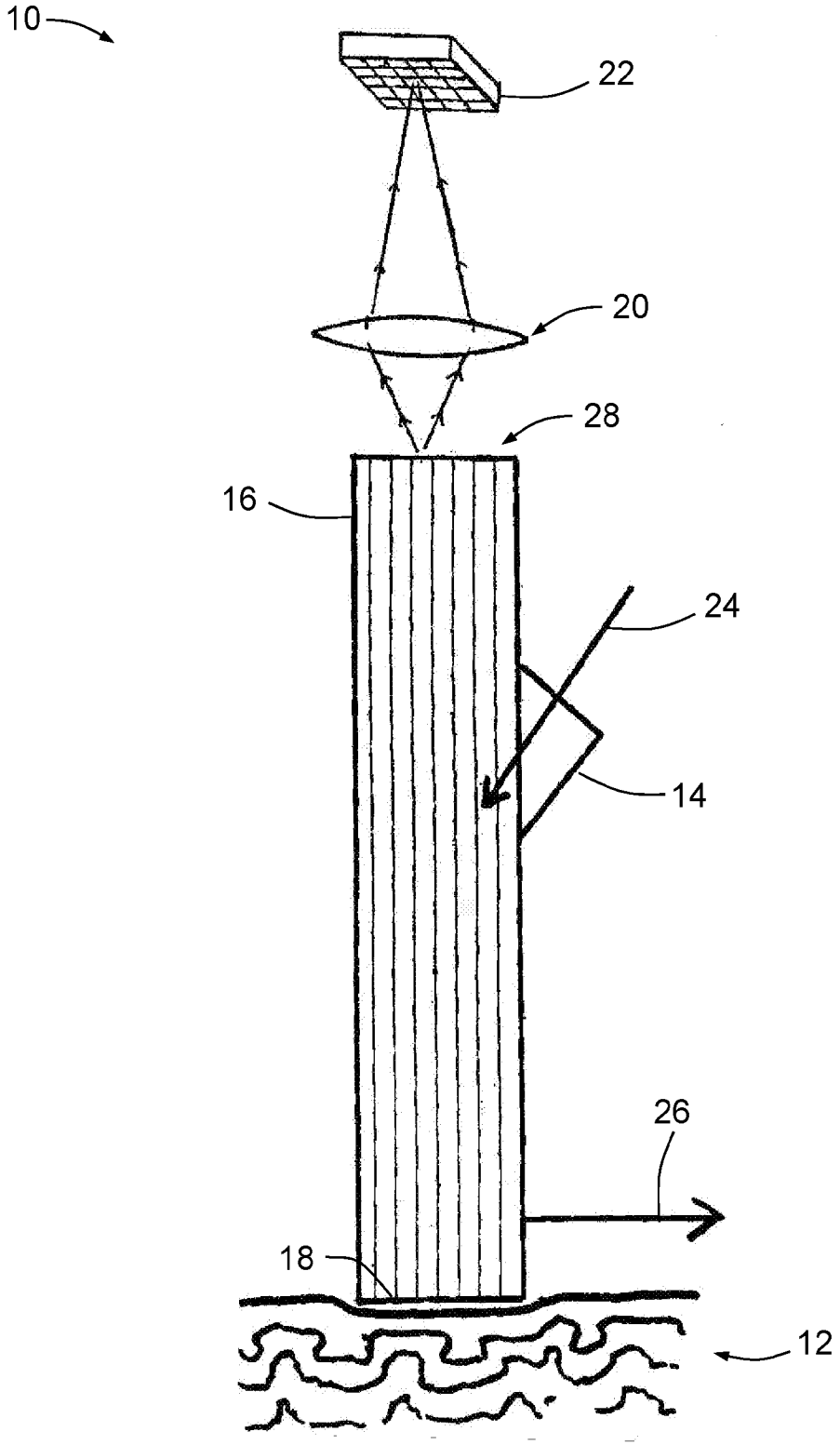


Figure 2

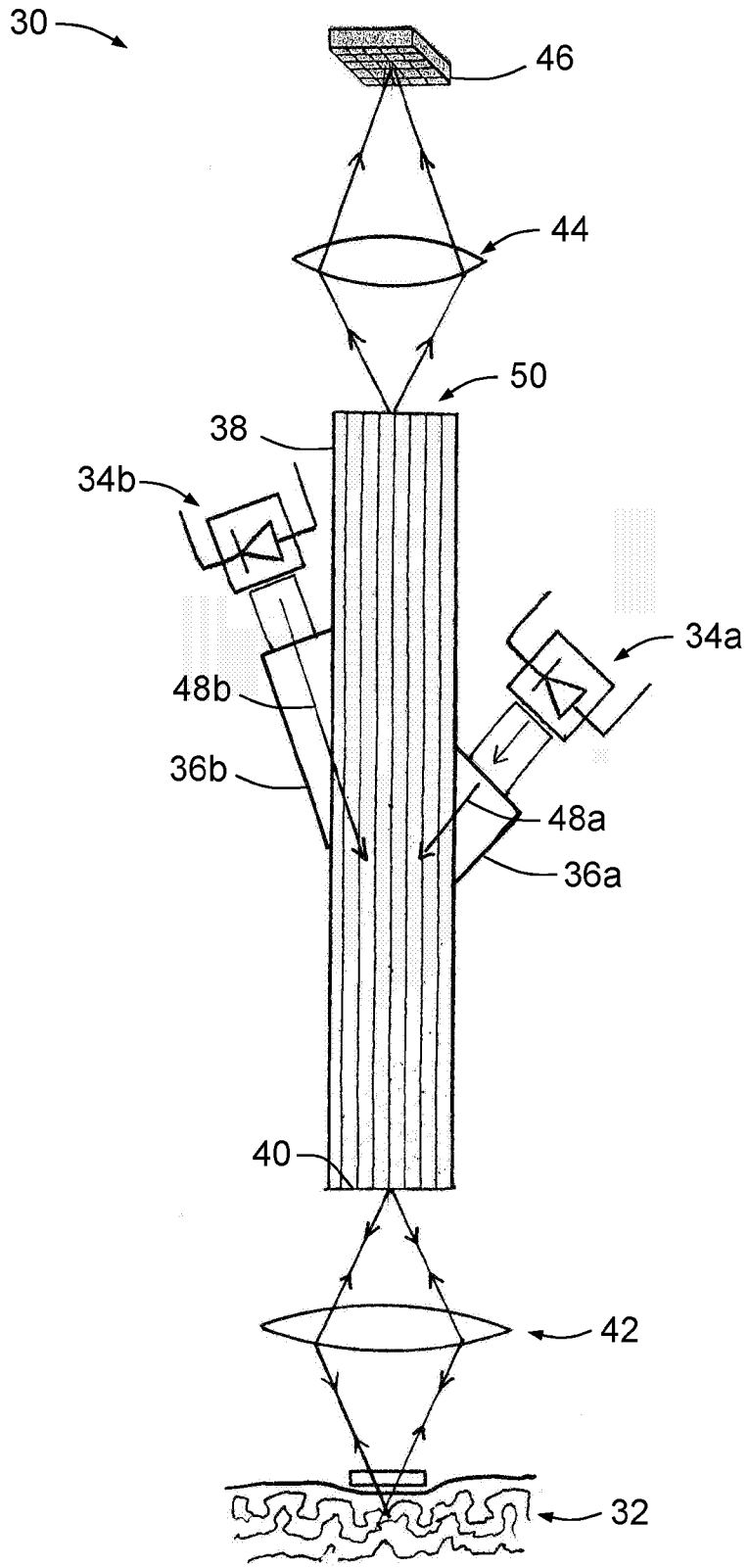


Figure 3

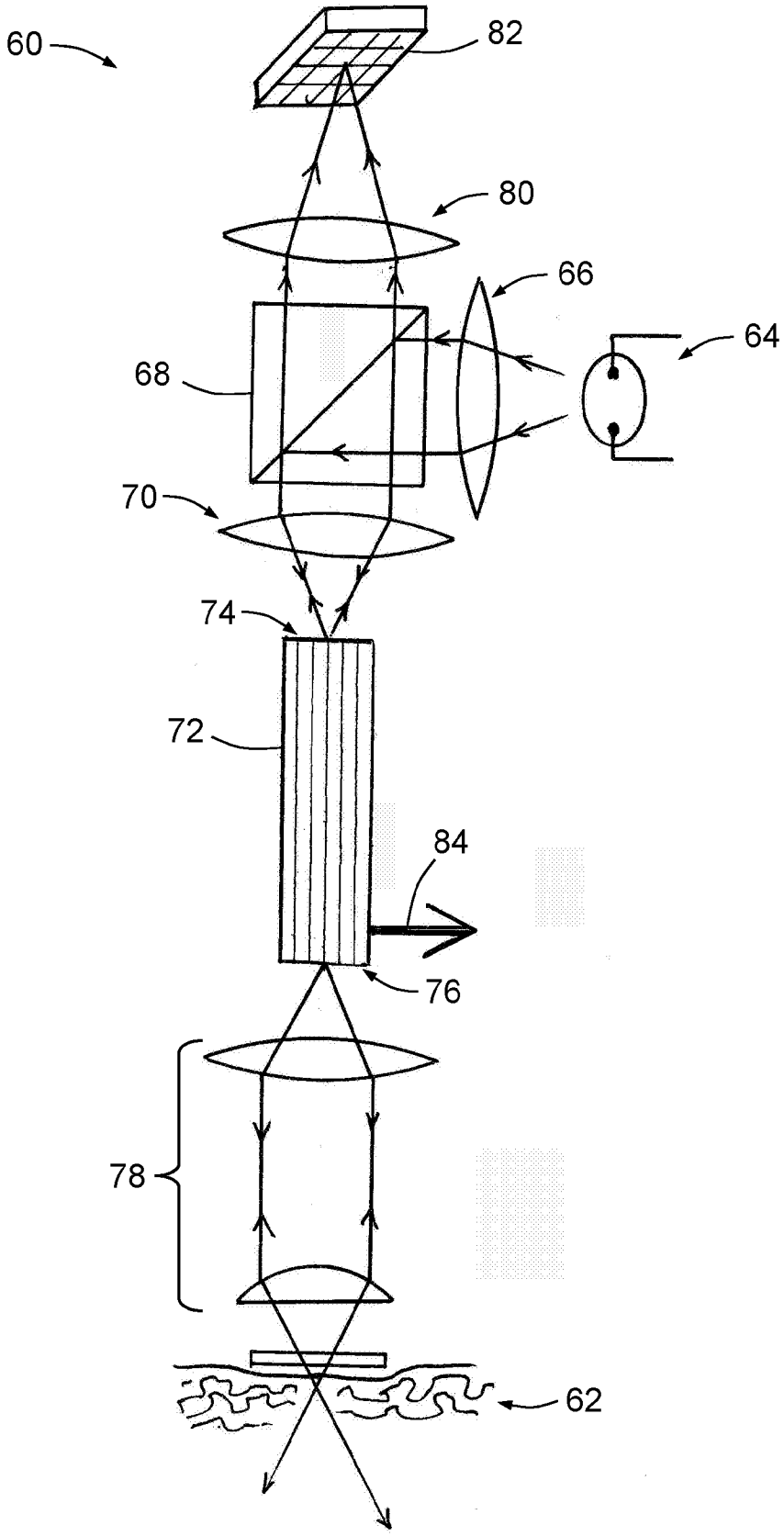


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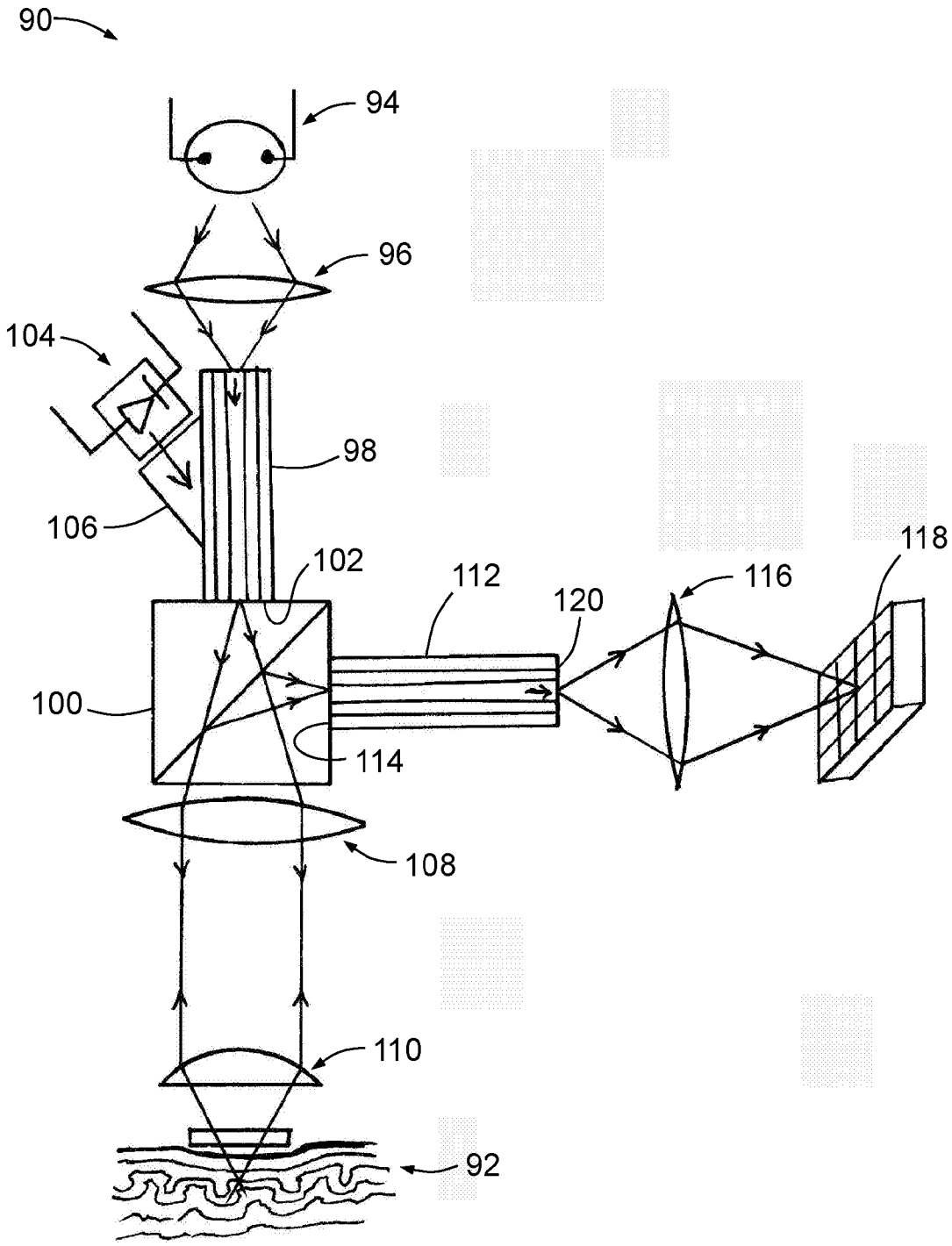


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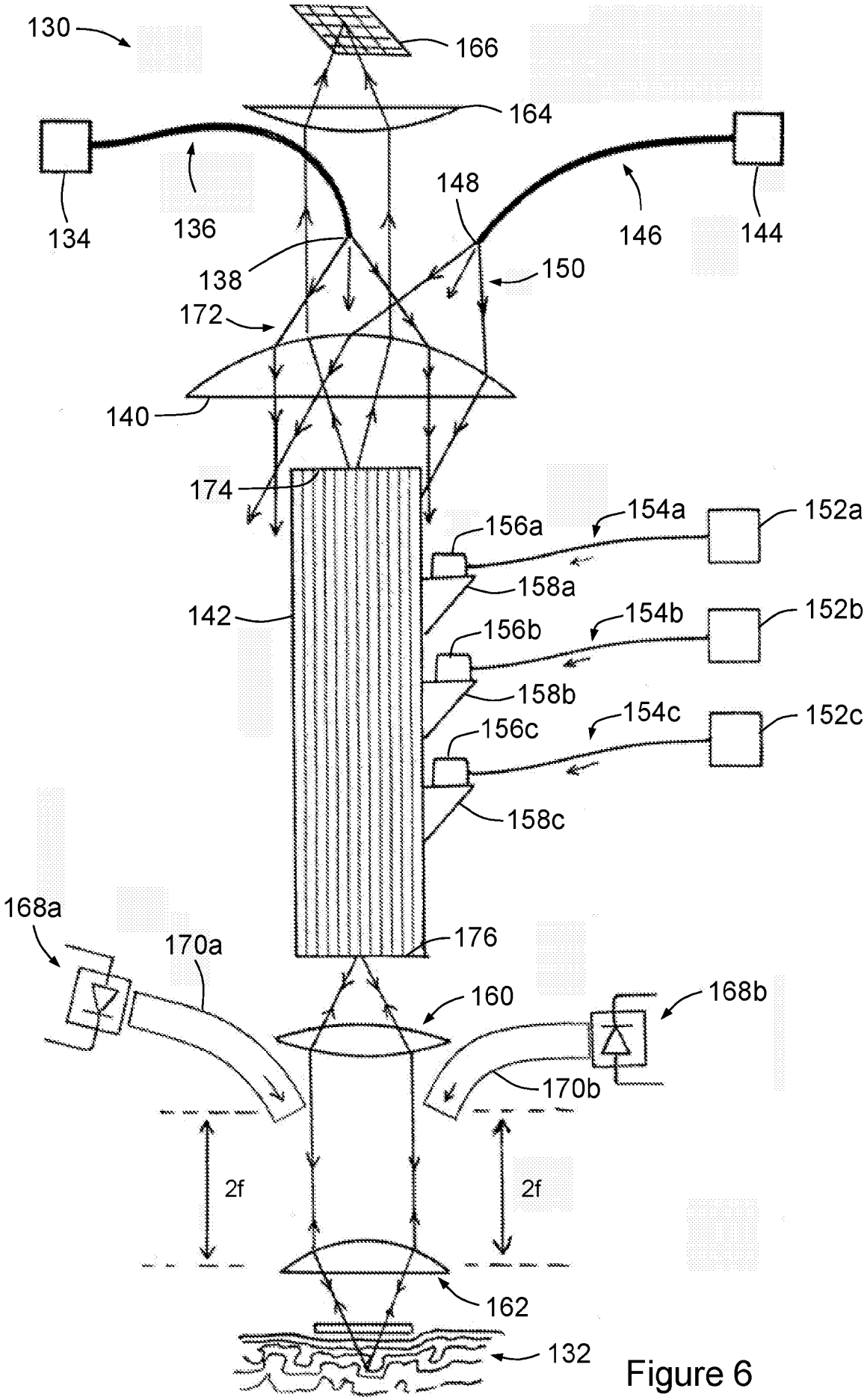

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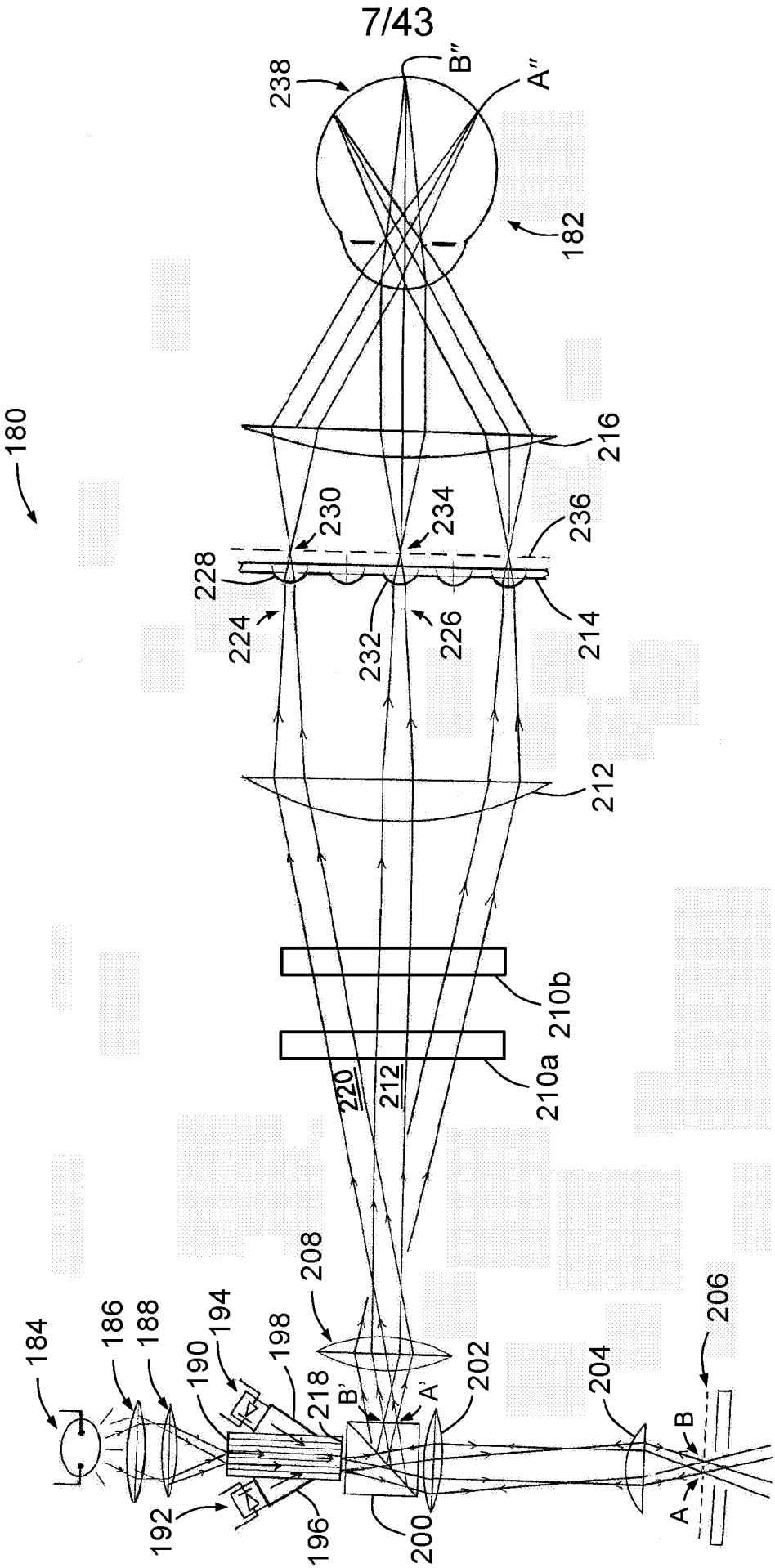

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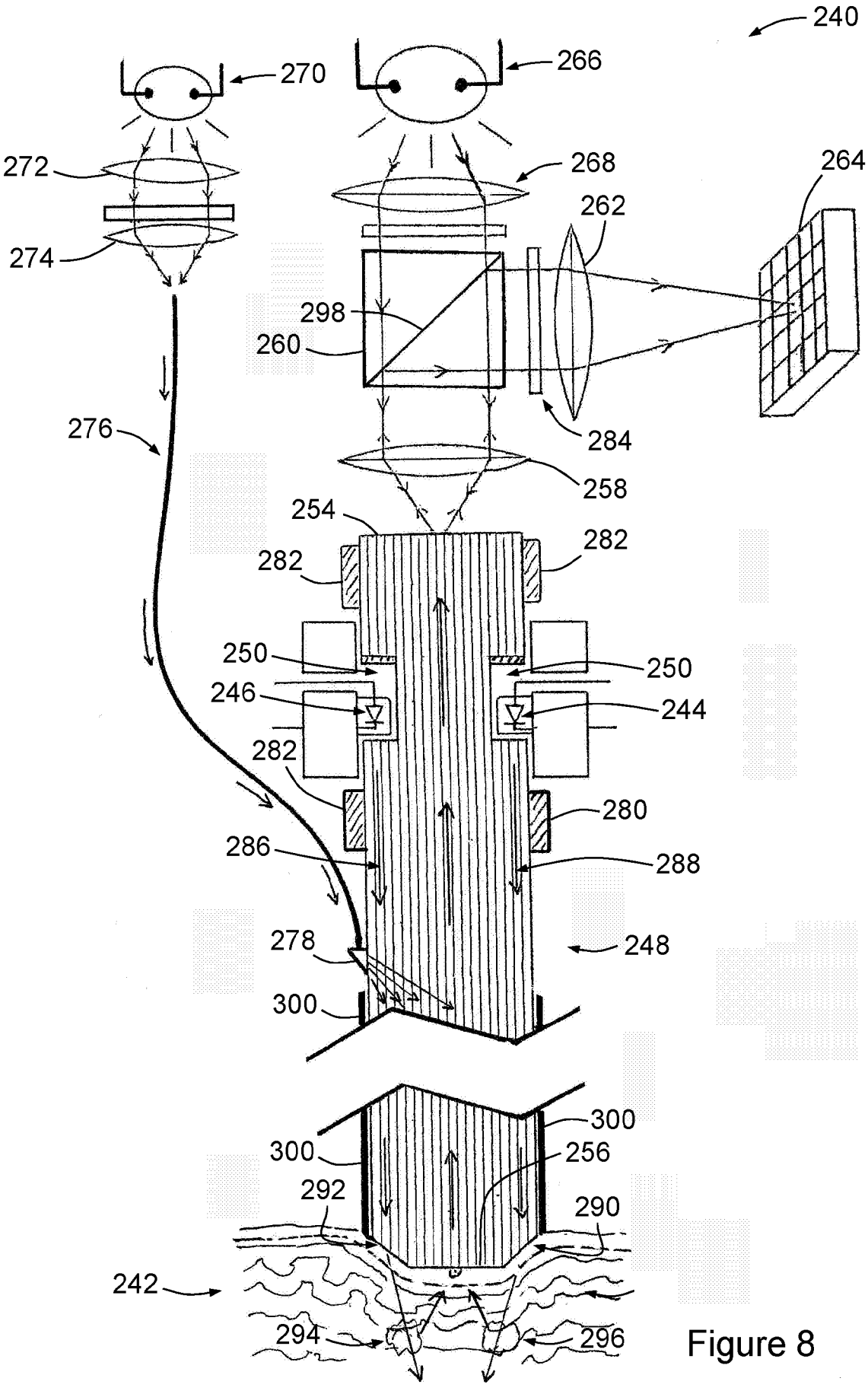

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Figure 8

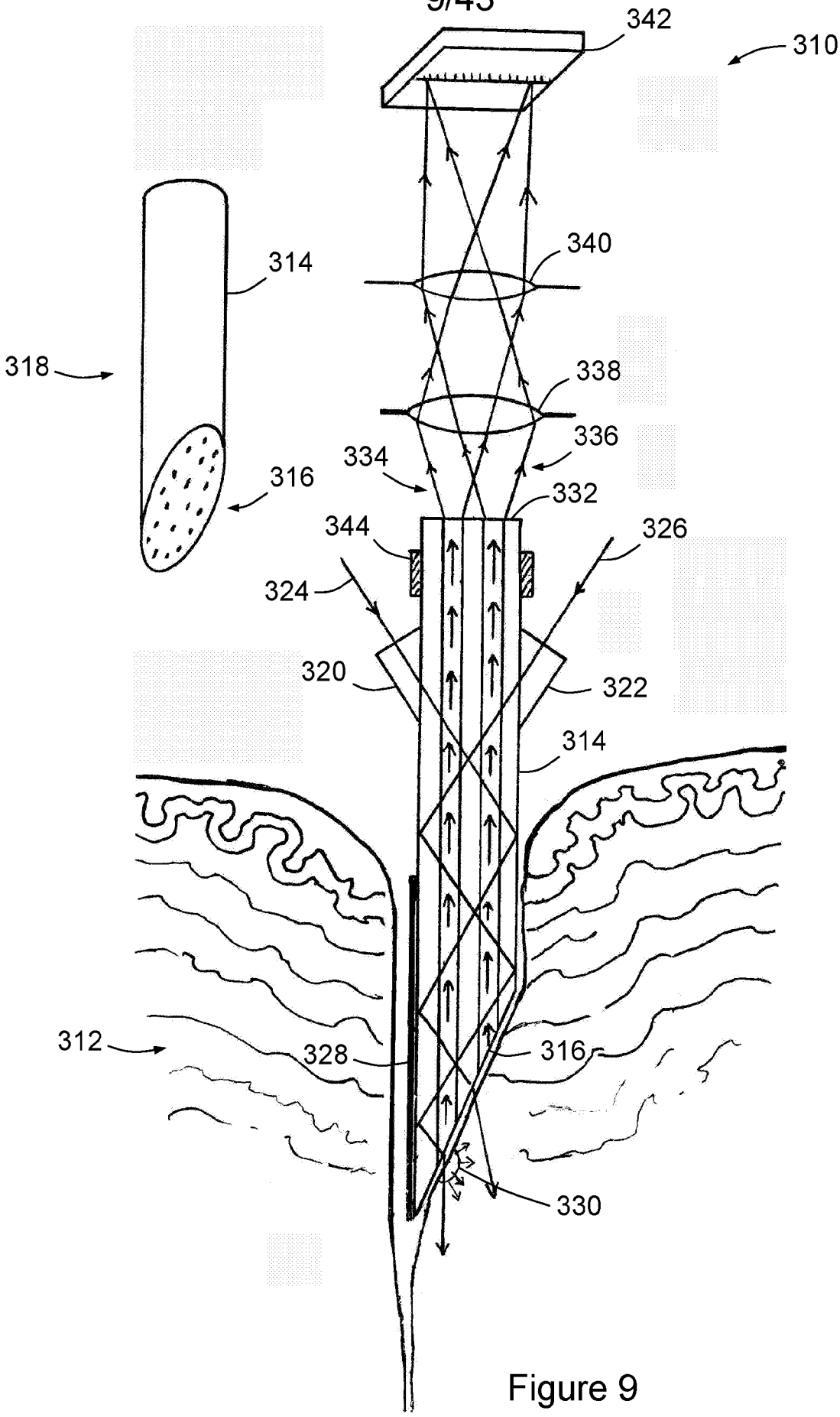

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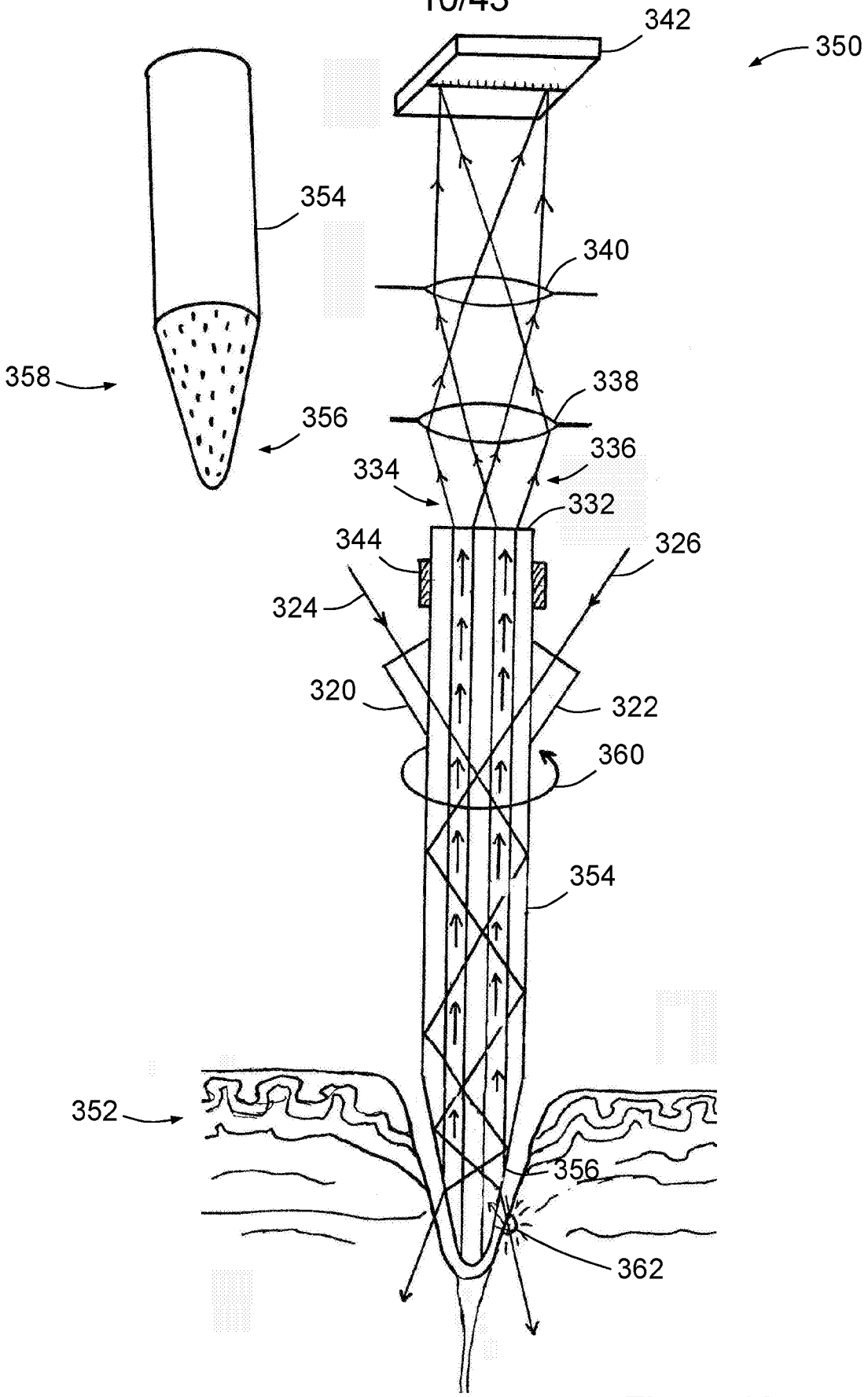

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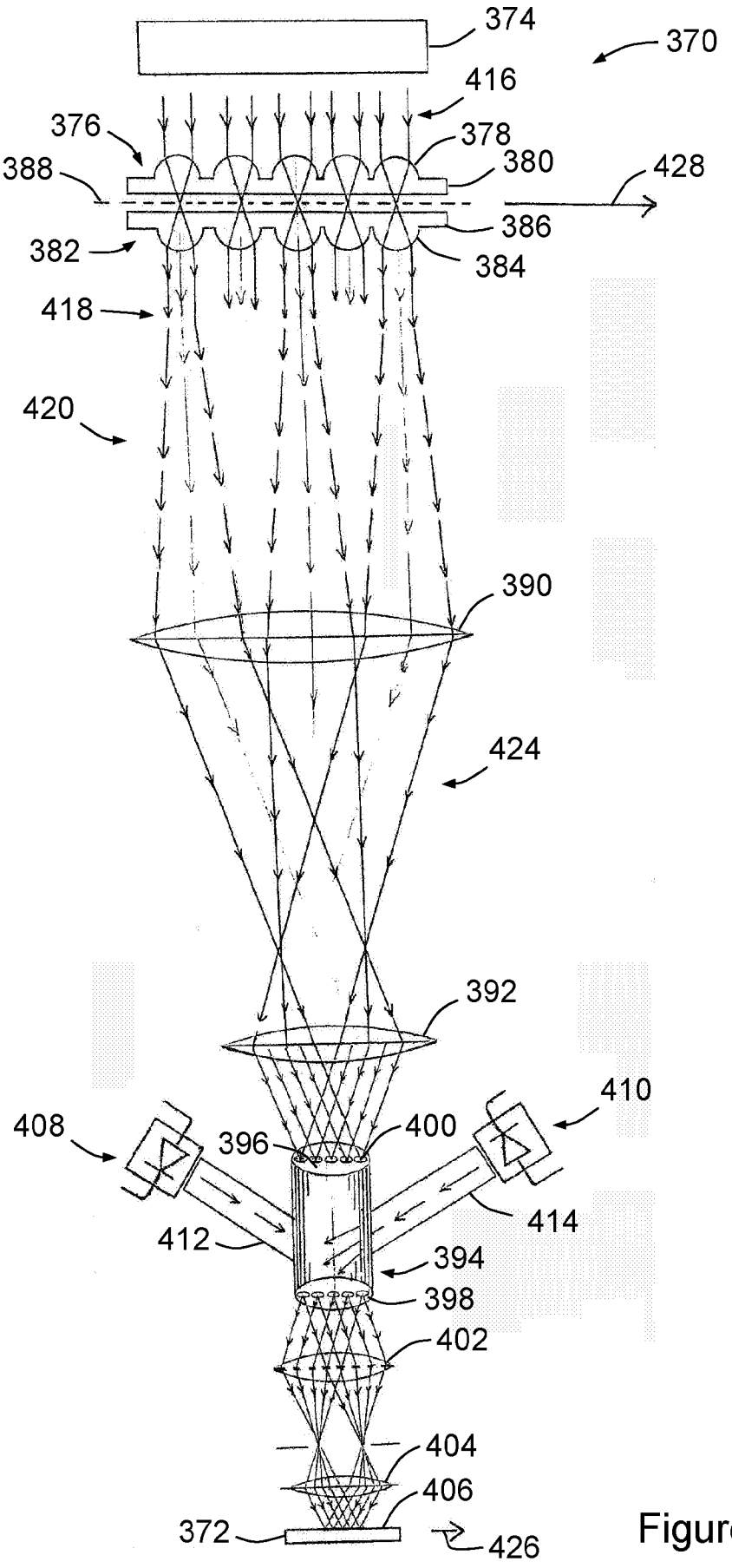

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Figure 11

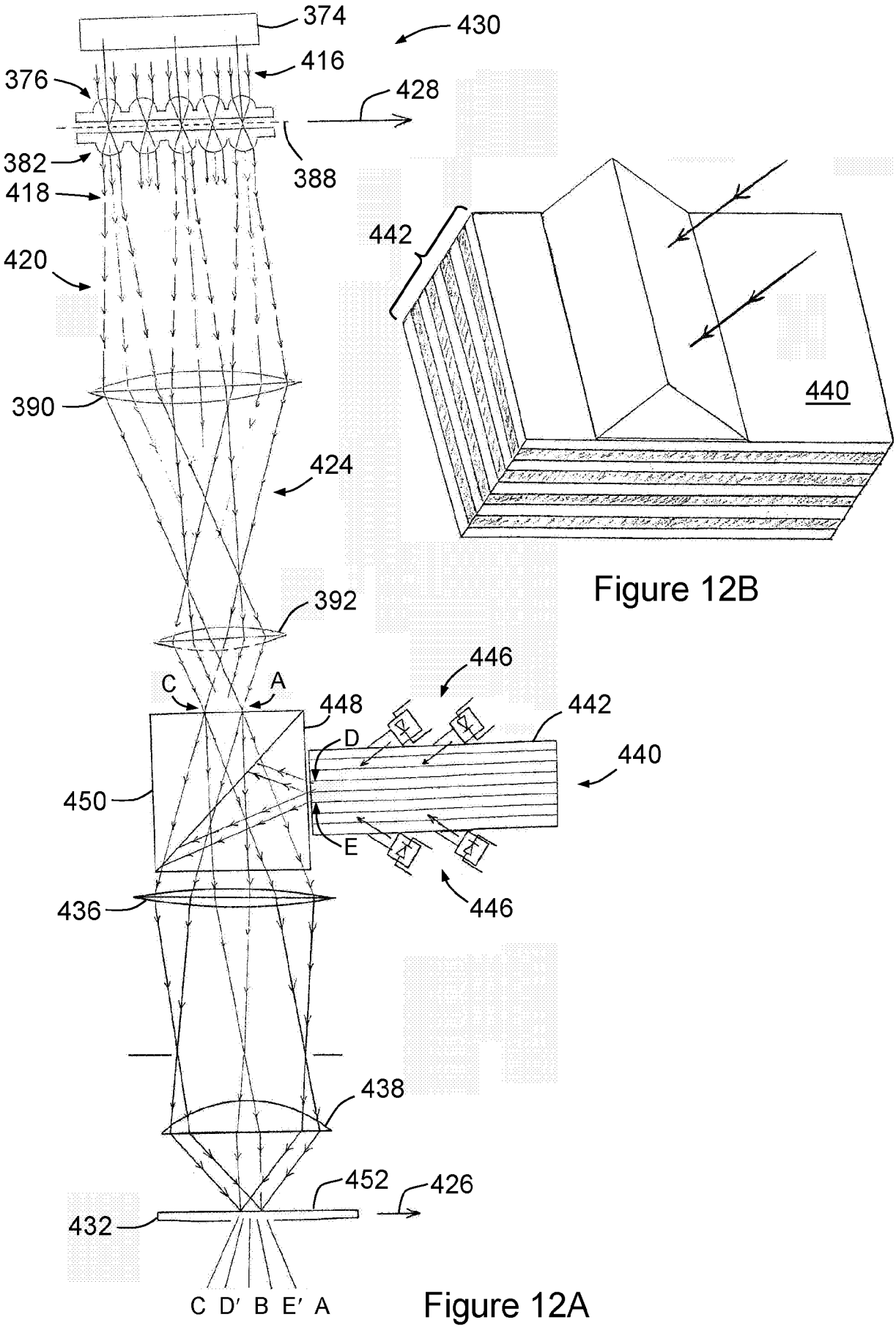


Figure 12B

Figure 12A

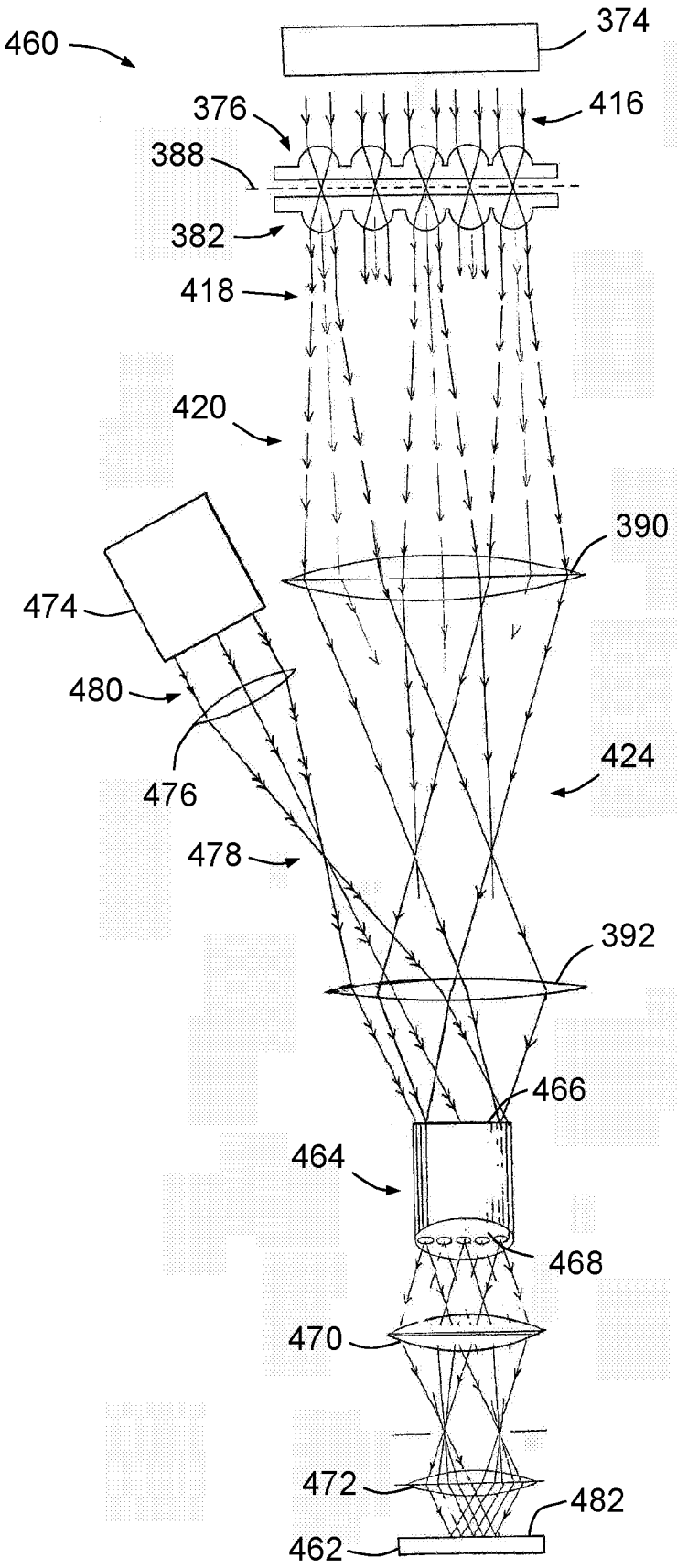


Figure 13A

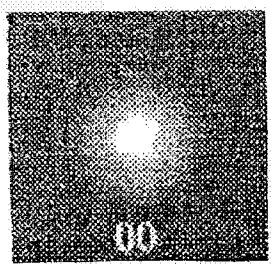


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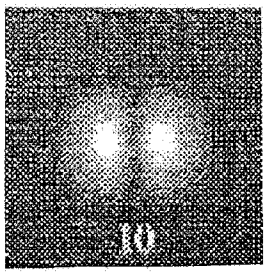


Figure 13C

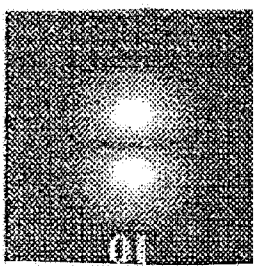


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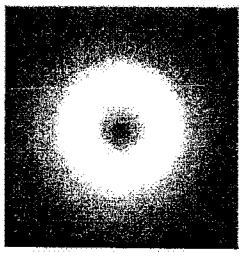


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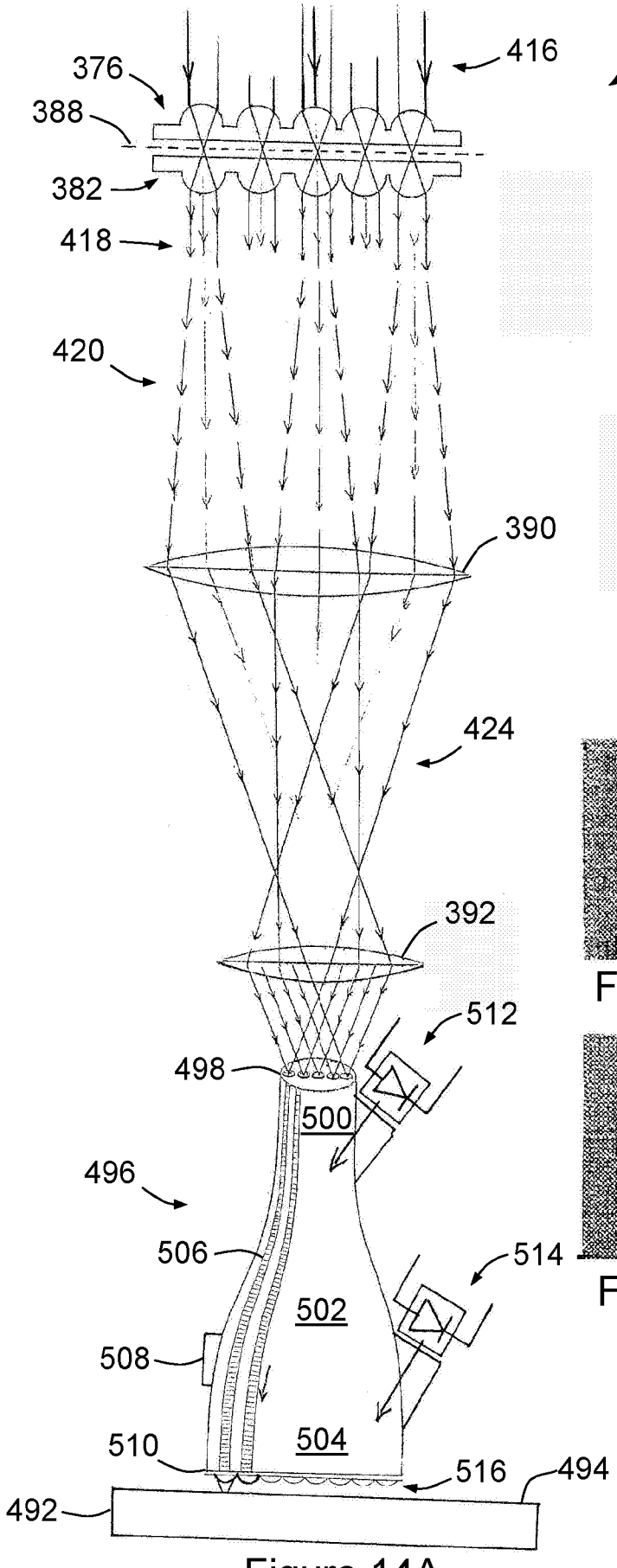


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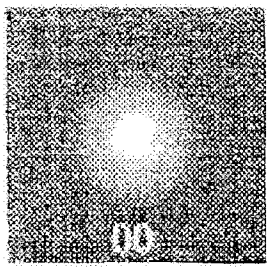


Figure 14C

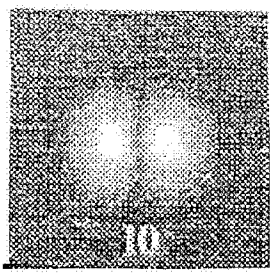


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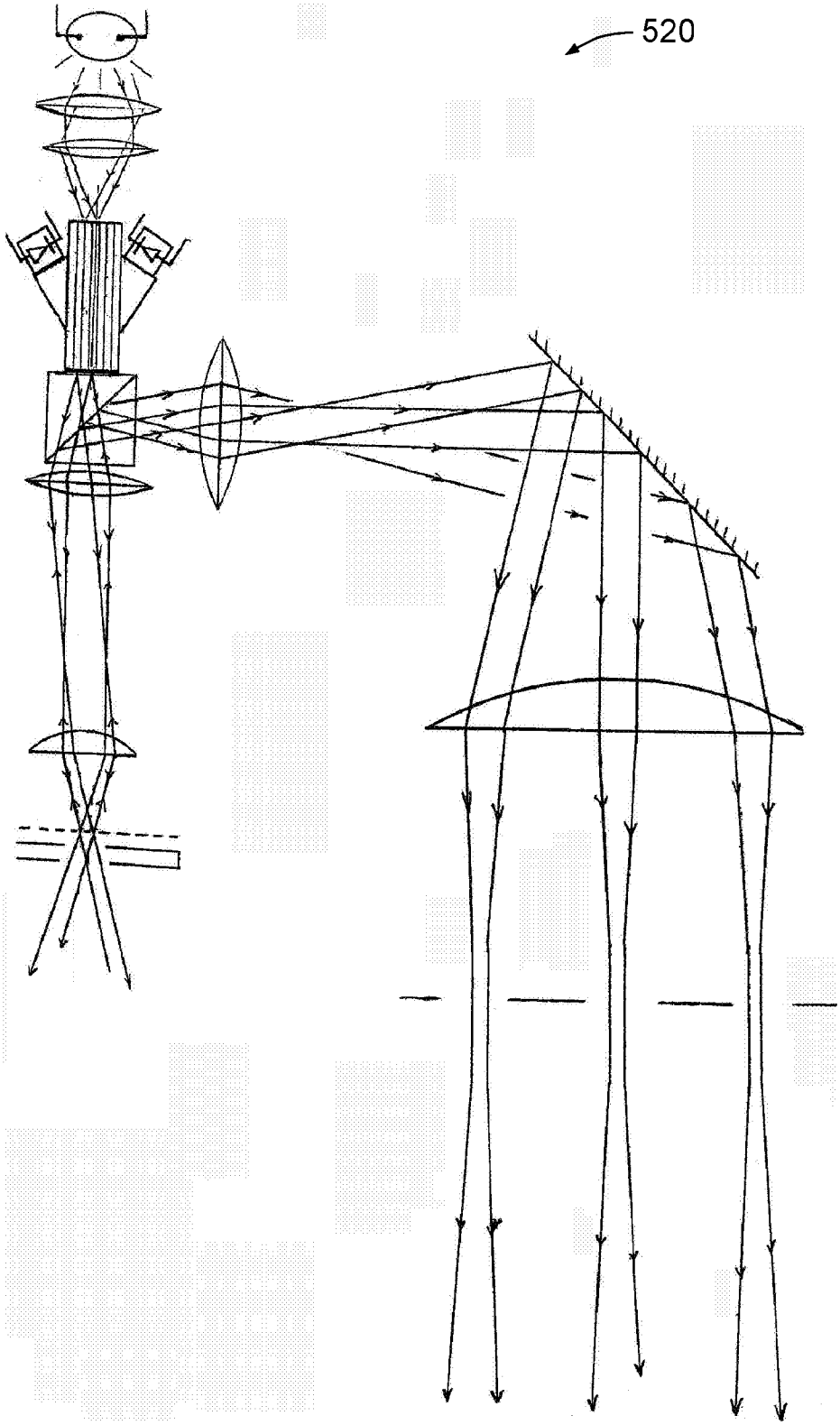


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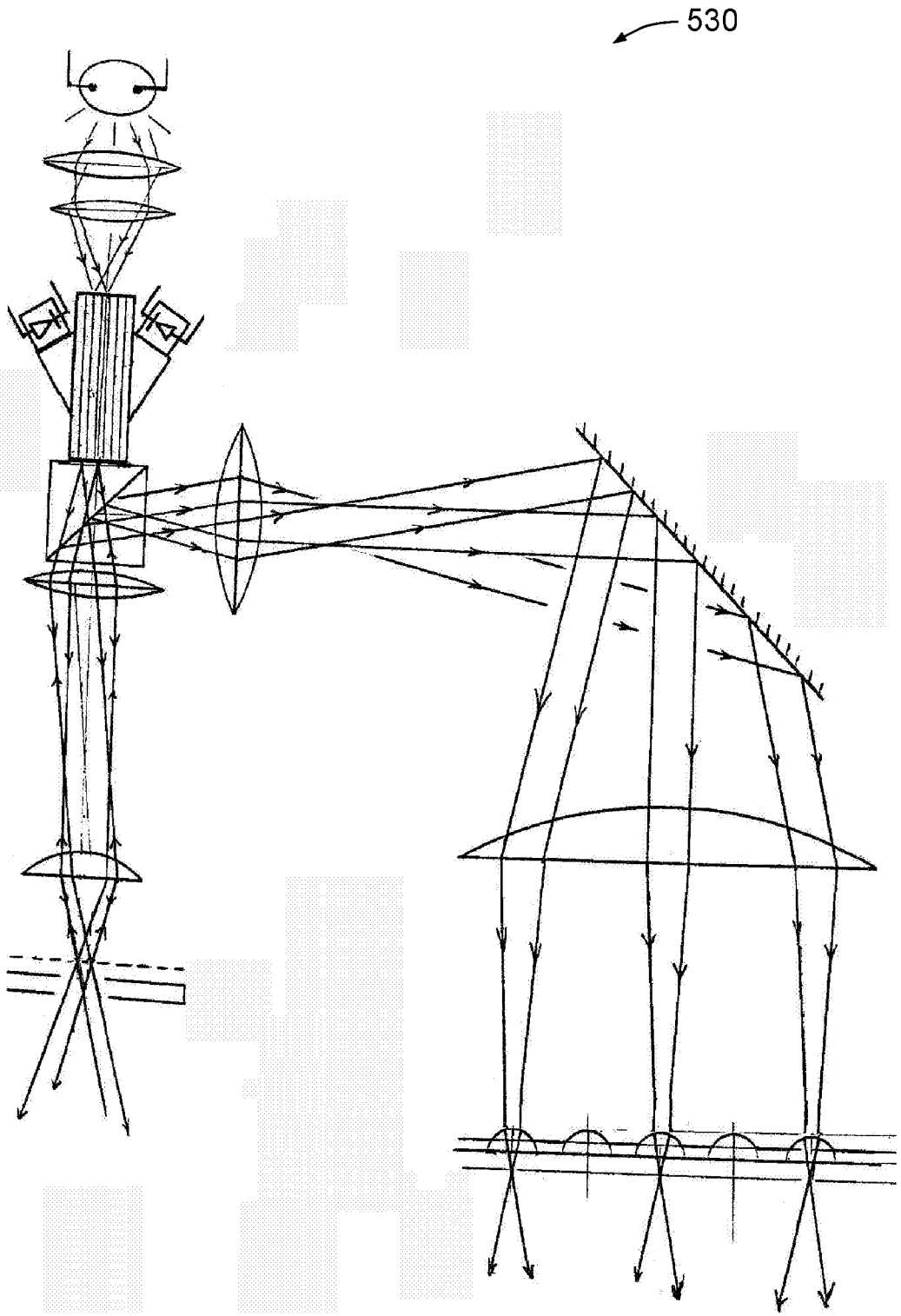


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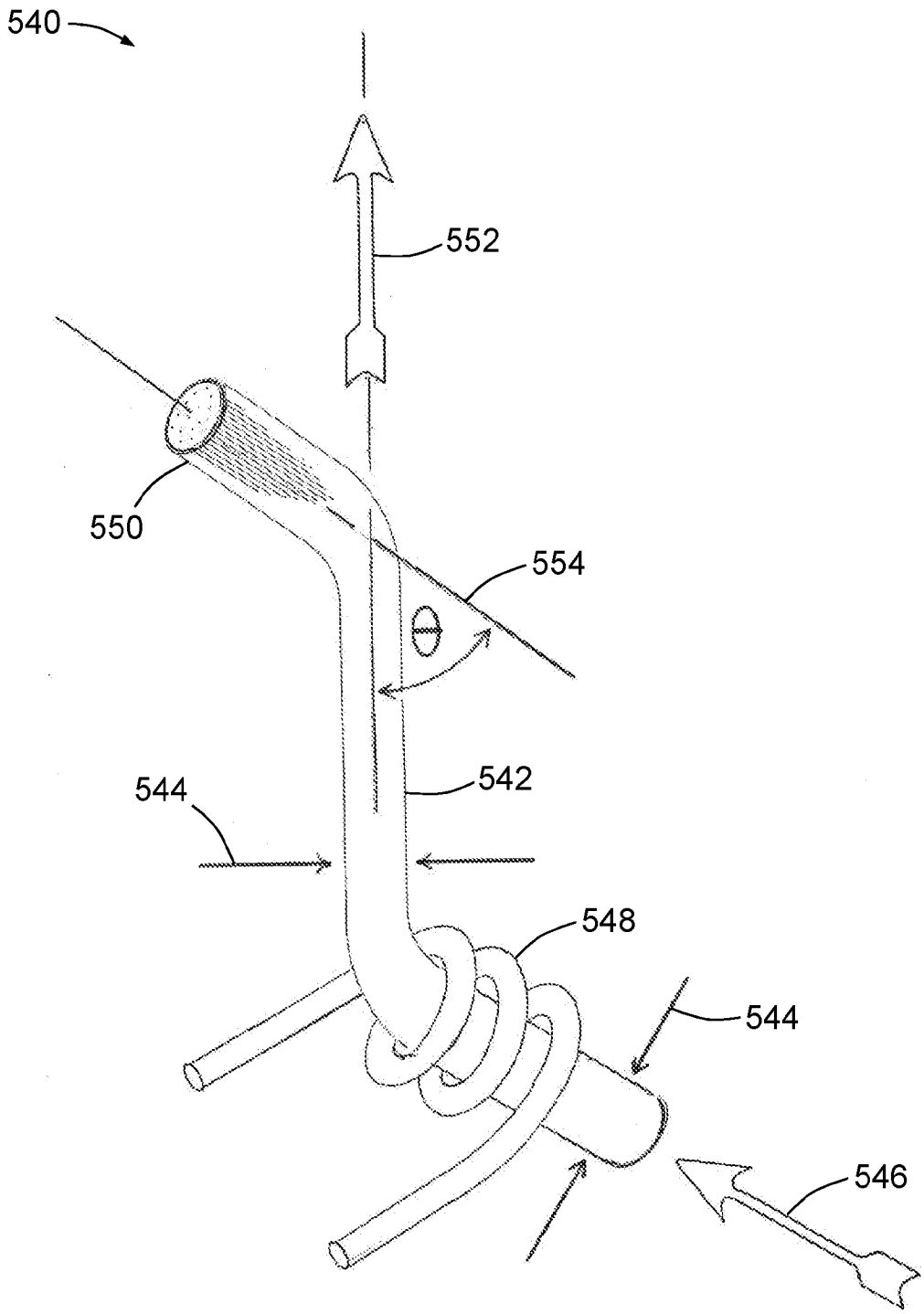


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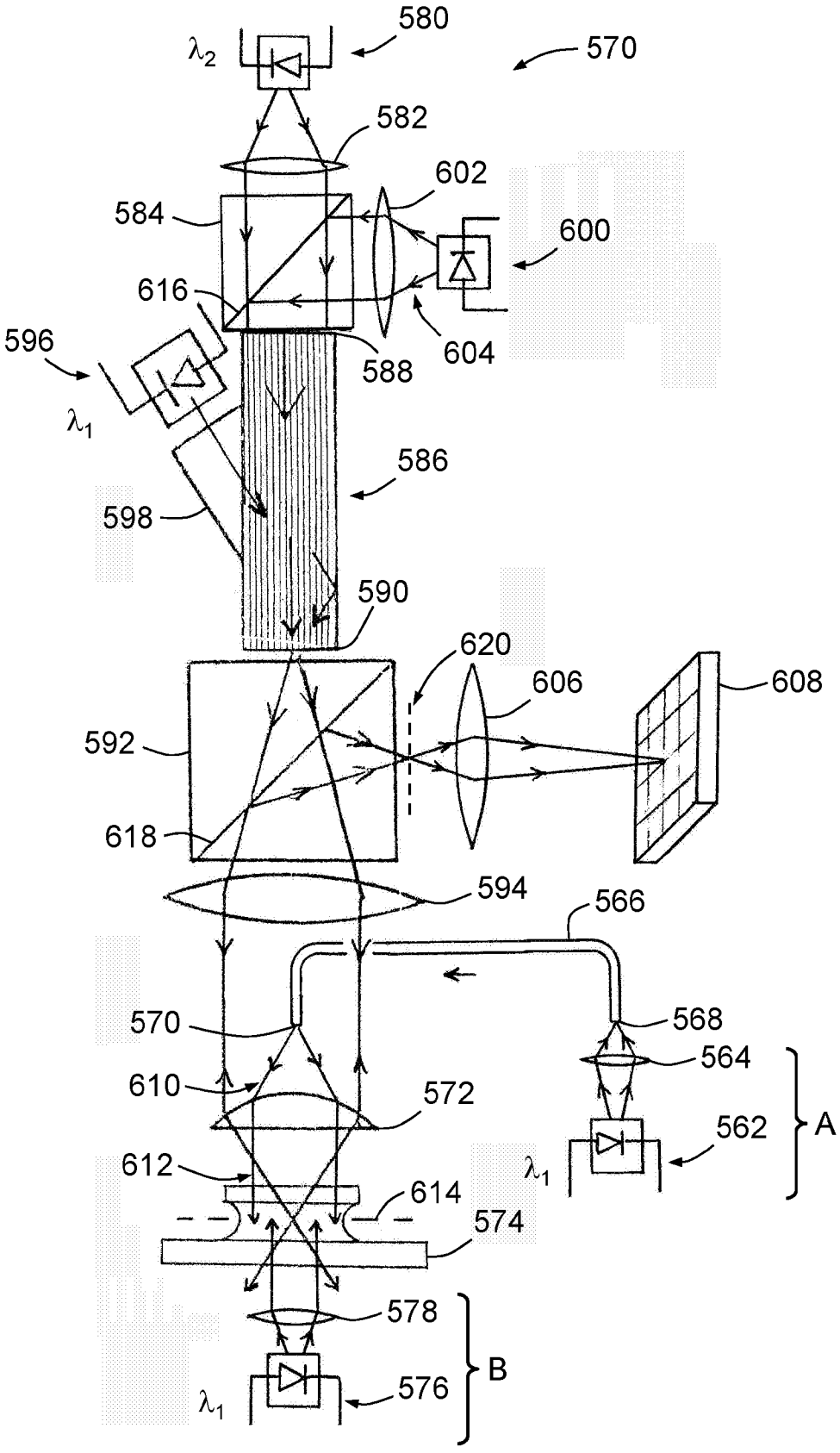

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λ_1

Figure 18

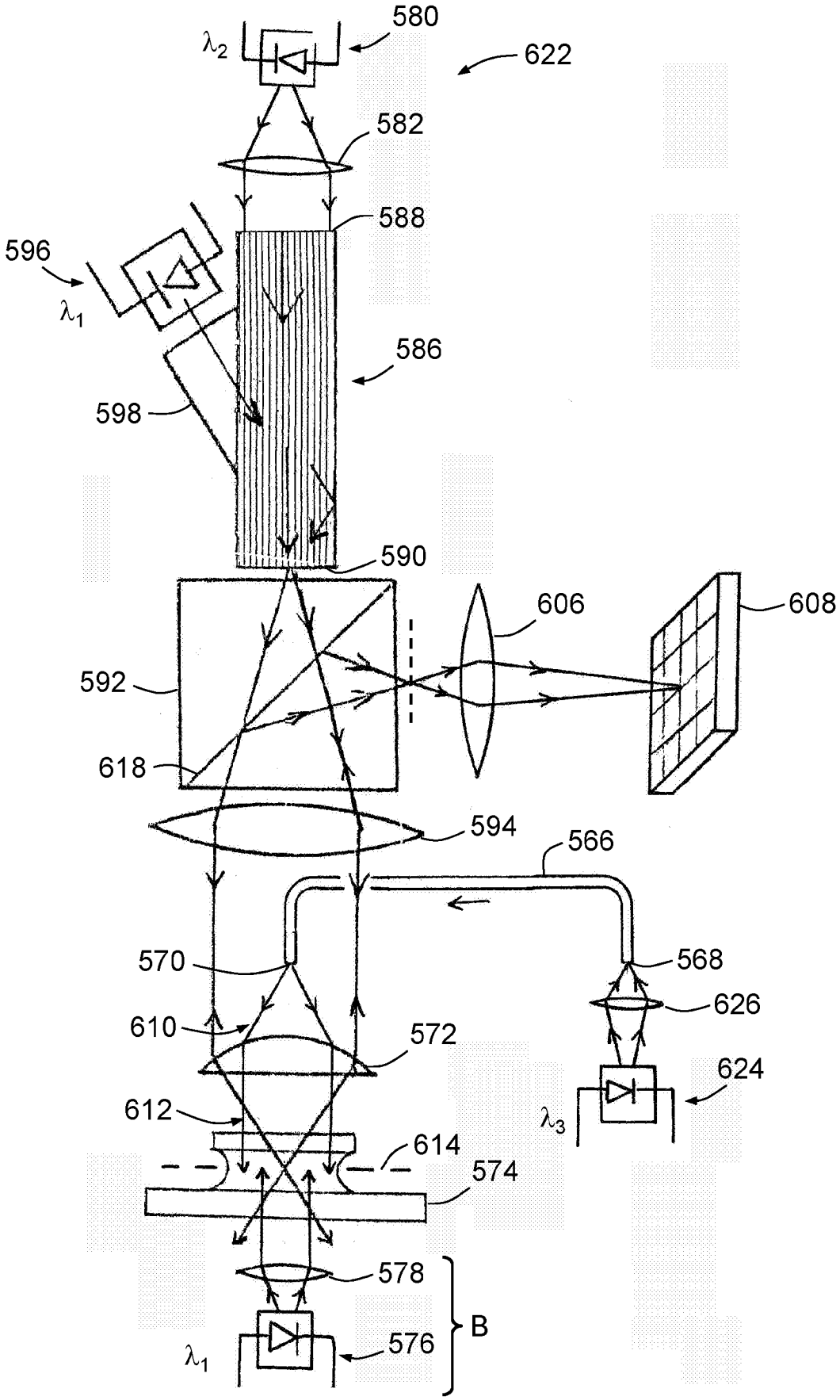

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λ_1

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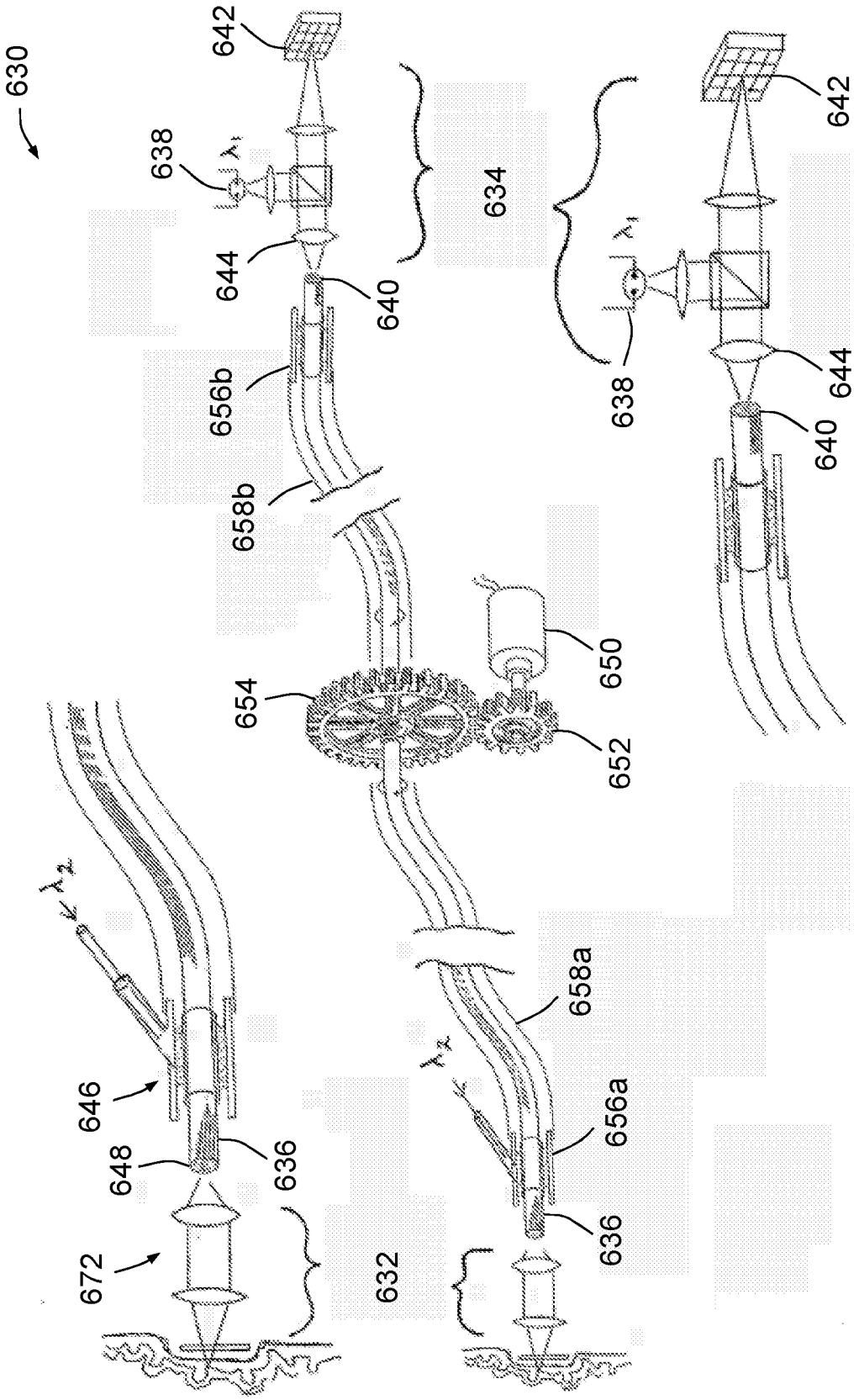

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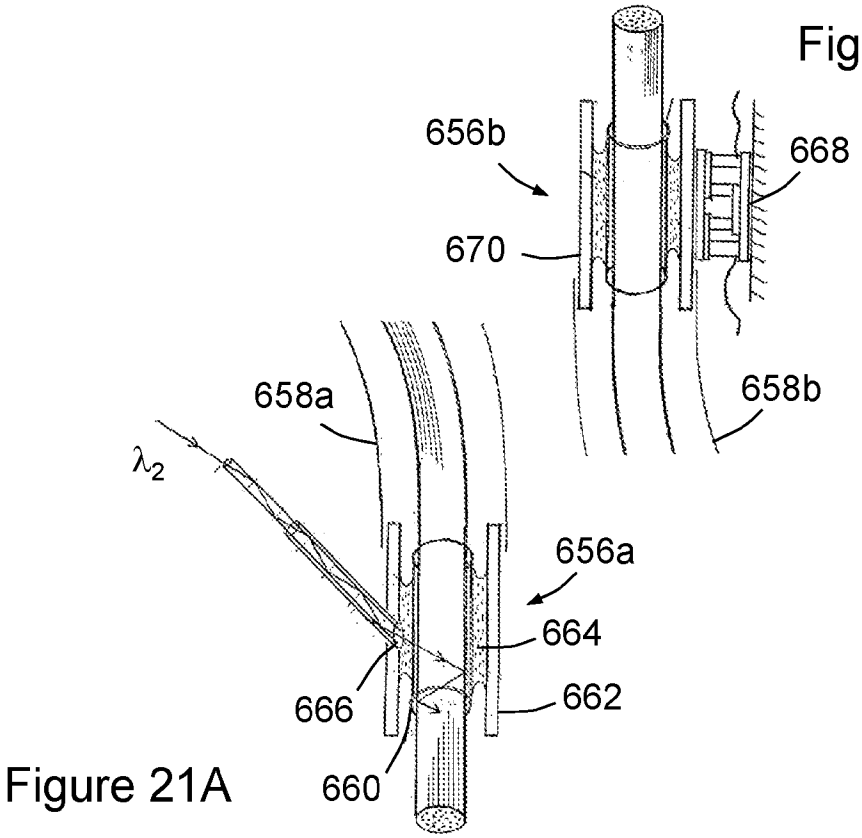


Figure 21A

Figure 21B

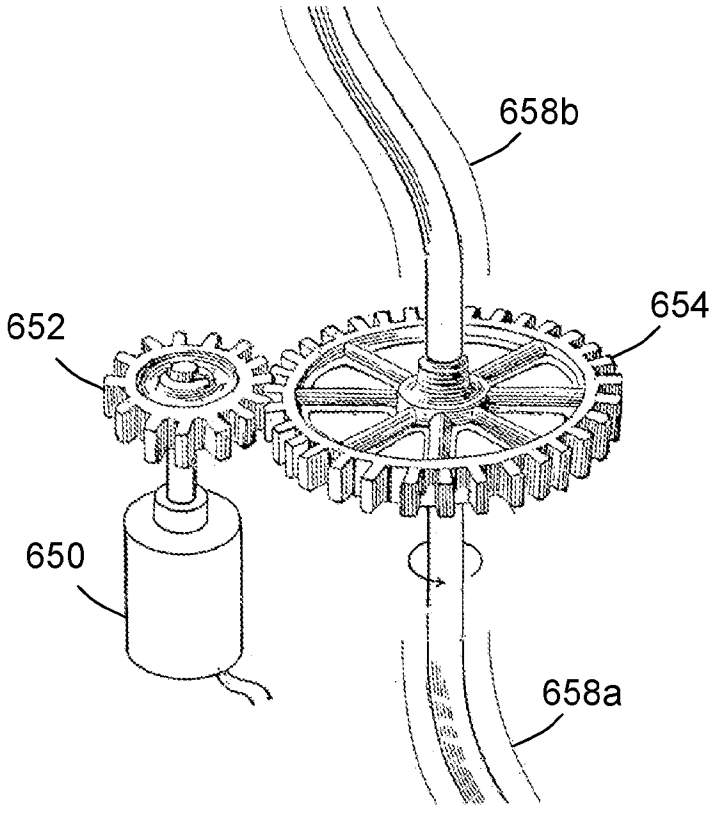


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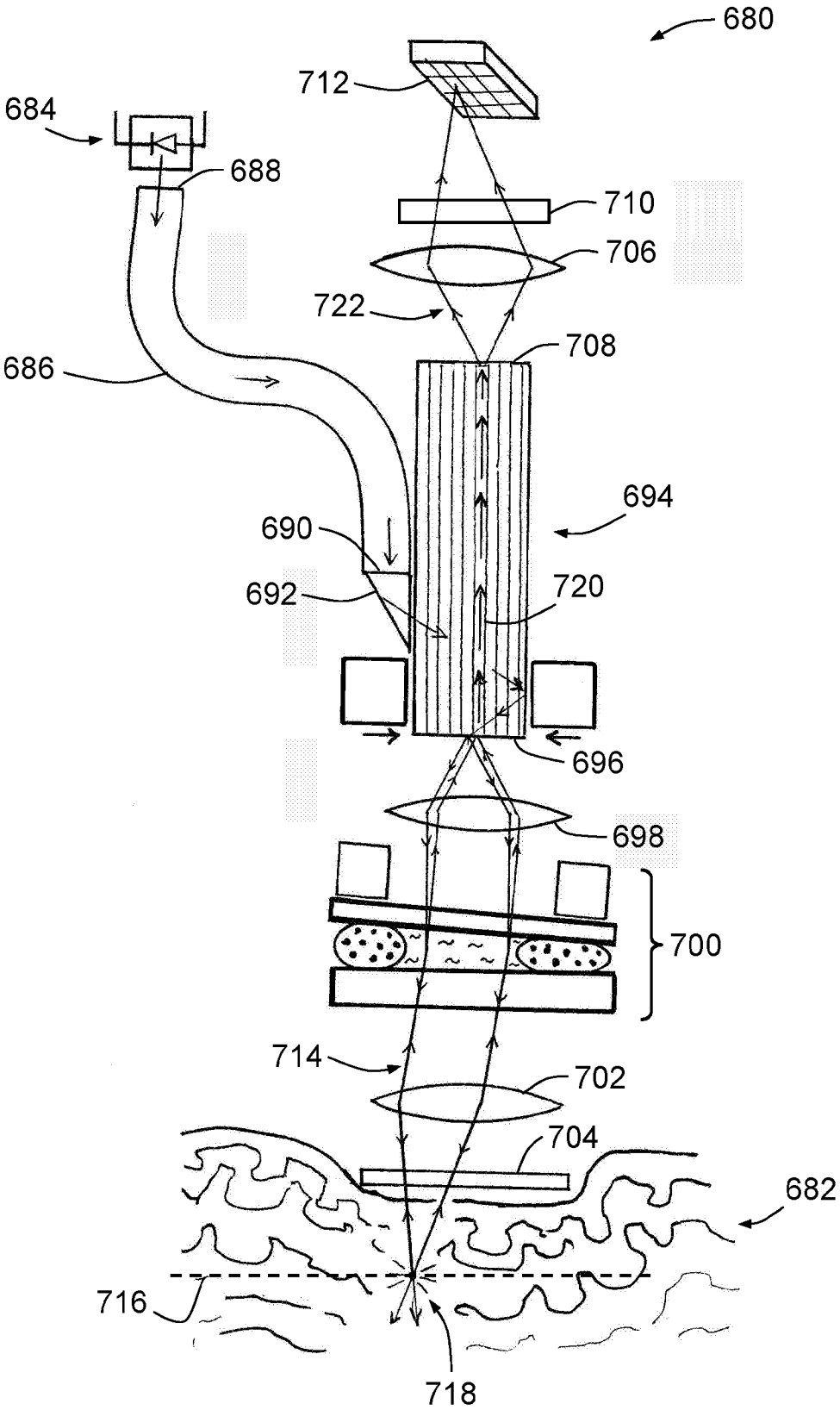

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Figure 22

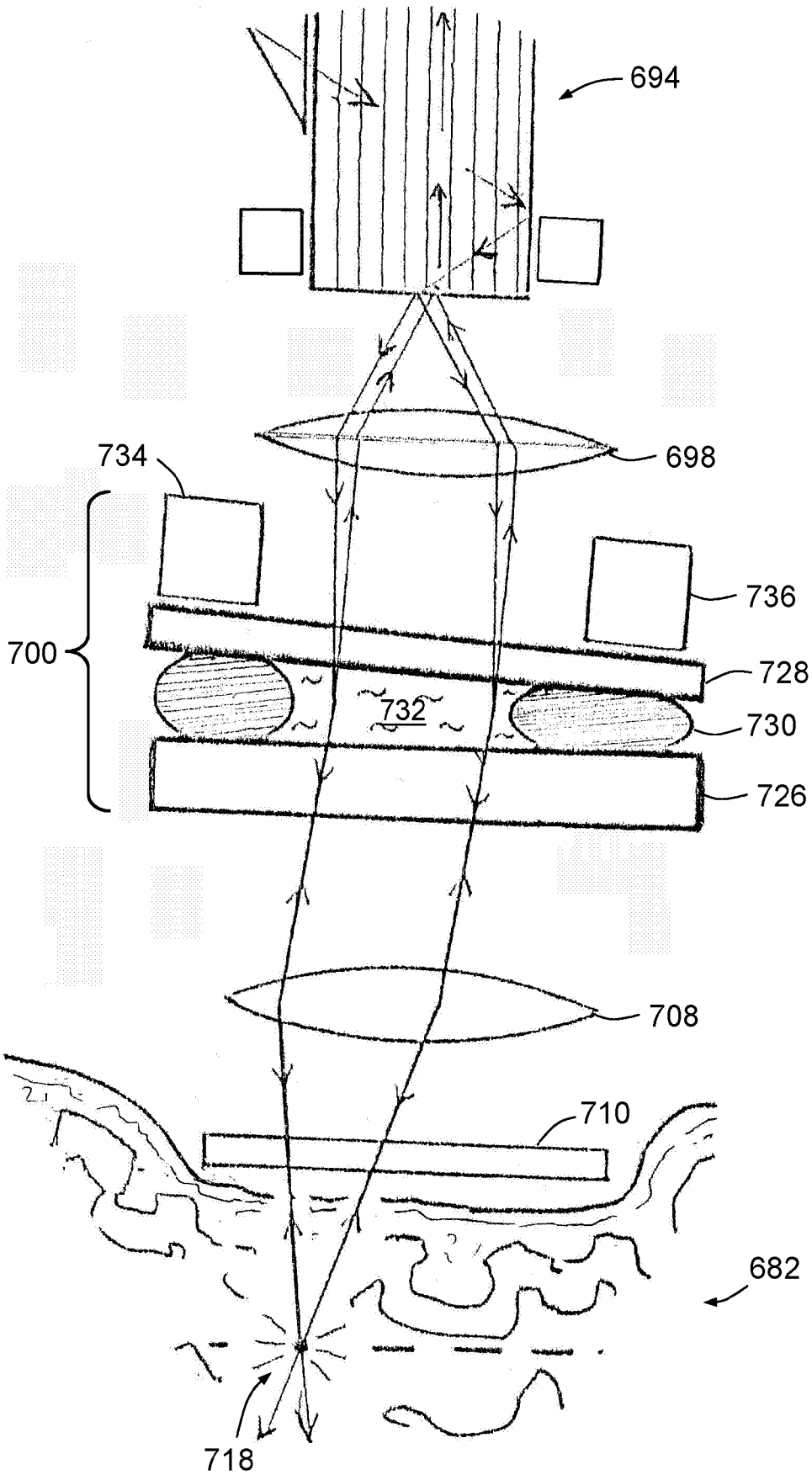


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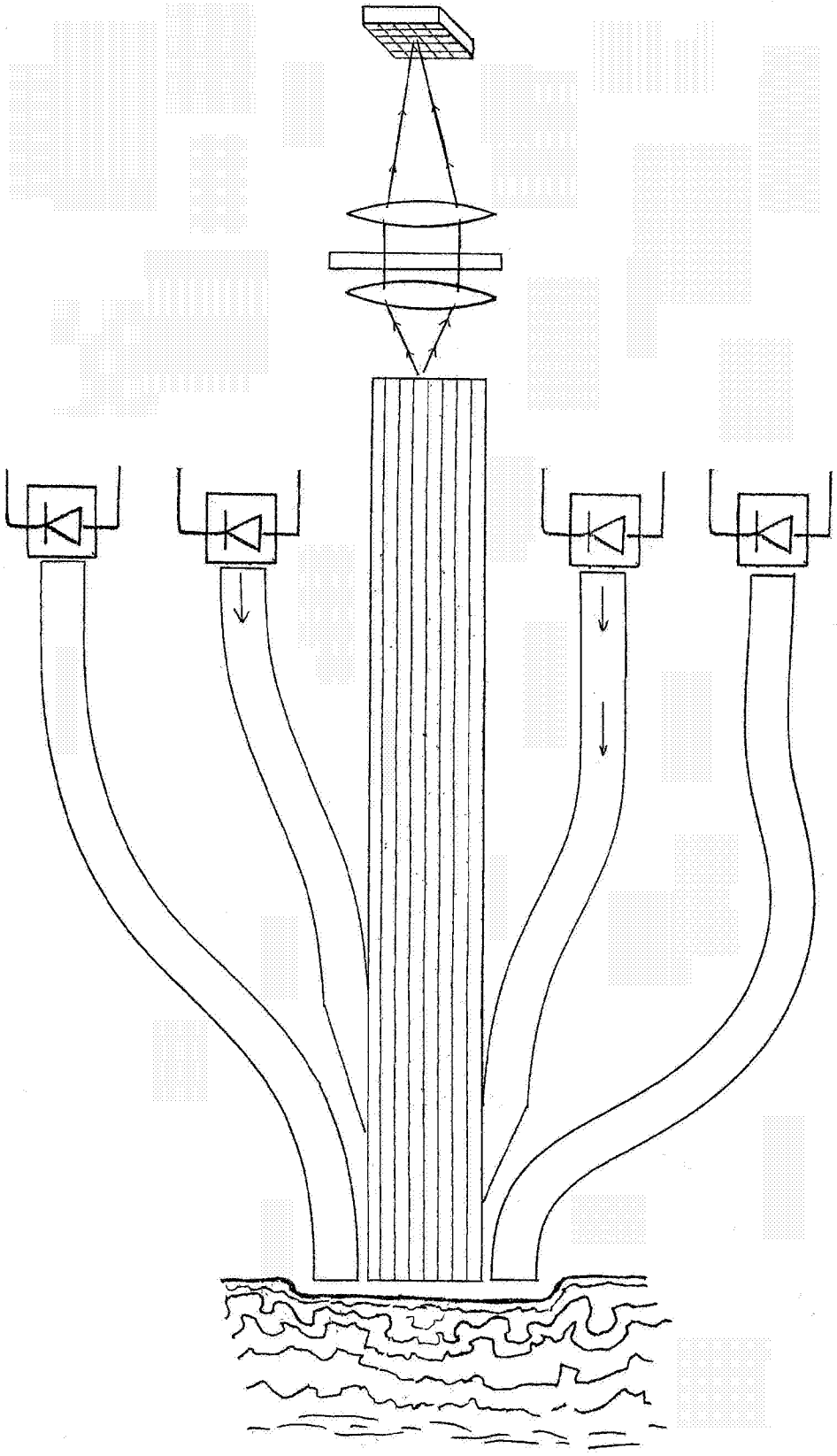


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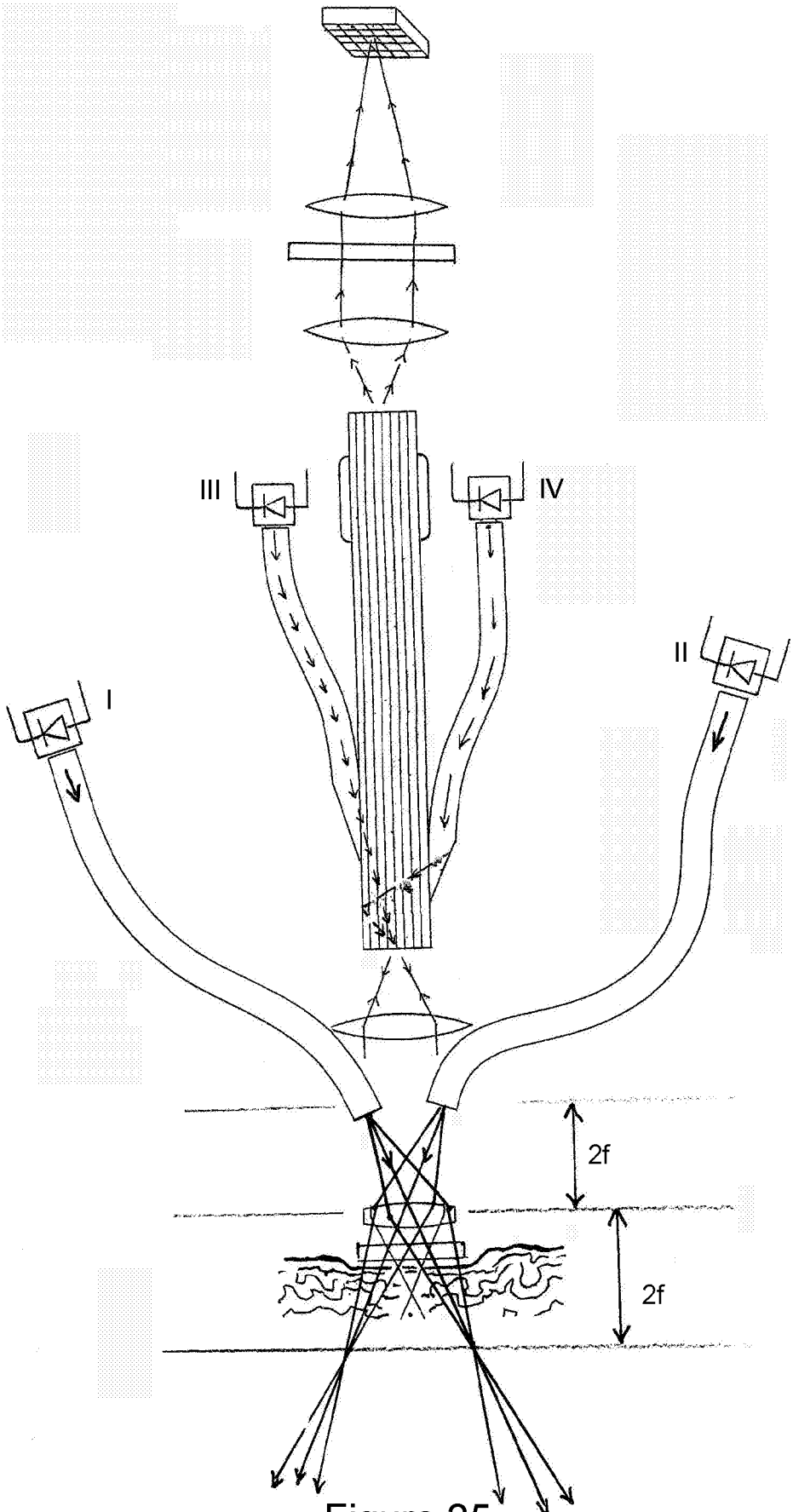


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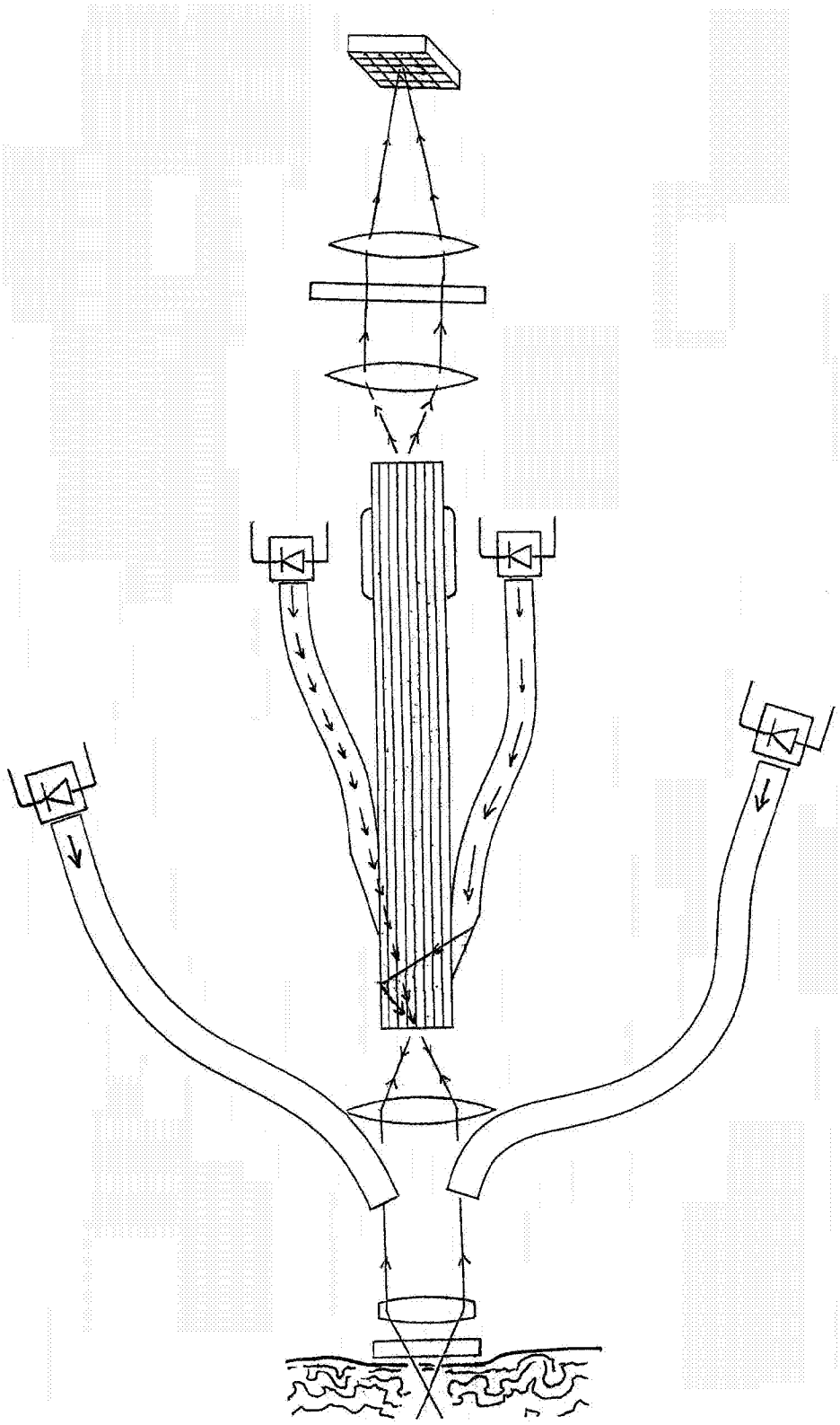


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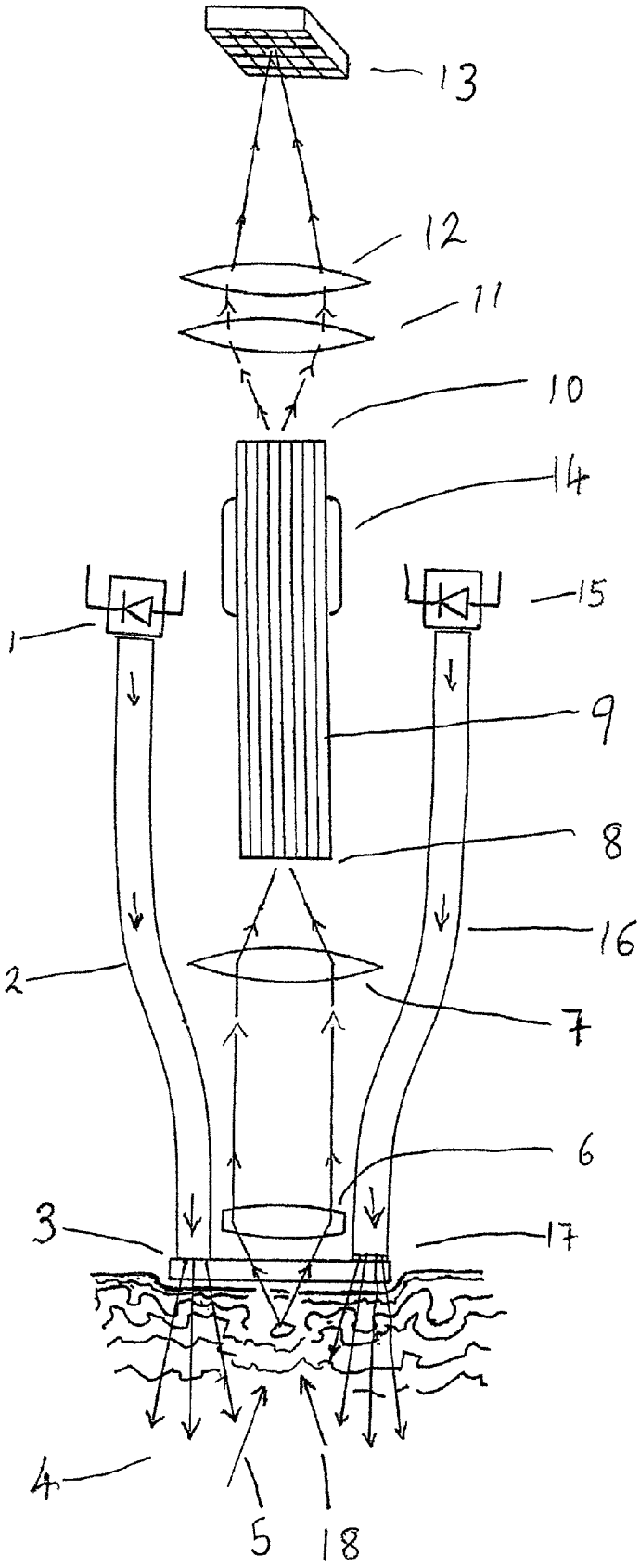


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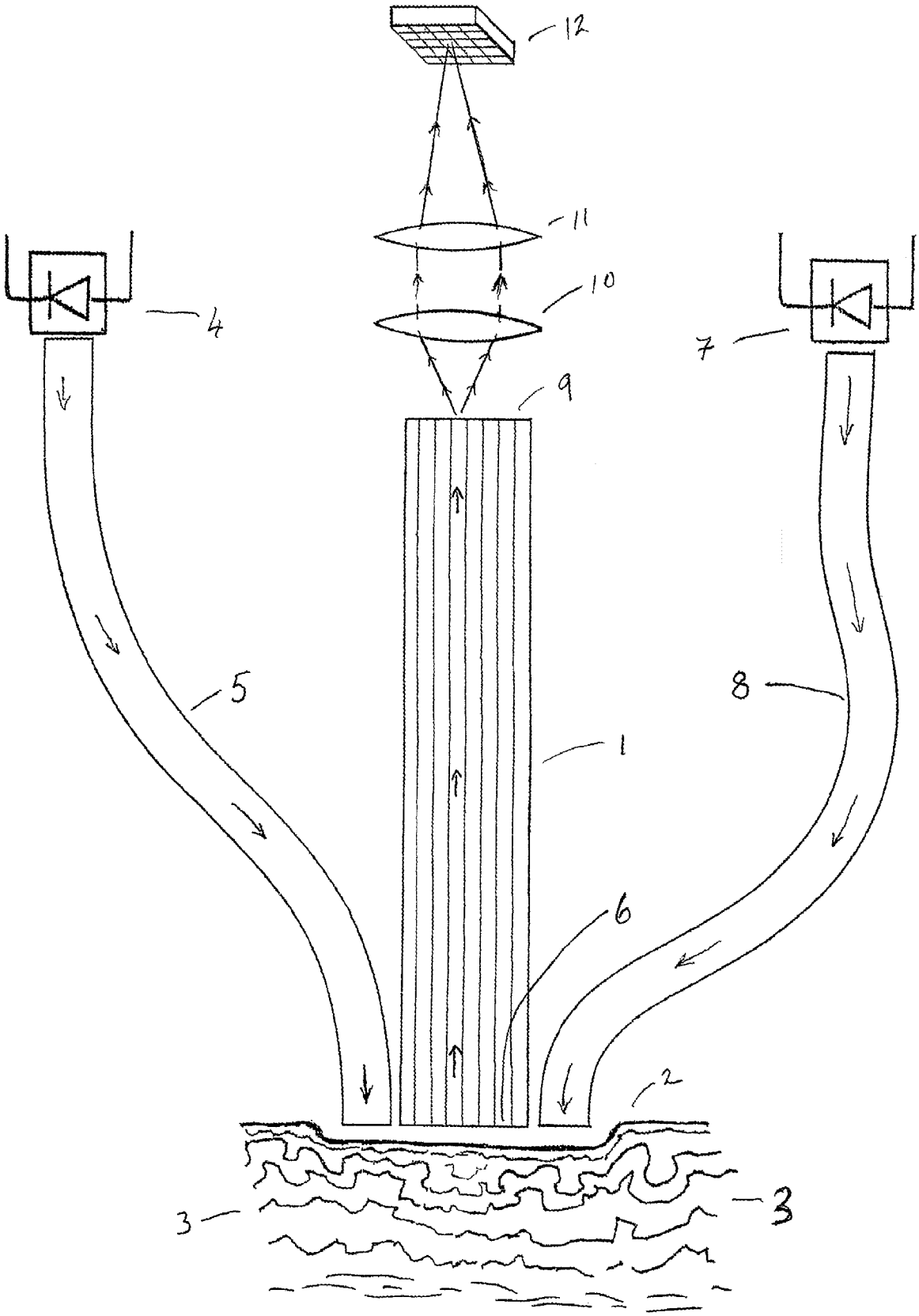


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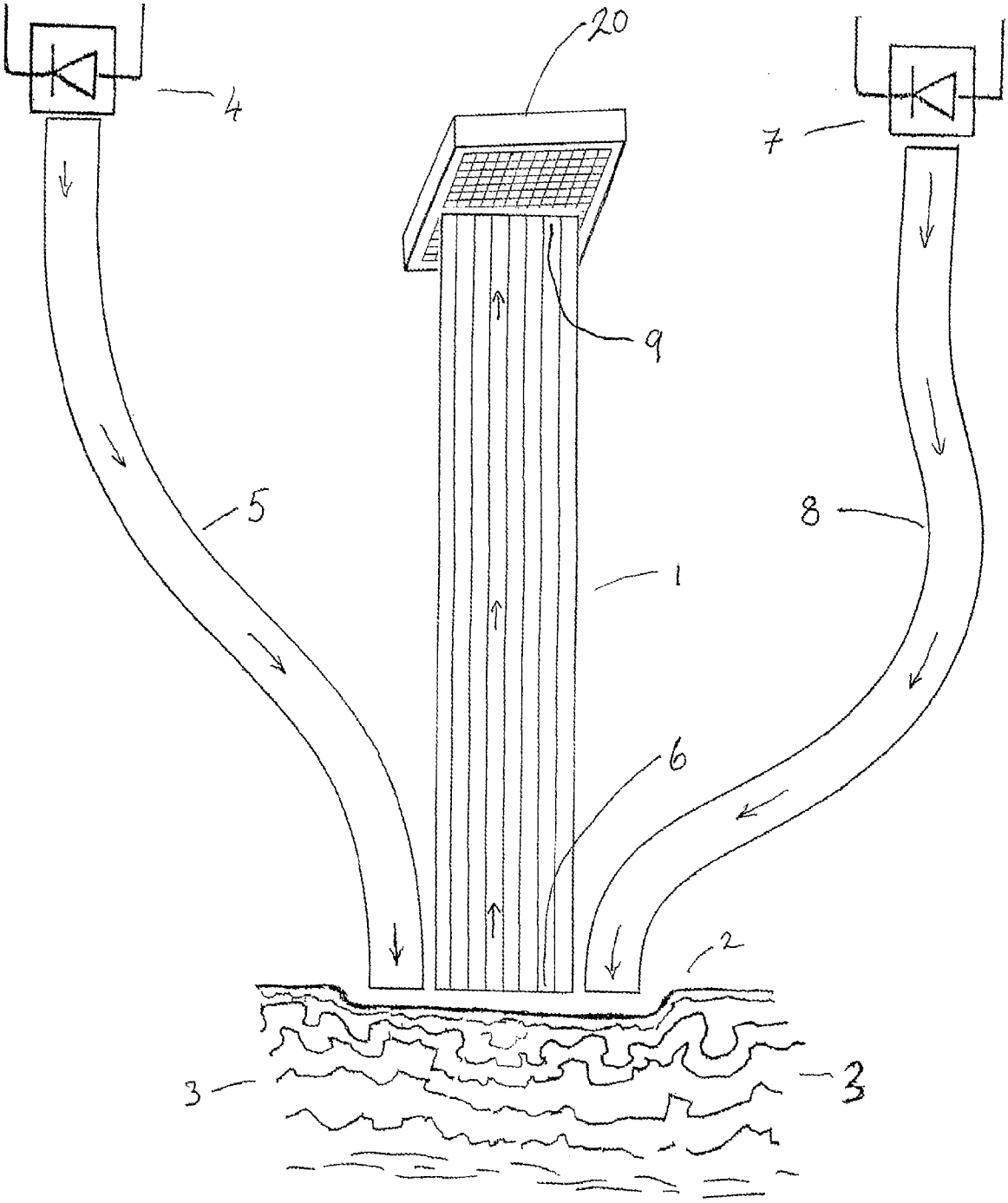


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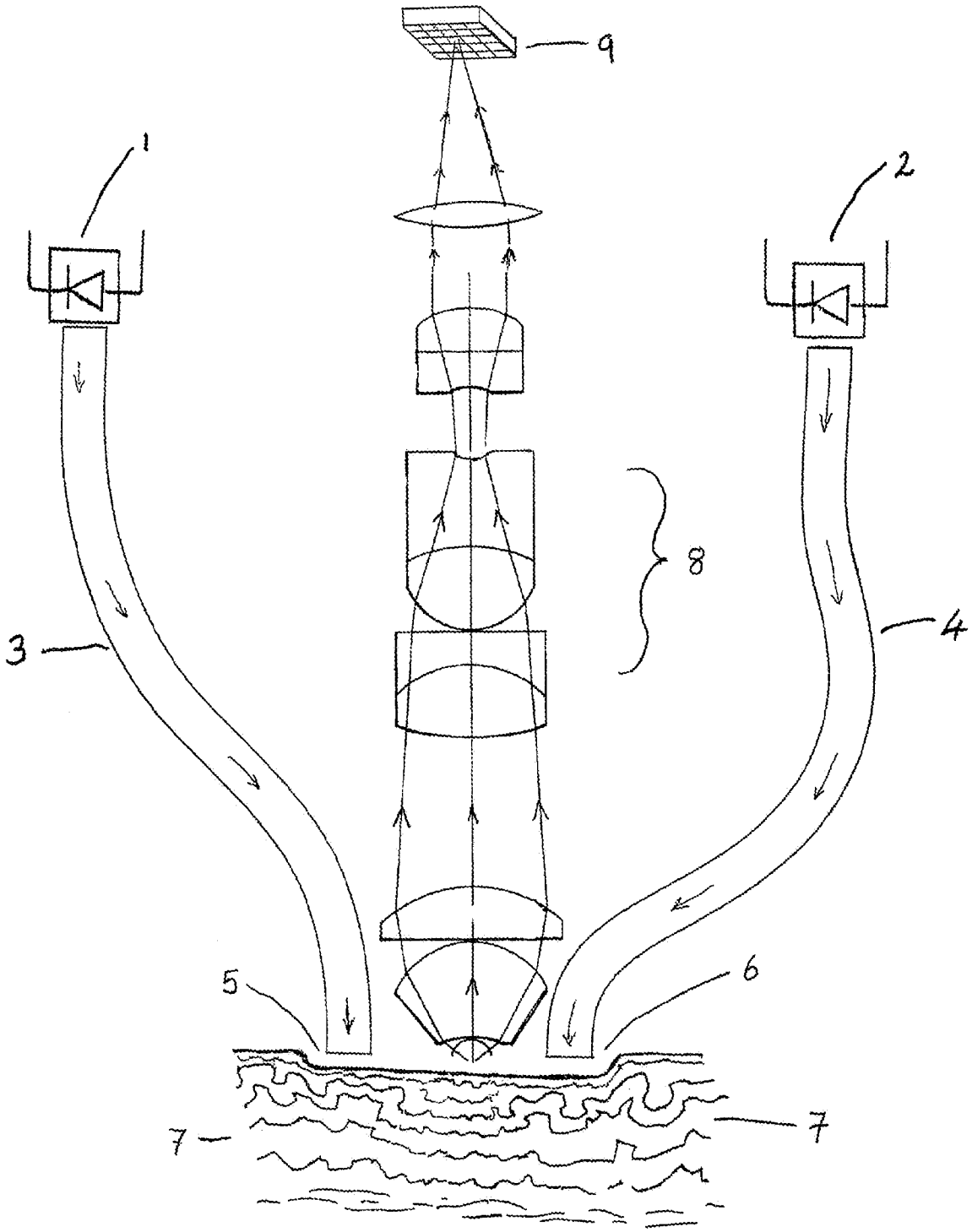


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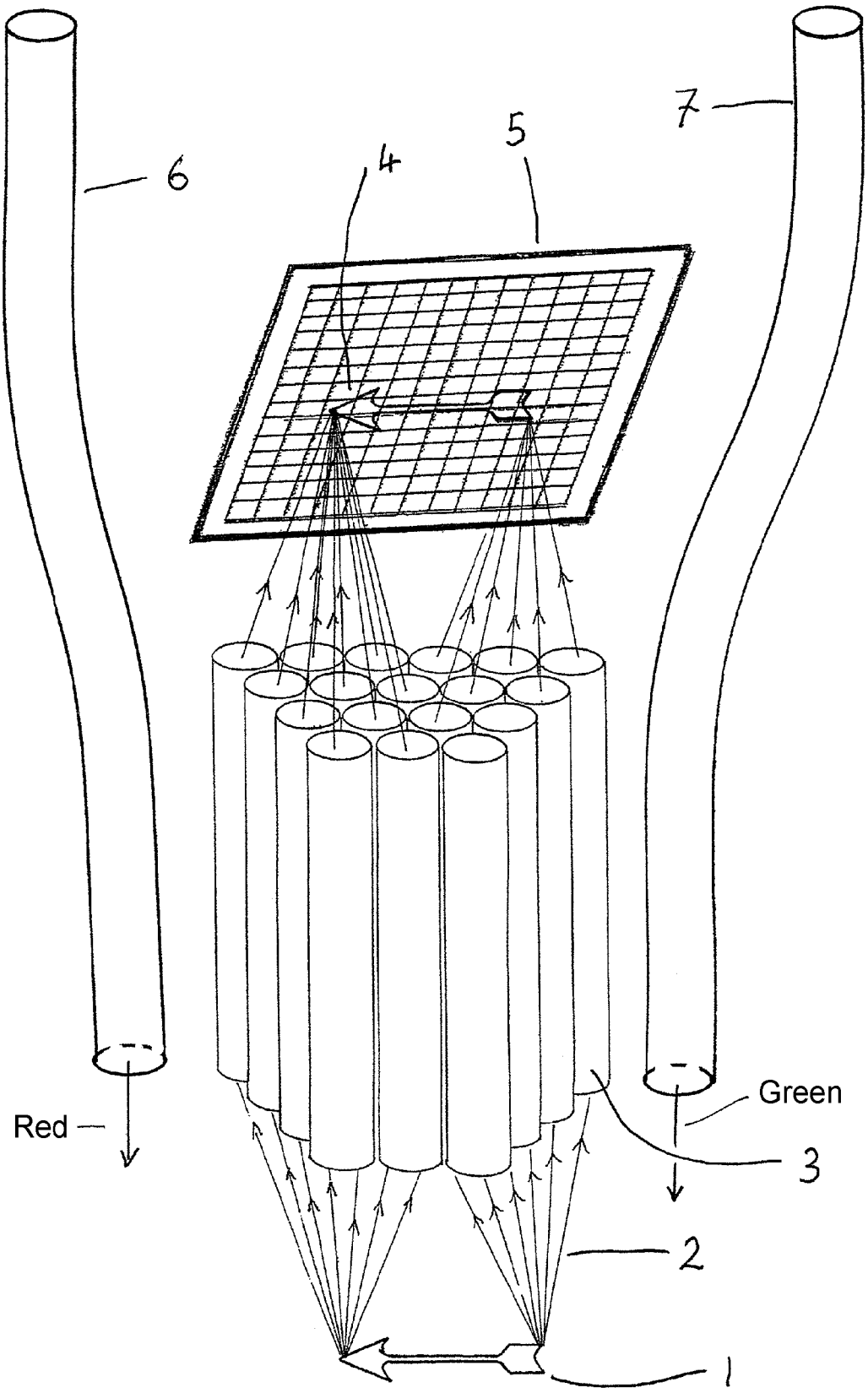


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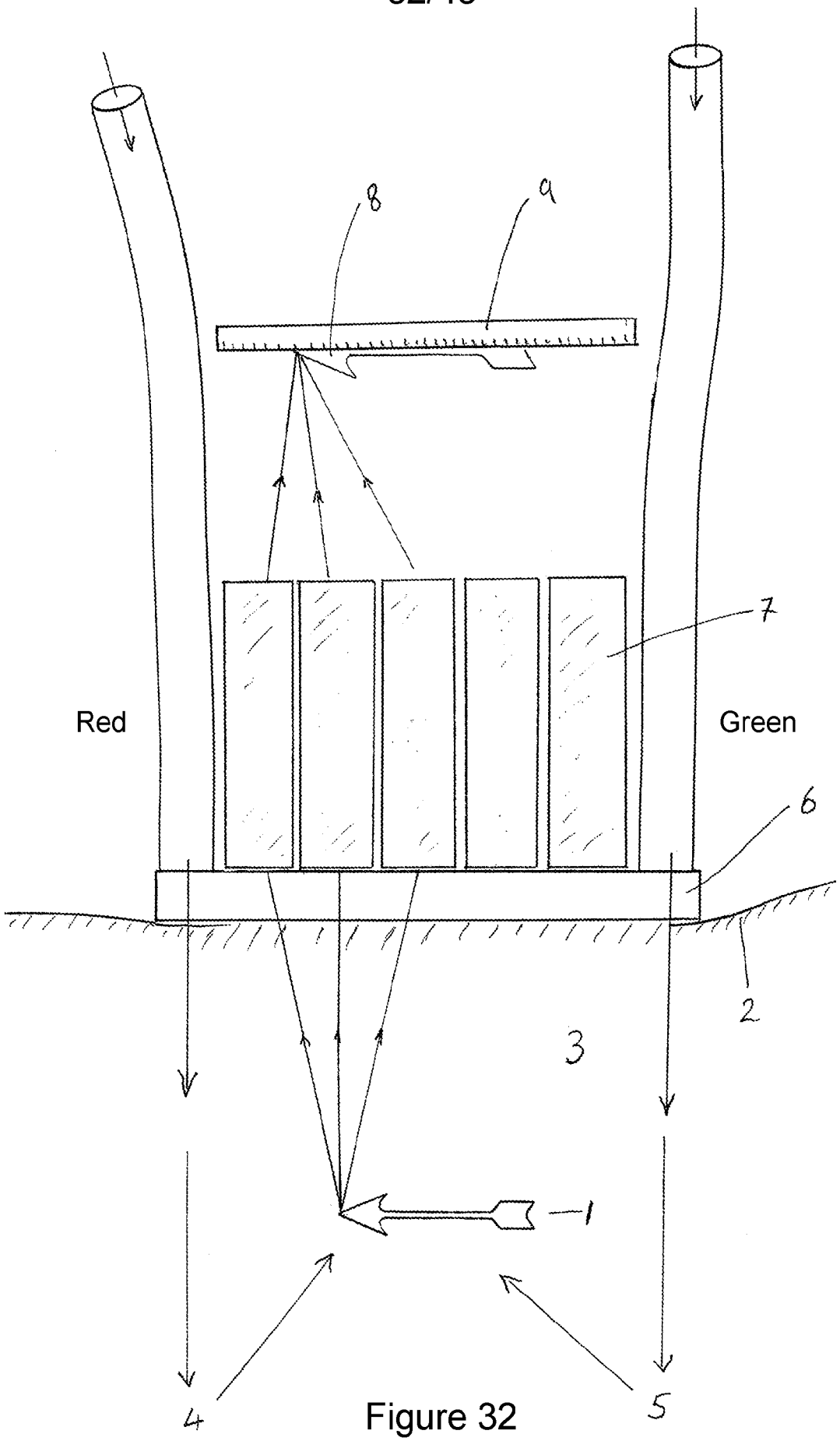


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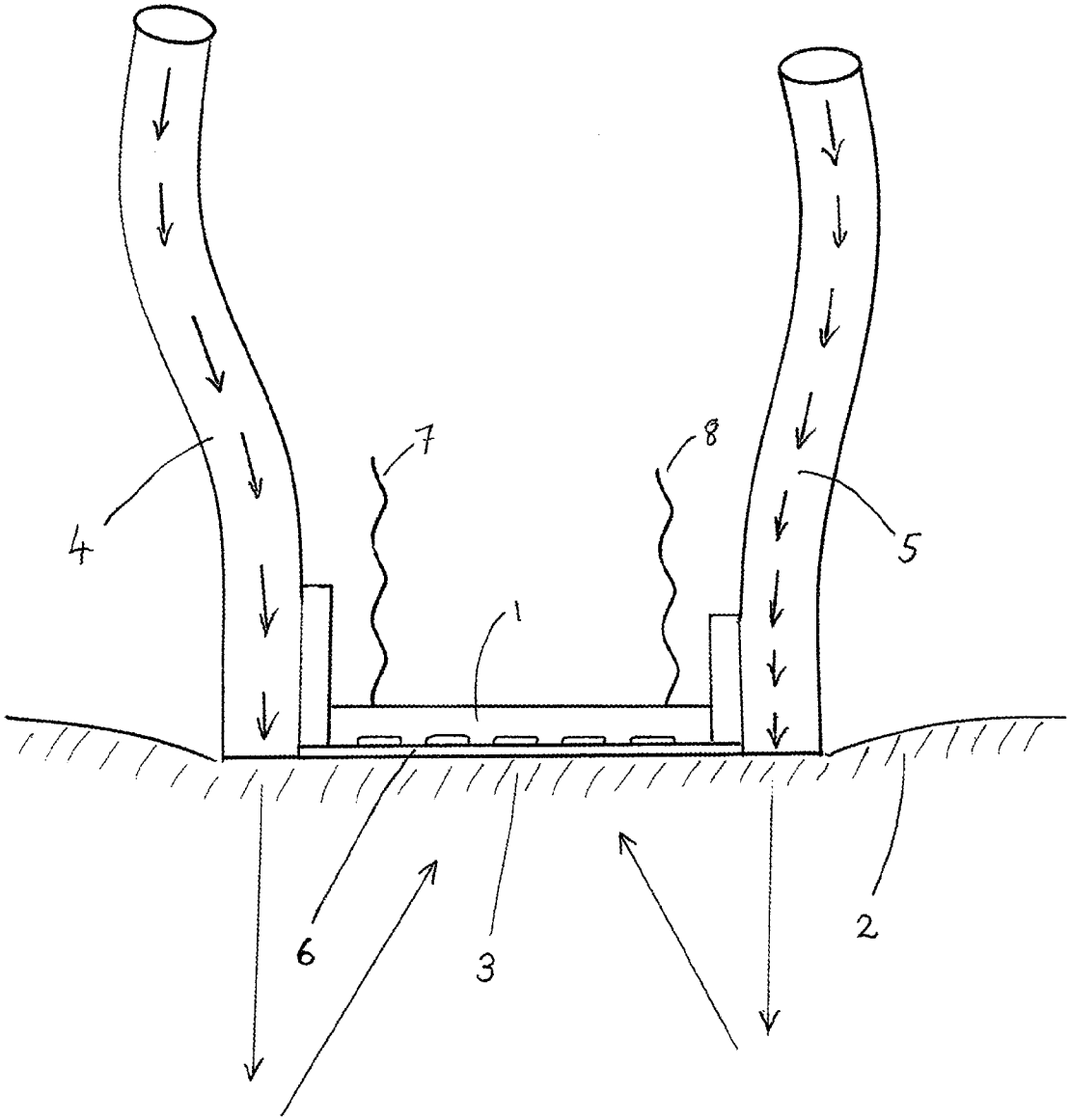


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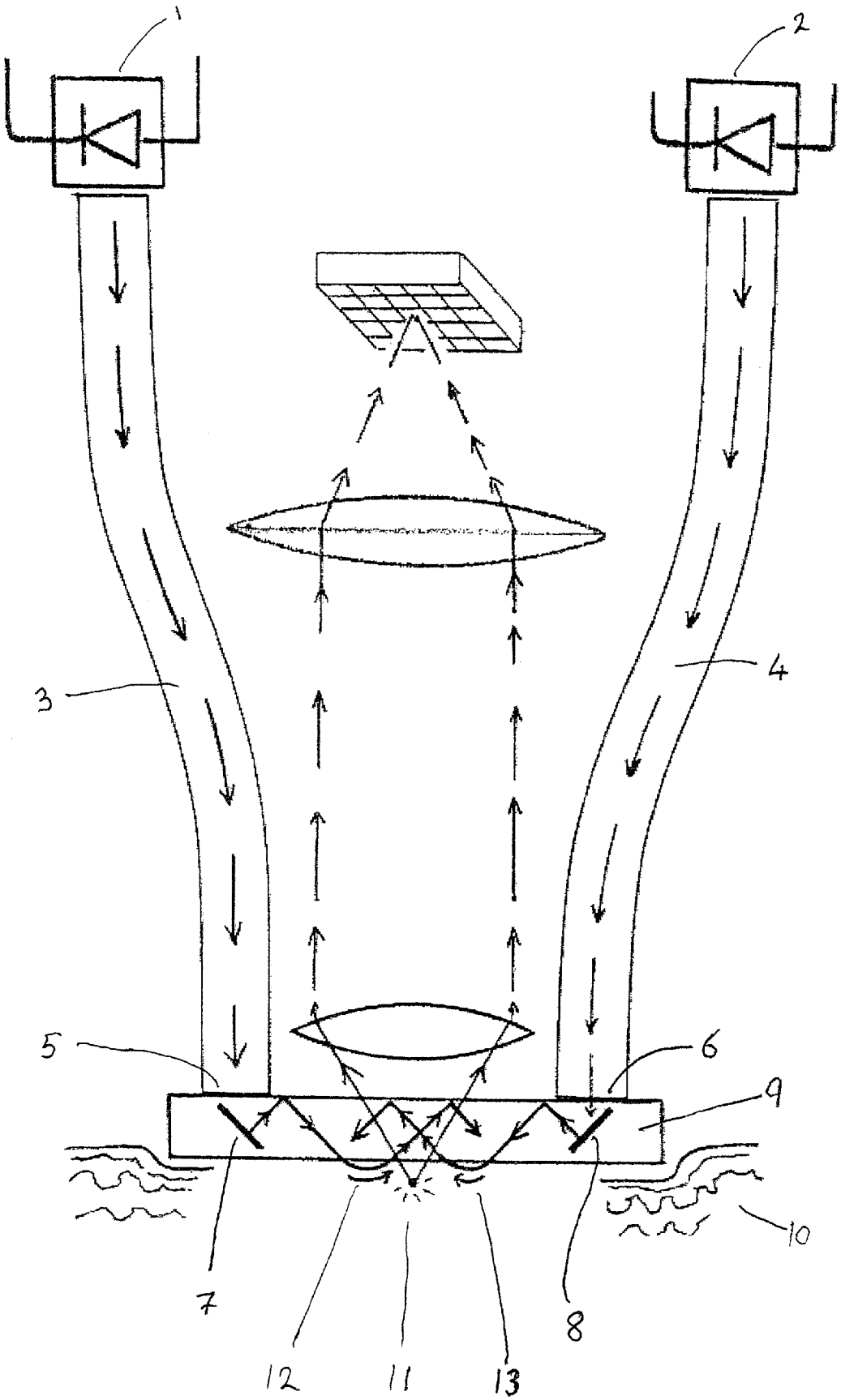


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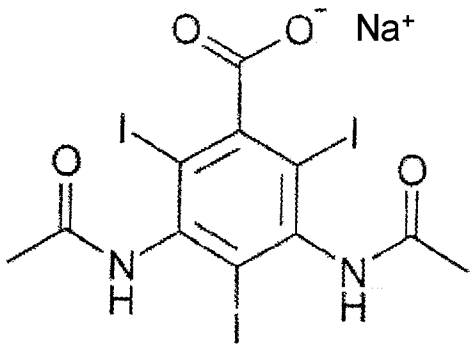


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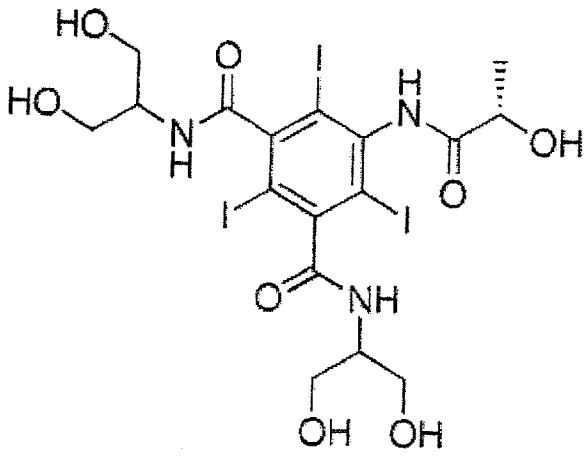


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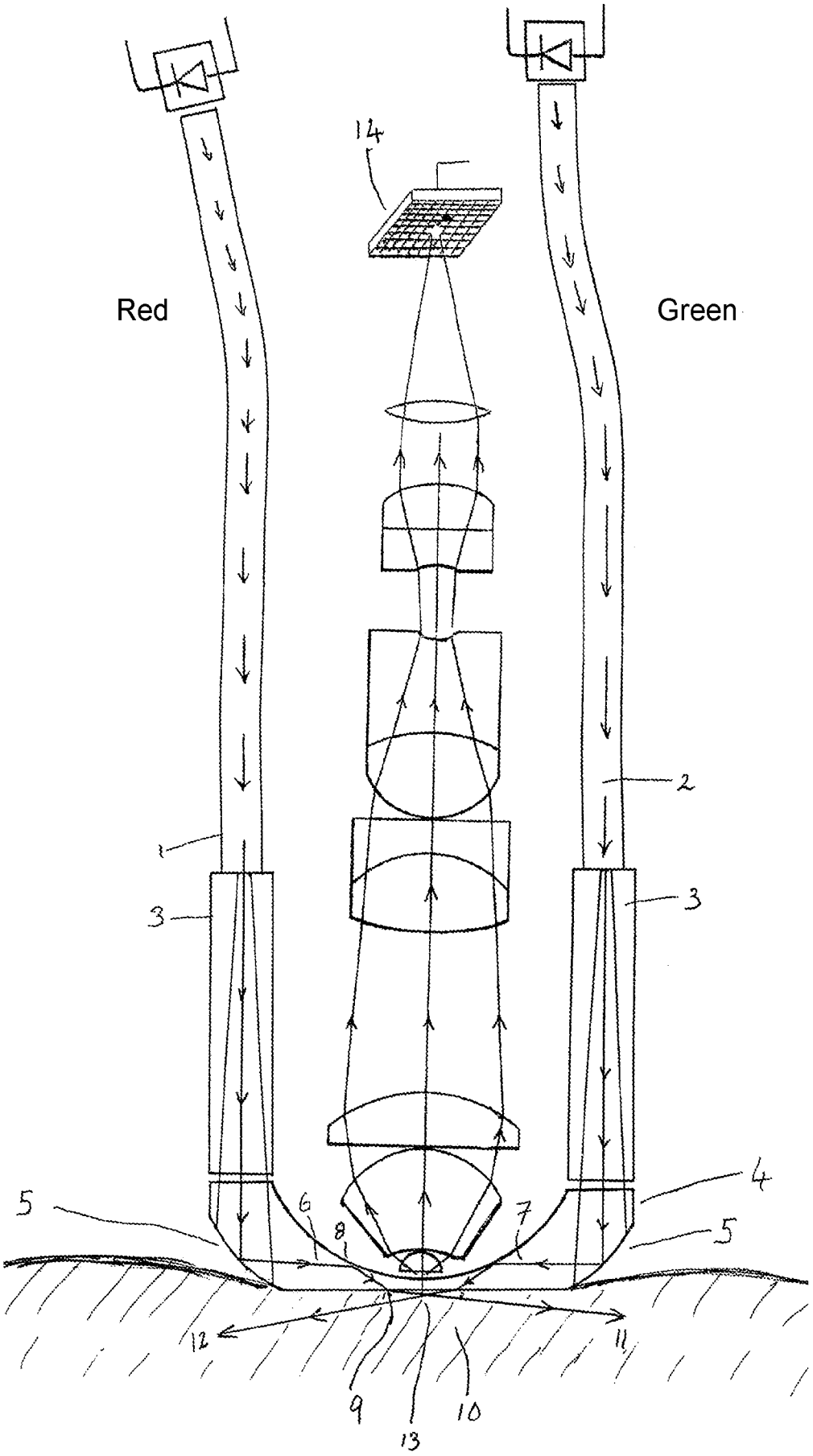


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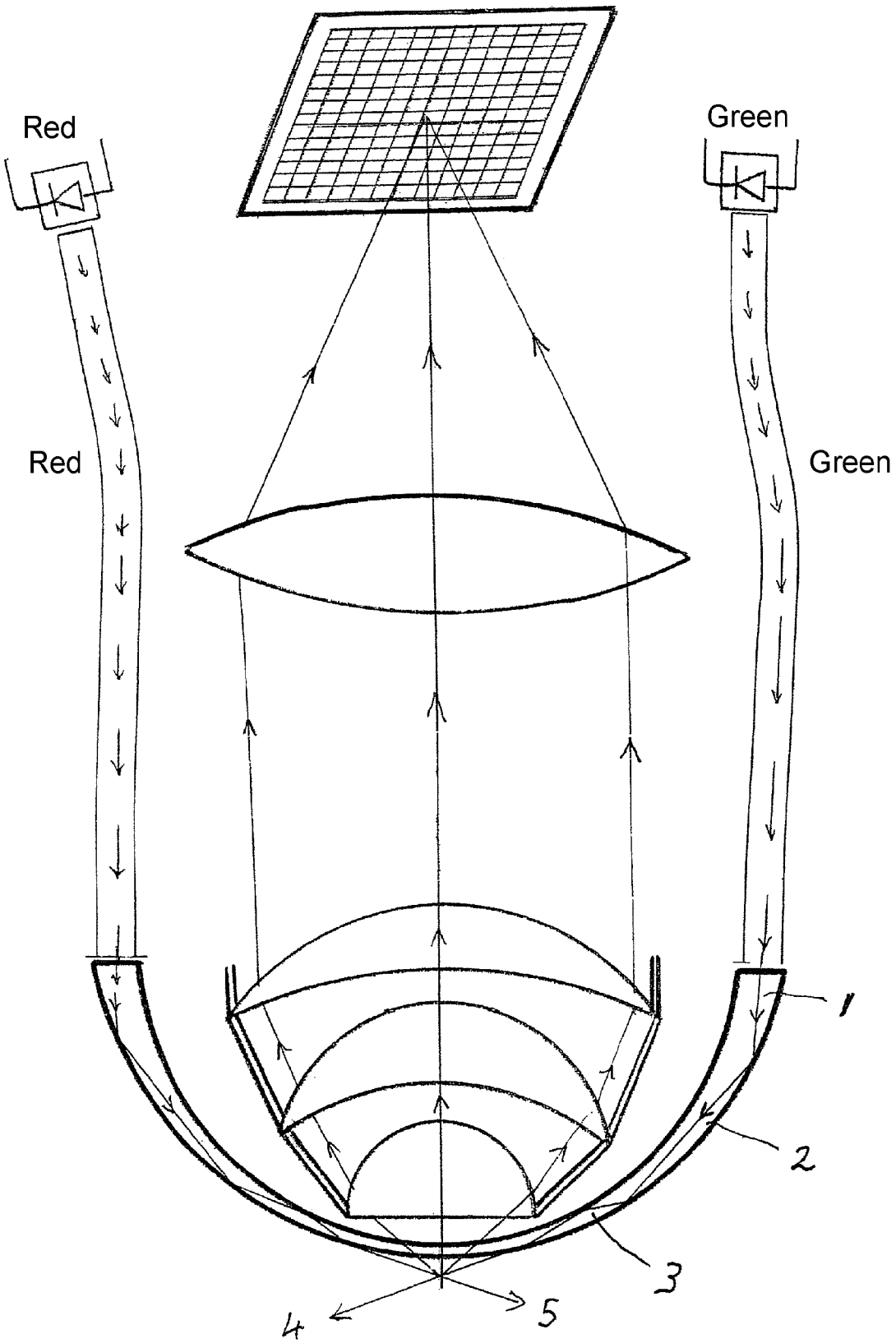


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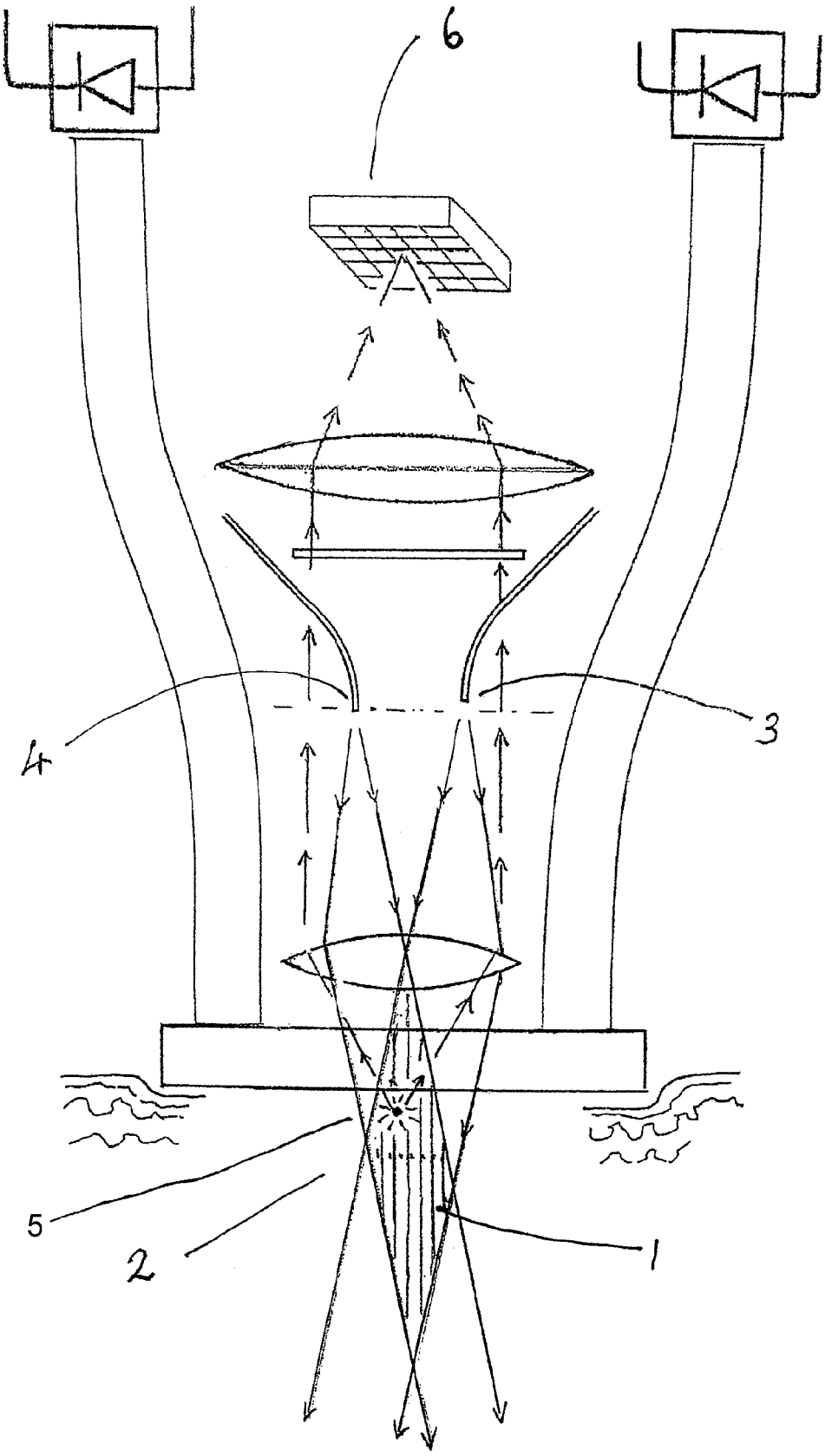


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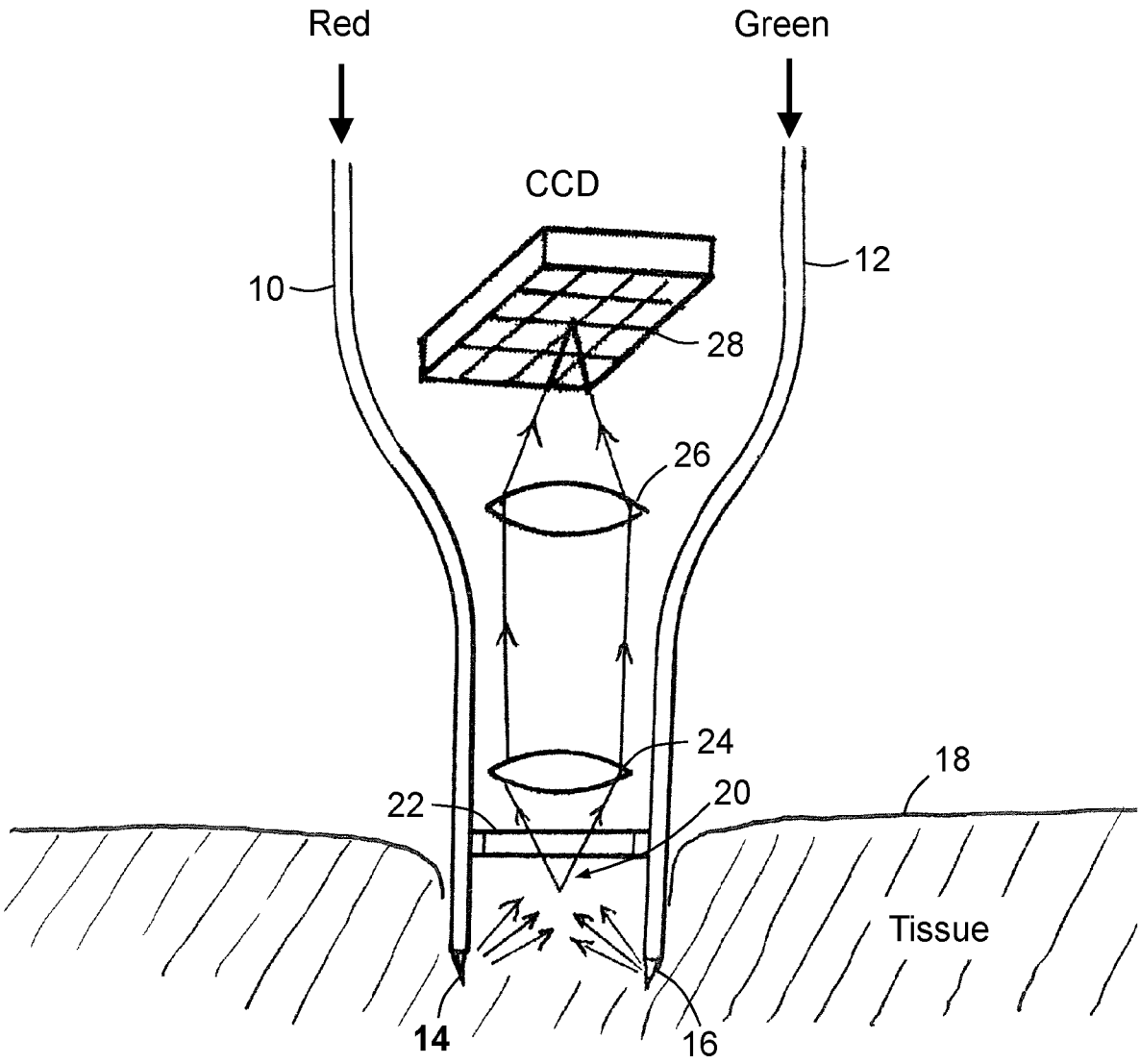


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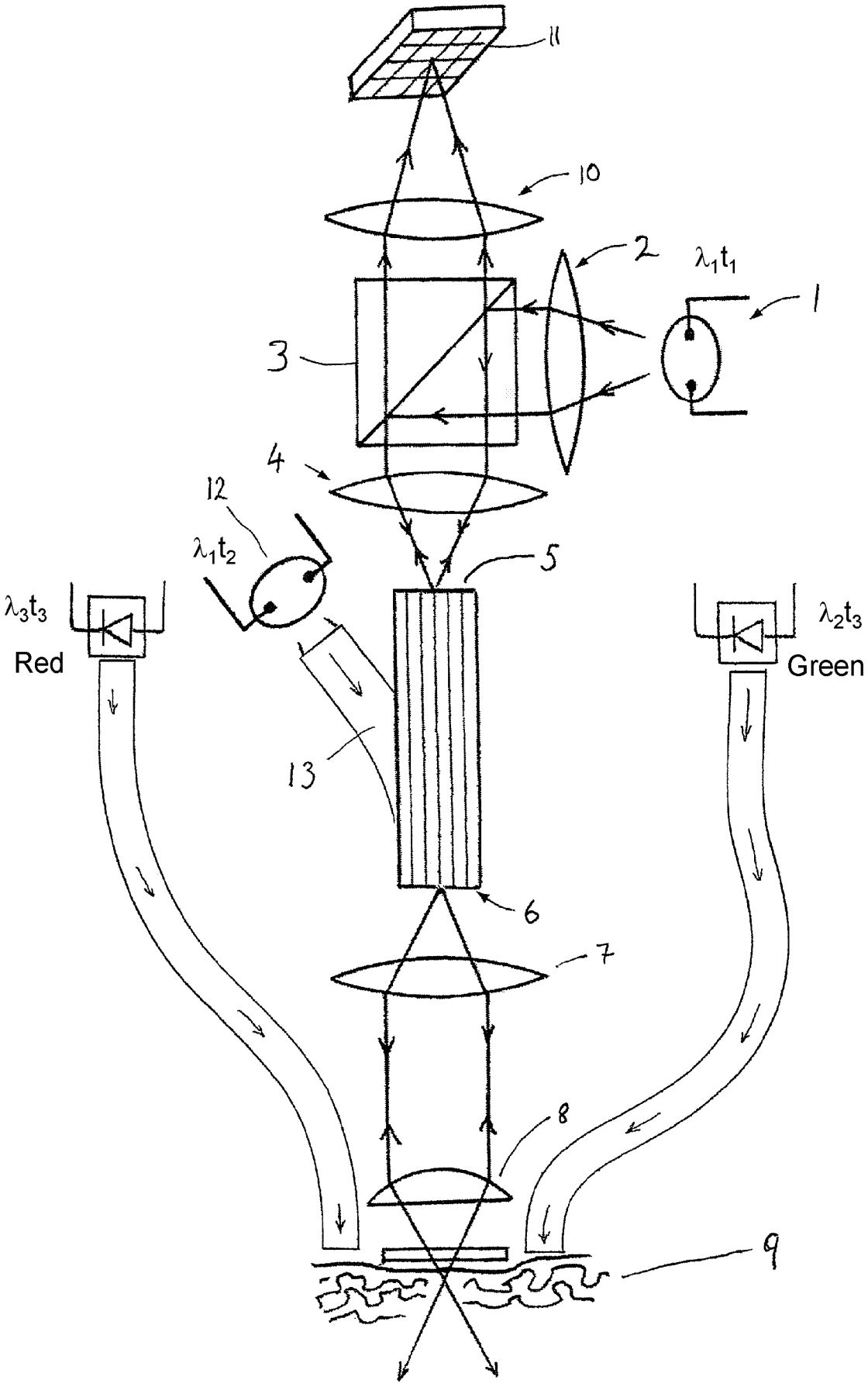

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Figure 40

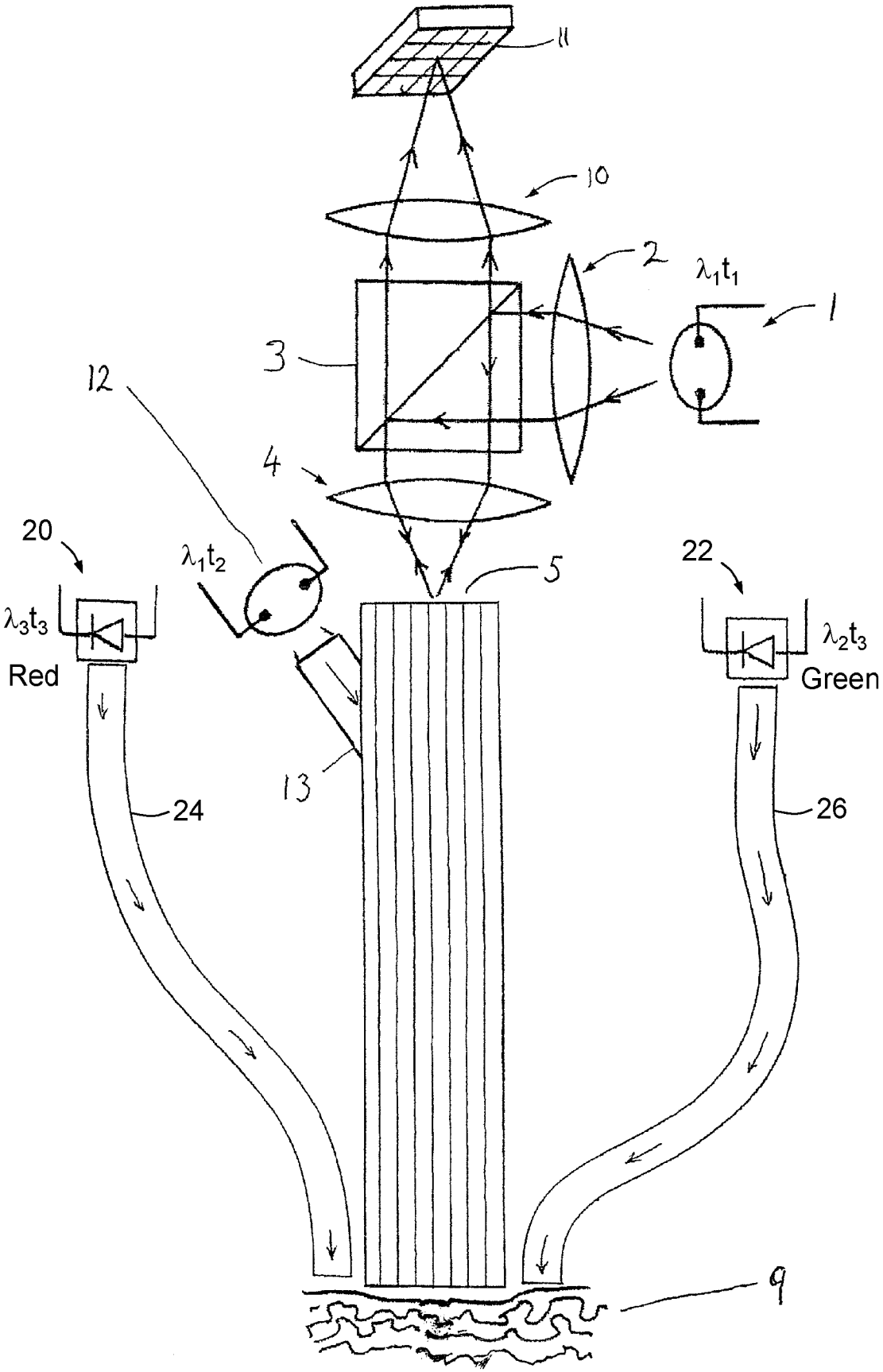

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Green
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Figure 41

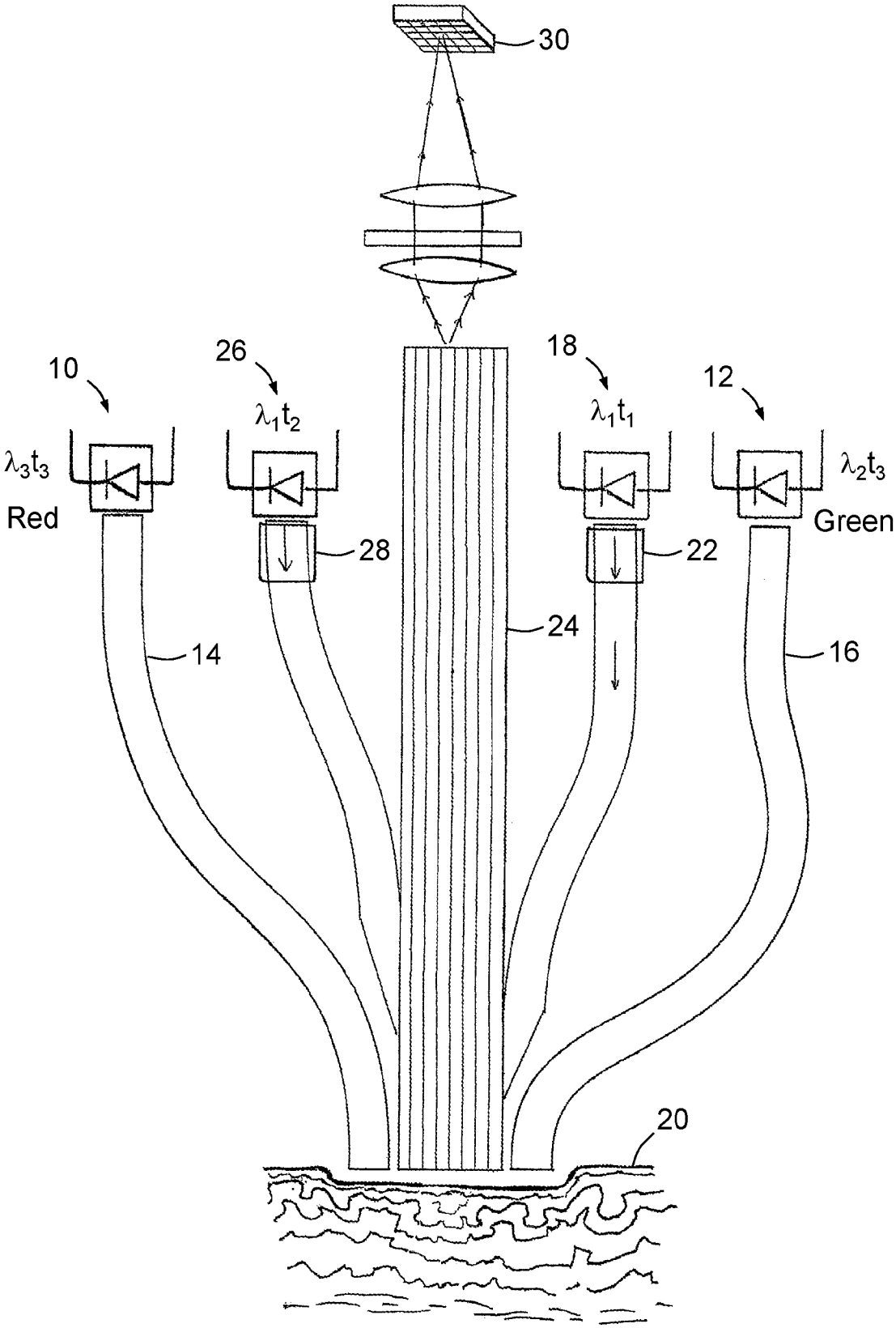

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Figure 42

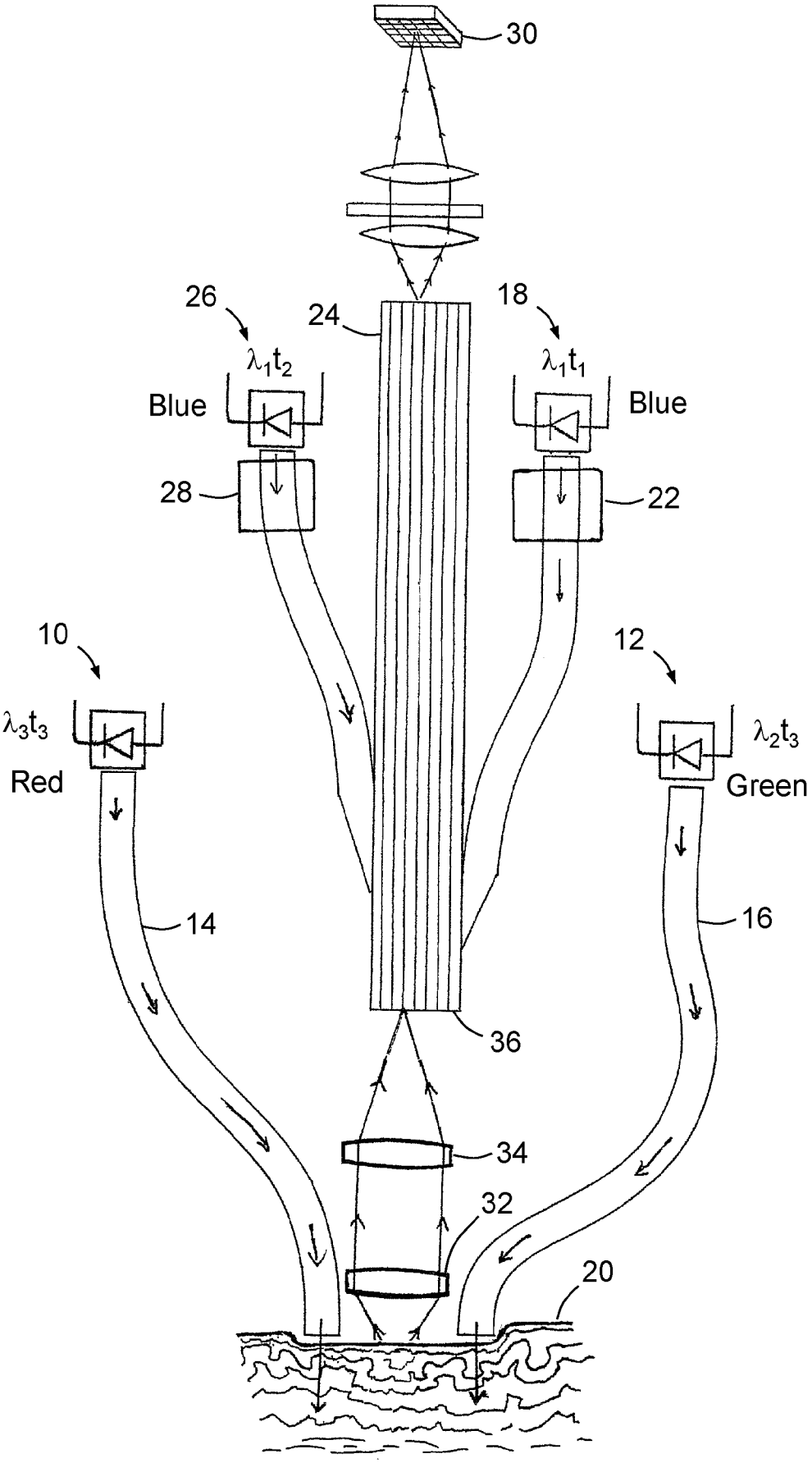

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Blue
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Green
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Figure 43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2013/000741

A. CLASSIFICATION OF SUBJECT MATTER

G02B 21/00 (2006.01) G01N 21/01 (2006.01) G01N 21/64 (2006.01) G02B 21/06 (2006.01) A61B 1/06 (2006.01)

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)

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)

WPI, EPODOC, INSPEC and TXTE
keywords: microscope, endoscope, light, illuminate, second, differentiate, phase gradient, fluorescence, oblique and similar terms
IPC marks: G01N21/64
Google Patents, Espacenet and Patent Lens keywords: microscope, endomicroscope, endoscope, light, LED, multiple, pattern, subtract, phase contrast, fluorescence, oblique and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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Date of the actual completion of the international search	Date of mailing of the international search report
23 August 2013	23 August 2013

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
Email address: pct@ipaustralia.gov.au
Facsimile No.: +61 2 6283 7999

Authorised officer

Susan Bellm
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. 0262832751

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2013/000741**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6650357 B1 (RICHARDSON) 18 November 2003 abstract; figure 2; col 3, lines 26-52; col 9, 5-67; col 10, lines 3-16; col 21, lines 61-67; col 22, lines 1-9	1-22
A	WO 2011/072175 A2 (DOUGHERTY et al.) 16 June 2011 page 1, lines 8-21	5, 6, 16, 17
A	US 2012/0069344 A1 (LUI) 22 March 2012 [0006]	5, 6, 16, 17
P,X	FORD, T. N., et al., 'Phase-gradient microscopy in thick tissue with oblique back-illumination", Nature Methods, 2012, Vol 9, pages 1195-1199. (Published online 28 October 2012) abstract, figure 1, pages 1195 to 1196	1, 6-12, 18-22

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2013/000741**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 6650357 B1	18 Nov 2003	CA 2322800 A1	15 Oct 1998
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End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.