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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

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(54) Title: POLARIZATION CONTROL SYSTEMS

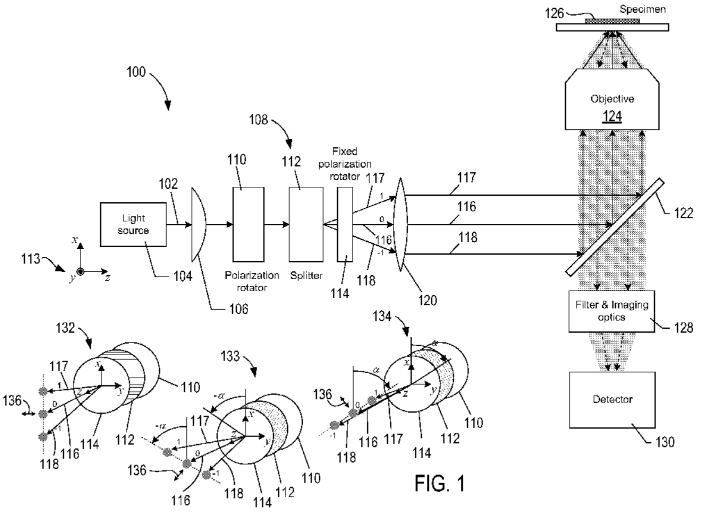

FIG. 1

(57) Abstract: Polarization rotation controls that can be incorporated in structured illumination microscopy instruments to rotate an interference fringe pattern through a desired angle of rotation and rotate the polarization through substantially the same rotation angle are described. In one aspect, a polarization rotation control includes at least one polarization rotator and a splitter. A beam of polarized coherent light is transmitted through one of the at least one polarization rotator and the splitter. The splitter splits the beam into at least three coherent beams of light that are focus by an objective lens of the microscopy instrument to form an interference fringe pattern, and the splitter is rotated to rotate the interference pattern through a desired rotation angle. The at least one polarization rotator rotates the polarization of the light through substantially the same rotation angle.

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POLARIZATION CONTROL SYSTEMS

CROSS-REFERENCE TO A RELATED APPLICATION

This application claims the benefit of Provisional Application No. 61/500,958; filed June 24, 2011.

TECHNICAL FIELD

This disclosure relates to structured illumination microscopy.

BACKGROUND

Three-dimensional structured illumination microscopy ("3D-SIM") achieves a factor of two improvement in lateral and axial resolution when compared to conventional wide-field fluorescence microscopes used in cell biology. 3D-SIM requires no specialized fluorescent dyes or proteins, unlike certain competing super-resolution techniques. Biologists achieve high resolution with 3D-SIM, but retain convenient and familiar fluorescence labeling techniques. Multiple images of the subject are made with a shifting and rotating illumination pattern. Higher resolution is achieved by solving a system of equations to restore the fine spatial detail normally blurred by diffraction.

A currently-available commercial 3D-SIM instrument uses a linearly polarized laser beam that is split into three or more beams by a binary phase grating. Each beam corresponds to a different diffraction order with most of the optical power concentrated in the first three diffraction orders (0^{th} and $\pm 1^{st}$, respectively). The 0^{th} and $\pm 1^{st}$ order beams are focused onto the back focal plane of the microscope objective and combined to form a three-dimensional interference fringe pattern in the sample volume. 3D-SIM image data are acquired by taking a fluorescence image excited by the fringe pattern. These images are used to recover a 3D optically sectioned image with approximately double the resolution obtained by conventional wide-field microscopy. In order to generate a high-contrast interference pattern, the polarization of the beam is also rotated to substantially match the rotation angle of the interference pattern. Typically the polarization is rotated to match the rotation angle of the grating by using an unpolarized source from which the polarization is selected with a polarizing plate or by using a polarized source and then rotating the polarization. However, systems that currently rotate the polarization along with the rotation of the grating are costly and add significant complexity to the instrument. For these reasons, engineers, scientists, and microscope manufacturers continue to seek lower cost, less complex systems and methods for rotating the polarization to substantially match the rotation angle of the interference pattern.

SUMMARY

Polarization rotation controls that can be incorporated in structured illumination microscopy instruments to rotate an interference fringe pattern through a desired angle of rotation and rotate the polarization through substantially the same rotation angle are described. In one aspect, a polarization rotation control includes at least one polarization rotator and a splitter. A beam of polarized coherent light is transmitted through one of the at least one polarization rotator and the splitter. The splitter splits the beam into at least three coherent beams of light that are focused by an objective lens of the microscopy instrument to form an interference fringe pattern, and the splitter is rotated to rotate the interference pattern through a desired rotation angle. The at least one polarization rotator rotates the polarization of the light through substantially the same rotation angle.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic representation of an example three-dimensional structured illumination microscopy instrument.

Figures 2A-2C show a representation of a three-dimensional structured-illumination pattern using three coherent beams.

Figures 3A-3E show bright lines of an interference pattern stepped through five periods of a spatial interval.

Figures 4A-4C show top views of an objective lens and three angular positions of an interference pattern.

Figures 5A-5B show an example representation of two polarization states associated with orthogonal polarized excitation beams.

Figure 6 shows an isometric view of an example polarization rotator.

Figure 7 shows an isometric view of an example implementation of a polarization rotator control.

Figures 8A-8C show use of the polarization rotator control shown in Figure 7.

Figure 9 shows an isometric view of an example polarization rotator control.

DETAILED DESCRIPTION

Various polarization rotation controls ("PRCs") are described along with a general description of three-dimensional structured illumination microscopy ("3D-SIM"). 3D-SIM achieves a factor of two improvement in lateral and axial resolution compared to conventional wide-field fluorescence microscopes used in cell biology. 3D-SIM requires no specialized fluorescent dyes or proteins, unlike certain competing super-resolution techniques. Biologists achieve high resolution with 3D-SIM, but retain convenient and familiar fluorescence labeling techniques. The illumination phase control provides for capturing multiple images of the subject by a shifting and rotating illumination pattern. Higher resolution can be achieved by solving a system of equations to restore the fine spatial detail normally blurred by diffraction.

Figure 1 shows a schematic representation of an example 3D-SIM instrument 100. There are many different types of SIM instruments and corresponding optical paths. Instrument 100 is not intended to represent the optical paths within all the different, well-known variations of instruments used in SIM microscopy, but is instead intended to illustrate the general principals of a SIM that includes a PRC. A high-intensity, substantially monochromatic beam 102 of coherent light is output from a light source 104. The light source 104 can be a laser that emits a polarized, high-irradiance, substantially monochromatic beam of excitation light selected to stimulate emission of fluorescent light from fluorophores of fluorescent probes that are designed to bind to particular components of a specimen 126. The beam 102 is transmitted through a lens or a series of lenses 106 that collimate the beam 102. The beam 102 then passes through a PRC 108 composed of a polarization rotator 110 and a fixed polarization rotator 114. As shown in Figure 1, a splitter 112 is located downstream of, and in close proximity to, the polarization rotater 110. Alternatively, the splitter 112 can be located upstream of, and in close proximity to, the polarization rotater 110. Figure 1 includes a Cartesian coordinate system 113 with the *z*-axis directed parallel to the direction light propagates along between the source 104 and a dichroic mirror 122. The splitter 112 can be a one-dimensional, transmissive diffraction grating that splits the beam into three divergent, co-planar (i.e., *xz*-plane) coherent beams 116-118 referred to as the 0^{th}, $+1^{st}$, and -1^{st} order diffracted beams, respectively. The splitter 112 can be any one of a variety of different types of transmissive gratings. For example, the splitter 112 can be a one-dimensional transmissive grating composed of a transparent plate of glass or plastic with a series of substantially parallel grooves formed in one surface of the grating or the splitter 112 can be an opaque plate with a series of substantially parallel thin slits. Alternatively, the splitter 112 can be two or more beamsplitters arranged to split the beam into three or more separate coherent beams. The three beams 116-118 pass through a lens or a series of lenses 120 which reorient the beams 116-118 so that the beams lie in the *xz*-plane and the $+1^{st}$ and -1^{st} order diffracted beams 117 and 118 are nearly parallel to the 0^{th} order diffracted beam 116. The beams 116-118 are reflected off of the dichroic mirror 122 to enter an objective lens 124. In the example of Figure 1, the beams 116-118 are focused within the specimen 126 so that the beams interfere with one another to generate a high-contrast, three-dimensional structured-illumination, interference-fringe pattern within a volume of the specimen 126.

Figures 2A-2C show generation of a three-dimensional structured-illumination, interference-fringe pattern using three coherent beams. As shown in Figure 2A, the beams 116-118 are transmitted into the back of the objective lens 124. Figures 2A-2C include a Cartesian coordinate system 200 with the *z*-axis directed parallel to the direction the beams 116-118 propagate along into the objective lens 124. Because the beams 116-118 originate from a coherent light source 102, the beams 116-118 have plane waves in which the phases of component waves of the beams are identical across any plane, such as plane 202, normal to the beam direction. While the beams 116-118 are coherent, each beam may have a different phase displacement than the other two beams. The objective lens 124 focuses the incident beams to a focal point 204, which changes the direction of the two non-axial beams 117 and 118, as shown in Figure 2A. As a result, the three plane waves are no longer parallel with wave vectors having different directions and the three sets of plane waves intersect to form a three-dimensional, interference-fringe pattern of bright lines, due to constructive interference, surrounded by dark regions, due to destructive interference. In other words, as shown in the example of Figure 2B, a stationary interference pattern 206 is located in the focal plane of the objective lens 124. Lines 208 represent bright lines of excitation light separated by darker regions. The lattice of bright lines of the excitation light comprise the interference pattern 206 and cause fluorescent emission of light from fluorophores in the specimen 126 (not shown). Figure 2C shows a side view of the objective lens 124 and an end-on view of the bright lines comprising the three-dimensional interference pattern 206. Open circles 210 represent an end-on view of the bright lines of excitation light separated by darker regions 212. Each bright line excites fluorescence of fluorophores attached to components of the specimen 126 that intersect the bright line. Fluorophores attached to components of the specimen 126 that are located in the dark regions 212 between the bright lines 210 do not fluoresce.

Returning to Figure 1, the objective lens 124 captures and directs a portion of the fluorescent light emitted from the fluorophores to the dichroic mirror 122. The fluorescent light passes through the dichroic mirror 122 to filter and imaging optics 128, which filters stray excitation light and focuses the fluorescent light onto a sensor array of a detector 130. For example, the detector 130 can be a photodetector array, CCD camera, or a CMOS camera.

In 3D-SIM, the orientation of the interference pattern 206 can be obtained by physically rotating the splitter 112. The polarization of the beams 116-118 is also rotated to match the orientation of the splitter 112 in order to achieve maximum contrast between bright lines and dark regions of the interference pattern. In the example of Figure 1, the splitter 112 and polarization rotator 110 can be disposed on a rotatable stage that is connected to a motor (not shown) that physically rotates the splitter 112 and polarization rotator 110 through a desired angle about the central 0th order diffracted beam 116 axis (i.e., *z*-axis). Figure 1 also includes isometric views 132-134 of the PRC 108 and the splitter 112 to illustrate three different rotational orientations for the beams

116-118. In views 132-134, directional arrow 136 represents the electric field component, or polarization direction, of the beams 116-118. In view 132, the splitter 112 and polarization rotator 110 are oriented so that the beams 116-118 lie within the xz-plane and the polarization associated with each beam is at 0° with respect to the y-axis (i.e., lies in the yz-plane). In view 133, the splitter 112 and polarization rotator 110 are rotated through an angle $-\alpha$ about the central 0^{th} order diffracted beam axis (i.e., z-axis). As a result, the beams 117 and 118 are rotated through the angle $-\alpha$ about the 0^{th} order diffracted beam 116 axis with respect to the x-axis, and the polarizing rotator 110 and fixed polarization rotator 114 in combination select the polarization of the beams 116-118 to substantially match the angle $-\alpha$ with respect to the y-axis. In view 134, the splitter 112 and polarization rotator 110 are rotated through an angle $+\alpha$ about the central 0^{th} order beam 116 axis (i.e., z-axis). As a result, the beams 117 and 118 are rotated through the angle $+\alpha$ about the 0^{th} order diffracted beam axis with respect to the x-axis, and the polarizing rotator 110 and fixed polarization rotator 114 in combination select the polarization of the beams 116-118 to substantially match the angle $+\alpha$ with respect to the y-axis.

Using a 3D interference fringe pattern to acquire images of a region of a specimen are now described with reference to Figures 3 and 4. 3D-SIM image data is acquired by taking a fluorescence image excited by the interference pattern, moving the interference pattern by $1/n^{th}$ of a period perpendicular to the optical axis of the objective lens 124, followed by taking another image, and repeating these steps for a total of n images. The splitter 112 can be translated in the x-direction, shown in Figure 1, to step the interference pattern perpendicular to the optical axis of the objective lens by $1/n^{th}$ of a period. Figures 3A-3E show an example of stepping the interference pattern 206 through five periods of a spatial interval centered about the optical axis 302 of the objective lens 124. In the example of Figures 3A-3E, the bright lines of the interference pattern 206 are directed substantially perpendicular to the z-axis, as described above with reference to Figure 2. Marks labeled 1-5 in Figures 3A-3E, represent five periods of a spatial interval centered about the optical axis 302. Dotted line 304 identifies the center of the interference pattern 206. Figures 3A-3E represent five discrete steps in which the interference pattern 206 is translated substantially perpendicular to the optical axis 302.

For example, in Figure 3A, the interference pattern 206 is in the first period denoted by "1," and in Figure 3B, the interference pattern 206 is stepped to the second period denoted by "2." At each of the five periods represented in Figures 3A-3E, a fluorescence image excited by the interference pattern 206 is captured. The final step rewinds to reset the interference pattern for the next cycle.

The interference pattern 206 is then rotated in the xy-plane, and the n-step image process described above with reference to Figure 3 is repeated, followed by another rotation and the capture of another n images, for a total of $3n$ images. After the $3n$ images are obtained, the sample is stepped to a new focal plane in the z-direction and another set of $3n$ images is obtained. The interference pattern is rotated through a particular rotation angle by rotating the splitter through the same angle. Figures 4A-4C show top views (i.e., xy-plane views) of the objective lens 124 and the interference pattern 206. In Figure 4A, the bright lines of the interference pattern 206 are initially angled at $-60°$ with respect to the y-axis. The interference pattern 206 is stepped through five periods of a spatial interval oriented substantially perpendicular to the bright lines, as described above with reference to Figure 3, with a fluorescent image captured at each step. In Figure 4B, the interference pattern 206 is rotated through 60° so that the bright lines are angled at 0° with respect to the y-axis. The interference pattern 206 is again stepped through five periods of a spatial interval oriented substantially perpendicular to the bright lines, as described above with reference to Figure 3, with a fluorescent image captured at each step. In Figure 4C, the interference pattern 206 is finally rotated through an additional 60° so that the bright lines are angled at 60° with respect to the y-axis. The interference pattern 206 is again stepped through five periods of a spatial interval oriented substantially perpendicular to the bright lines, as described above with reference to Figure 3, with a fluorescent image captured at each step. Figures 4A-4C represent the interference pattern 206 rotated 3 times in the xy-plane by 60°, with each rotation followed by the capture of 5 images for a total 15 images. These fluorescent images are used to solve a system of linear equations to recover a three-dimensional optically sectioned image with approximately double the resolution obtained by conventional wide-field microscopy.

The beam 102 can be output from the source 104 with any polarization. In particular, the beam 102 can be output with either p- or s-polarization. P- and s-polarization is determined relative to the propagation direction of the light through the instrument 100. The figures include a Cartesian coordinate system that is referenced to describe the relative orientation of the instrument 100 components, directions along with light travels through the instrument 100, and is now referenced to describe the polarization states of the beams 102 and 116-118. As shown in Figure 1, the z-axis represents the propagation direction of the beam 102 output from the light source 104 and the path the light propagates along through the PRC 108. Figures 5A-5B show an example representation of p- and s-polarization states for a beam of light traveling in the z-direction through the instrument 100 with respect the coordinate system 113 shown in Figure 1. In Figure 5A, the polarization state of a beam is represented with an electric field component, $\vec{E}_x$, that oscillates in the xz-plane. The beam is referred to as p-polarized or having p-polarization, as represented by double-headed directional arrow 502 directed parallel to the x-axis. On the other hand, the polarization state of a beam represented in Figure 5B has an electric field component, $\vec{E}_y$, that oscillates in the yz-plane. The beam is referred to as s-polarized or having s-polarization, as represented by double-headed directional arrow 504 directed parallel to the y-axis.

As described above with reference to Figure 1, the PRC 108 is implemented with polarization rotators 110 and 114. Figure 6 shows an isometric view of an example polarization rotator 600 used to rotate linearly polarized light. The polarization rotator 600 is an optical device that rotates the polarization of a linearly polarized light by a selected angle. In the example of Figure 6, the rotator 600 has a fast axis represented by a line 602 oriented parallel to the y-axis and has a slow axis (not shown) oriented parallel to the x-axis. Directional arrow 604 represents linearly polarized light traveling in the z-direction with a linear polarization angle θ with respect to the fast axis. The polarization rotator 600 rotates the light through a total angle of 2θ and the light emerges from the polarization rotator 600 traveling in the z-direction, but with a polarization angle of $-\theta$ with respect to the fast axis. In other words, the incident light is oscillating in the first and third quadrants of the xy-plane and the light emerges from the polarization rotator is oscillating in the second and fourth quadrants of the xy-

plane. When linearly polarized light is incident with a polarization angle of $\theta = 0°$, the polarization of the light is not rotated.

The polarization rotators 110 and 114 of the PRC 108 can be implemented with half-wave plates. Figure 7 shows an isometric view of an example implementation of a PRC 700. The PRC 700 is composed of a half-wave plate 702 and a fixed-position half-wave plate 706. The half-wave plate 702 is rotated on the same stage as a 1D grating 704 and using the same motorized mechanical means. The half-wave plates 702 and 706 each create a $\lambda/2$ retardance in a beam of light traveling along the z-axis, where λ is the wavelength of the light. With reference to Figure 1, the grating 704 corresponds to the splitter 110, the plate 702 corresponds to the polarization rotator 110, and the plate 706 corresponds to the fixed-position polarization rotator 114. In the example of Figure 7, the plates 702 and 706 both have corresponding fast axes 708 and 710 that are parallel to the y-axis. The lines or grooves comprising the 1D line pattern of the grating 704 are also oriented substantially parallel to the y-axis.

Figures 8A-8C show how use of the PRC 700 is used to rotate three diffracted beams with rotation angles 0°, 60° and -60° and rotate the polarization to substantially match the rotation angles. This is done using a single rotation mechanical means with both the waveplate 702 and grating 704 rotated through the same angle of rotation using the same motorized rotational mechanical means. In the example of Figures 8A-8C, the fast axes of the plates 702 and 706 and the grating 704 are arranged so that an incident beam 802 traveling along the z-axis with s-polarization is split into three diffracted 0 and ±1 beams 804-806. In Figure 8A, the fast axes 708 and 710 of the plates 702 and 706, respectively, are parallel to the y-axes and lines or grooves of the 1D grating 704 are also parallel to the y-axis. Because the beam 802 is s-polarized, the polarization of the beam 802 is parallel to the fast axis of the half-wave plate 702. As a result, the beam 802 passes through the plate 702 with no change in polarization. The grating 704 splits the beam 802 into the beams 804-806 which lie within the xz-plane. The beams 804-806 have s-polarization and because the fast axis of the plate 706 is also parallel to the y-axis, the beam 804-806 pass through the plate 706 with s-polarization preserved. As a result, the orientation of the half-wave plate 702 and grating 704 shown in Figure 8A corresponds to an interference pattern with a 0° rotation with respect to y-

axis and a matching 0° rotation in the polarization with respect to the y-axis. In Figure 8B, the stage (not shown) upon which the plate 702 and grating 704 are disposed in rotated about the z-axis through an angle of 60° with respect to the y-axis. As the beam 802 passes through the plate 702, the polarization of the beam 802 is rotated from 0° (i.e., s-polarization) to 120° with respect to the y-axis. The grating 704 splits the beam 802 into the beams 804-806 which lie within a plane rotated to 60° with respect to the x-axis. As the beams 804-806 emerge from the grating 704, the beams 804-806 have 120° polarization with respect to the y-axis. As the beams 804-806 pass through the fixed half-wave plate 706, the polarization of the beams 804-806 is rotated to 60° with respect to the y-axis. As a result, the orientation of the half-wave plate 702 and the grating 704 shown in Figure 8B produces an interference pattern with a 60° rotation with respect to the y-axis and a matching 60° rotation in the polarization with respect to the y-axis. In Figure 8C, the stage (not shown) upon which the plate 702 and grating 704 are disposed is rotated about the z-axis through an angle of -60° with respect to the y-axis. As the beam 802 passes through the plate 702, the polarization of the beam 802 is rotated from 0° (i.e., s-polarization) to -120° with respect to the y-axis. The grating 704 splits the beam 802 into the beams 804-806 which lie within a plane rotated to -60° with respect to the x-axis. As the beams 804-806 emerge from the grating 704, the beams 804-806 have -120° polarization with respect to the y-axis. As the beams 804-806 pass through the fixed half-wave plate 706, the polarization of the beams 804-806 is rotated to -60° with respect to the y-axis. As a result, the orientation of the half-wave plate 702 and the grating 704 shown in Figure 8C produces an interference pattern with a -60° rotation and a matching -60° rotation in the polarization.

In alternative embodiments, the polarization rotators can be double Fresnel rhombs. Figure 9 shows an isometric view of an example PRC 900. The PRC 900 is similar to the PRC 700 except the half-wave plate 702 has been replaced by a double Fresnel rhomb 902, also referred to as a half-wave retarder. The double rhomb 902 operates in the same manner as the plate 702 by creating a $\lambda/2$ retardance, but over a wider range of wavelengths than is typically available for birefringent waveplates. The rhomb 902 is rotated on the same stage as the grating 704, as described above with reference to Figure 8. The rhomb 902 can produce a $\lambda/2$ retardance for λ in the wavelength range of about 400nm to about 1500nm. The rhomb 902 is shaped so that light entering small face 904 is internally reflected four times: once from each of the four sloped faces before exiting through the other small face 906. The angle of internal reflection is the same for each internal reflection, and each reflection produces an approximately 45° ($\pi/4$ radians) phase delay between the s- and p- polarization components of the light with the p-polarization experiencing the larger phase delay. In still other embodiments, the fixed half-wave plate 706 can also be replaced by a fixed position double Fresnel rhomb.

The foregoing description, for purposes of explanation, used specific nomenclature to provide a thorough understanding of the disclosure. However, it will be apparent to one skilled in the art that the specific details are not required in order to practice the systems and methods described herein. The foregoing descriptions of specific examples are presented for purposes of illustration and description. They are not intended to be exhaustive of or to limit this disclosure to the precise forms described. Obviously, many modifications and variations are possible in view of the above teachings. The examples are shown and described in order to best explain the principles of this disclosure and practical applications, to thereby enable others skilled in the art to best utilize this disclosure and various examples with various modifications as are suited to the particular use contemplated. It is intended that the scope of this disclosure be defined by the following claims and their equivalents:

CLAIMS

1. A microscopy instrument comprising:

a light source to produce a polarized coherent beam of light;

a polarization rotation control to split the beam into at least three coherent beams of light; and

an objective lens to receive the at least three beams and focus the beams to form a three-dimensional, interference fringe pattern, wherein the control is to rotate the interference fringe pattern through a desired angle of rotation by rotating the beams through the same angle of rotation and to rotate the polarization of the interference fringe pattern through the same angle of rotation.

2. The instrument of claim 1, wherein the light source further comprises a laser to output a beam of excitation light.

3. The instrument of claim 1, wherein the polarization rotation control comprises

a first polarization rotator to receive the polarized beam of light output from the source and to rotate the polarization of the beam through twice the desired angle of rotation;

a splitter to split the beam into the at least three coherent beams; and

a second polarization rotator to receive the at least three beams and rotate the polarization back to desired angle of rotation.

4. The instrument of any of the above claims, wherein the first polarization rotator is a half-wave plate and the second polarization rotator is a half-wave plate.

5. The instrument of any of the above claims, wherein the first polarization rotator is a double Fresnel rhomb and the second polarization rotator is a double Fresnel rhomb.

6. The instrument of any of the above claims, wherein the first and second polarization rotators are a combination of a half-wave plate and double Fresnel rhomb.

7. The instrument of claim 1, wherein the splitter comprises a one-dimensional grating.

8. The instrument of claim 1, further comprising:

a dichroic mirror to reflect the at least three beams into the objective lens and to transmit collimated fluorescent light emitted from fluorescently labeled components of a specimen; and

a detector to receive the fluorescent light transmitted through the dichroic mirror.

9. A polarization rotation control to rotate beams of light through a desired angle of rotation and rotate the polarization through substantially the same angle of rotation, the control comprising:

a first polarization rotator to receive a polarized beam of light output from a light source and to rotate the polarization of the beam through twice a desired angle of rotation;

a splitter to split the beam into at least three beams; and

a second polarization rotator to receive the at least three beams and to rotate the polarization back to the desired angle of rotation.

10. The instrument of claim 10, wherein the first polarization rotator is a half-wave plate and the second polarization rotator is a half-wave plate.

11. The instrument of claim 10, wherein the first polarization rotator is a double Fresnel rhomb and the second polarization rotator is a double Fresnel rhomb.

12. The instrument of claim 10, wherein the first and second polarization rotators are a combination of a half-wave plate and double Fresnel rhomb.

13. The instrument of claim 10, wherein the splitter comprises a one-dimensional grating.

14. A method for rotating coherent polarized beams of light through a desired angle of rotation and rotating the polarization through substantially the same angle of rotation, the method comprising:

rotating the polarization of a polarized beam of light output from a light source through twice a desired angle of rotation using a first polarization rotator;

splitting the beam into at least three beams; and

rotating the at least three beams and the polarization associated with the at least three beams back to the desired angle of rotation using a second polarization rotator.

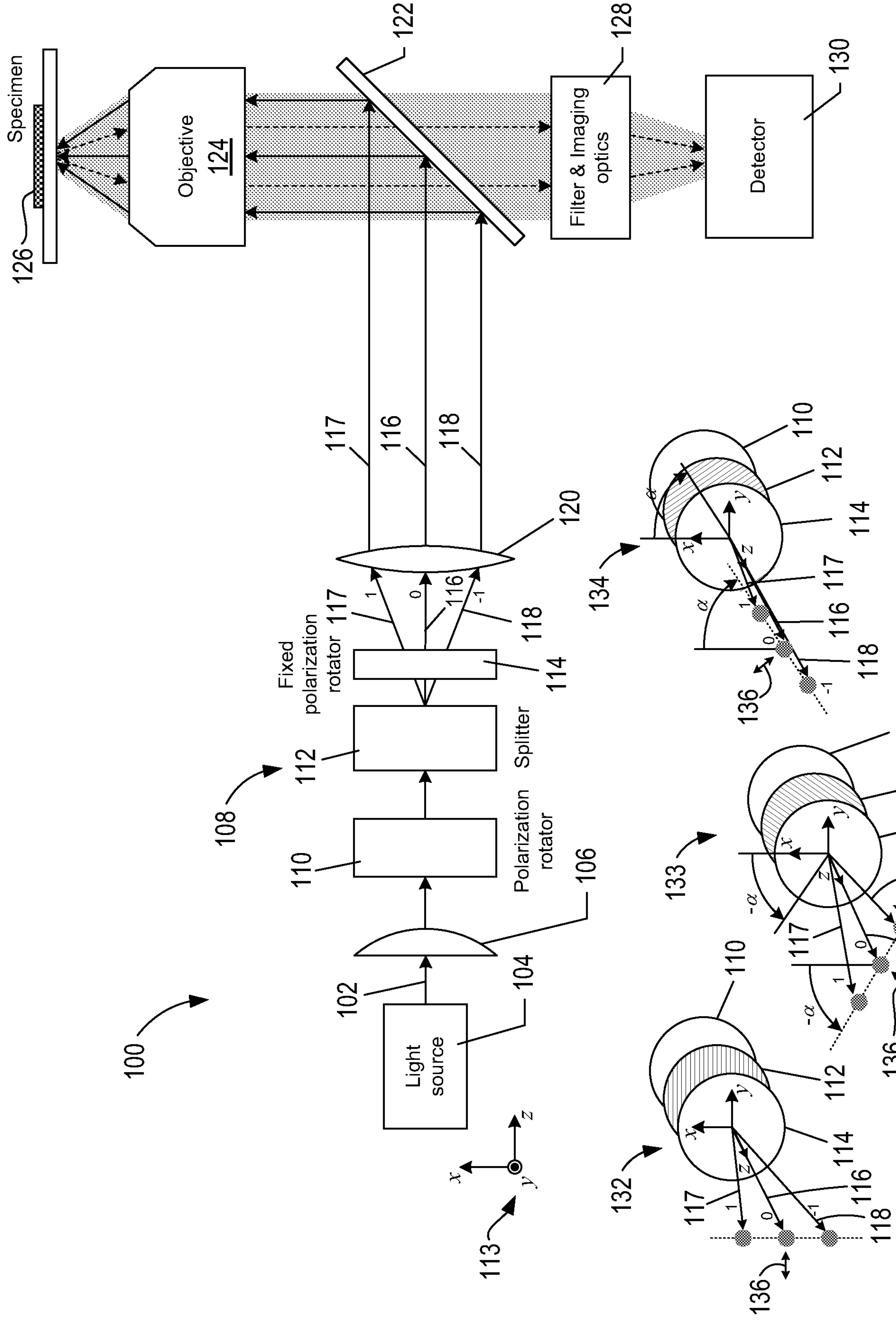
15. The method of claim 14, wherein rotating the polarization of the polarized beam output from the light source further comprises passing the beam through a half-wave plate positioned to rotate the polarization through twice the desired angle of rotation.

16. The method of claim 14, wherein rotating the polarization of the polarized beam output from the light source further comprises passing the beam through a double Fresnel rhomb positioned to rotate the polarization through twice the desired angle of rotation.

17. The method of claim 14, wherein splitting the beam into at least three beams further comprises passing the beam through a one-dimensional grating.

18. The method of claim 14, wherein rotating the at least three beams and the polarization associated with the at least three beams further comprises passing the beams through a half-wave plate to rotate the beams back to the desired angle of rotation.

19. The method of claim 14, wherein rotating the at least three beams and the polarization associated with the at least three beams further comprises passing the beams through a double Fresnel rhomb positioned to rotate the beams back to the desired angle of rotation.


Specimen
126
Objective
124
122
128
Filter & Imaging optics
130
Detector
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116
118
120
1
0
-1
Fixed polarization rotator
114
Splitter
112
108
110
Polarization rotator
106
102
104
Light source
100
x
y
z
113
132
133
134
136
α
-α

FIG. 1

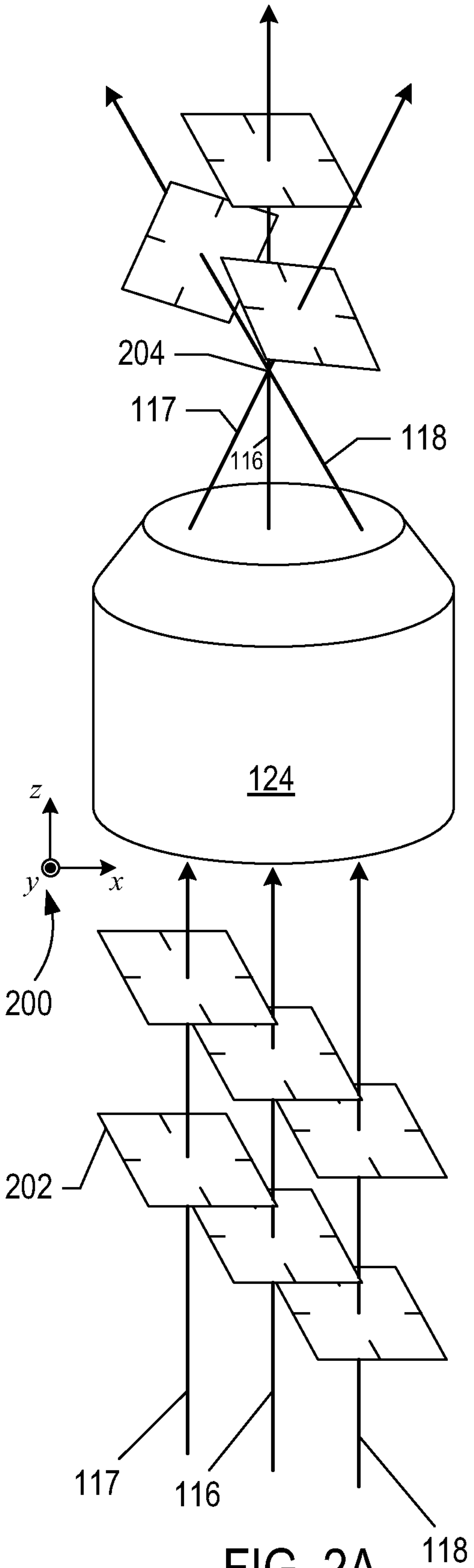

FIG. 2A

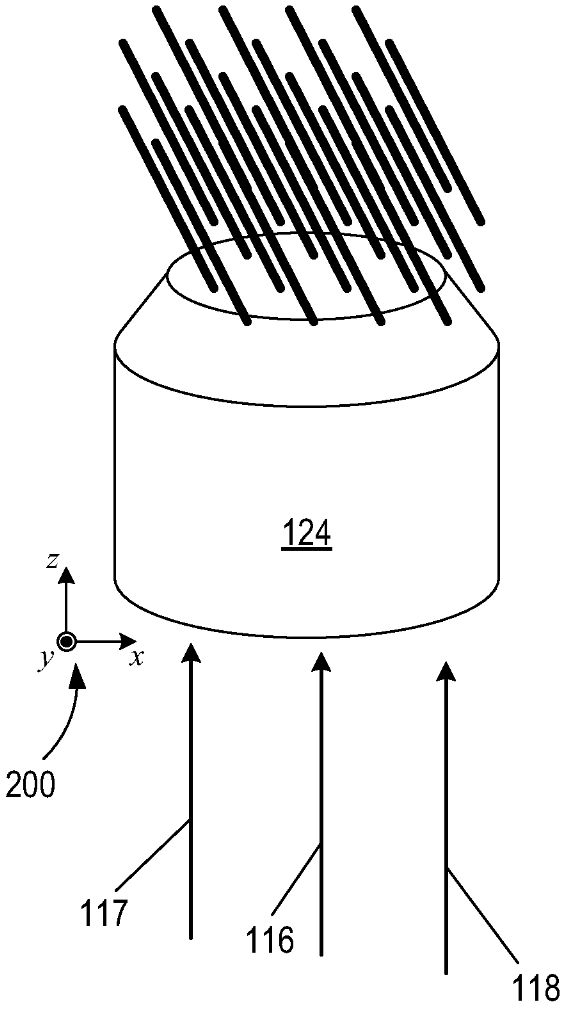

FIG. 2B

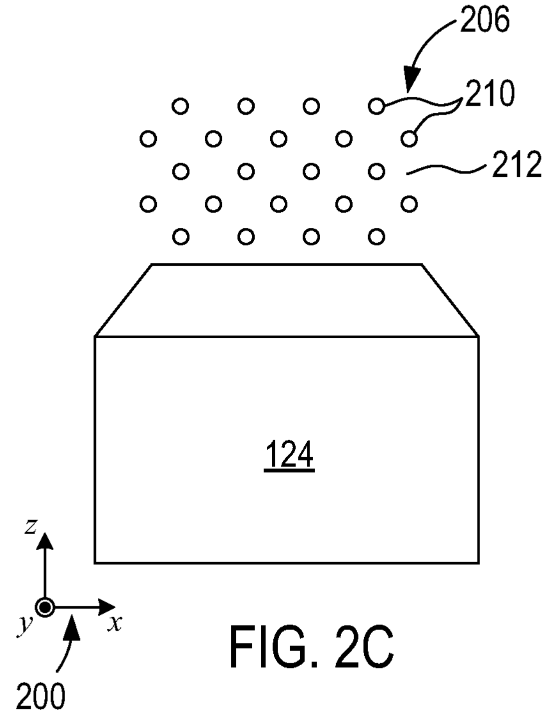

FIG. 2C

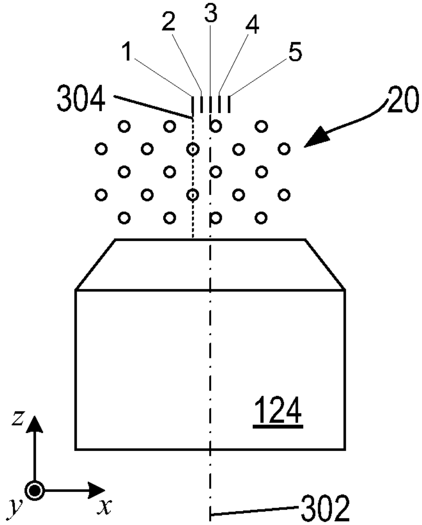

FIG. 3A

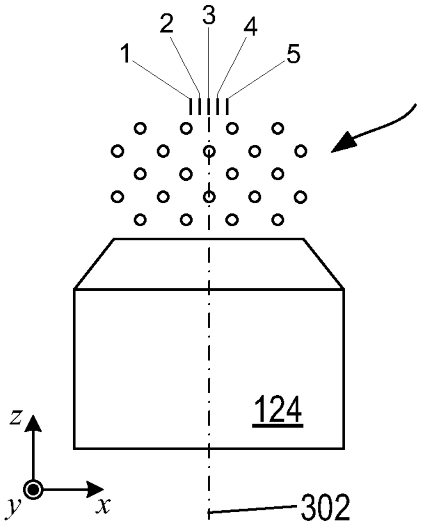

FIG. 3C

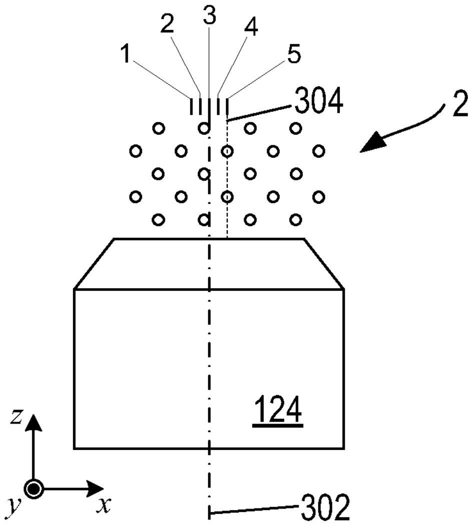

FIG. 3E

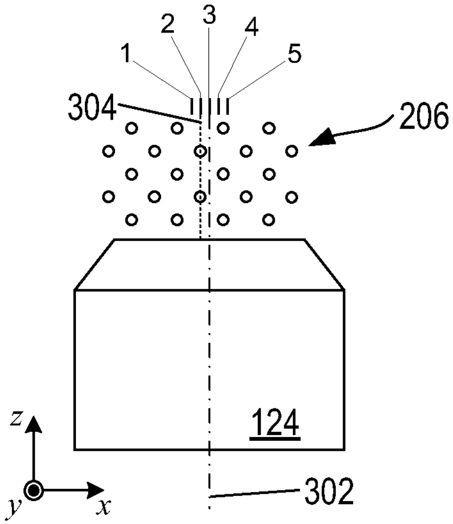

FIG. 3B

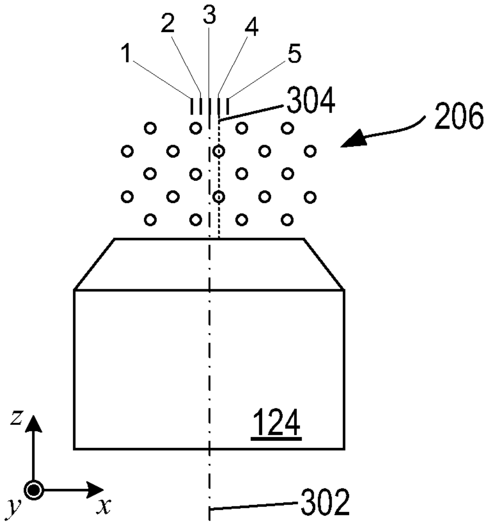

FIG. 3D

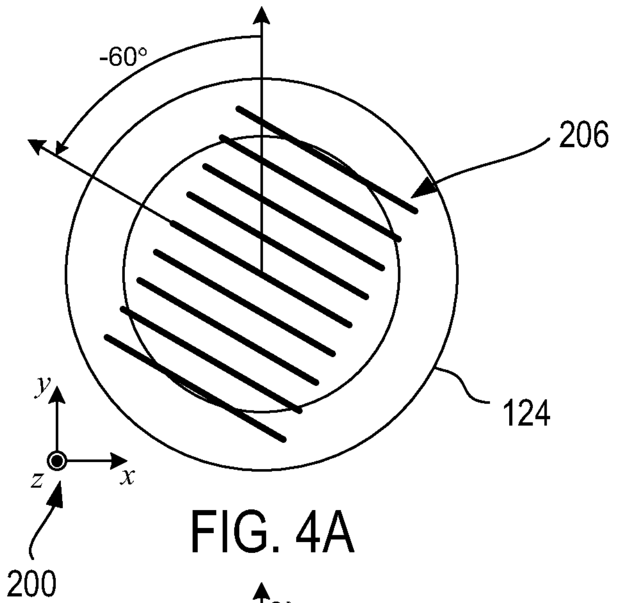

FIG. 4A

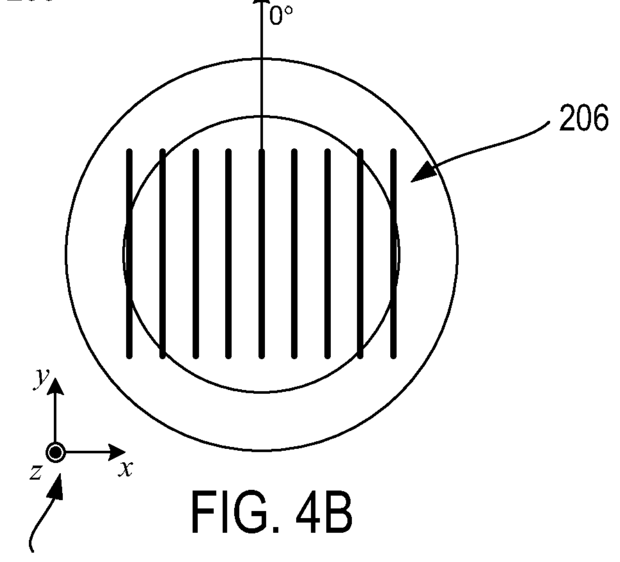

FIG. 4B

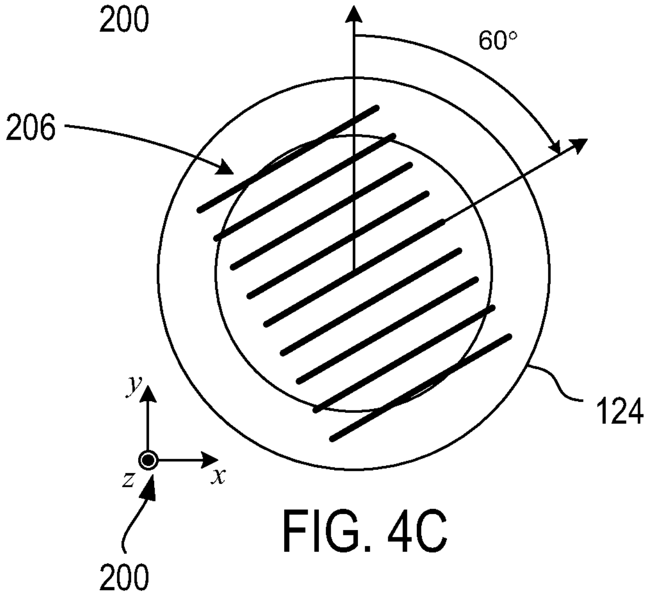

FIG. 4C

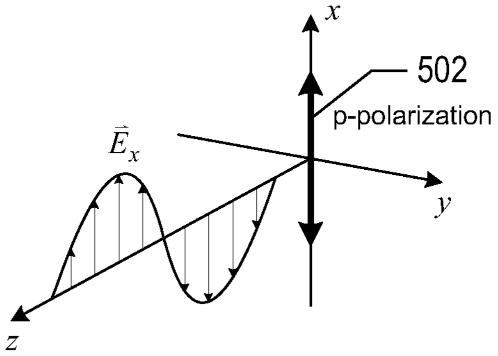

FIG. 5A

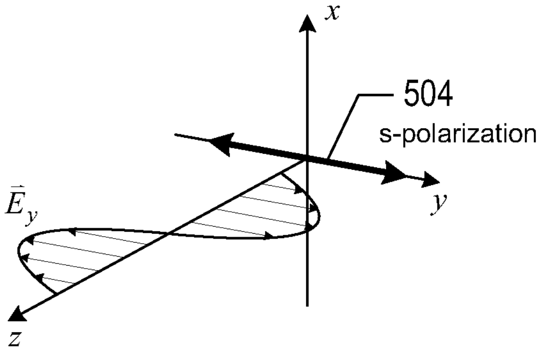

FIG. 5B

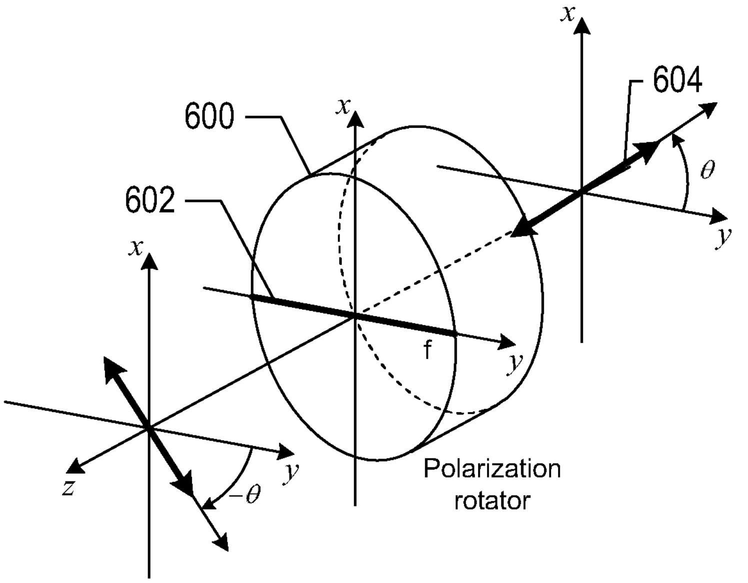

FIG. 6

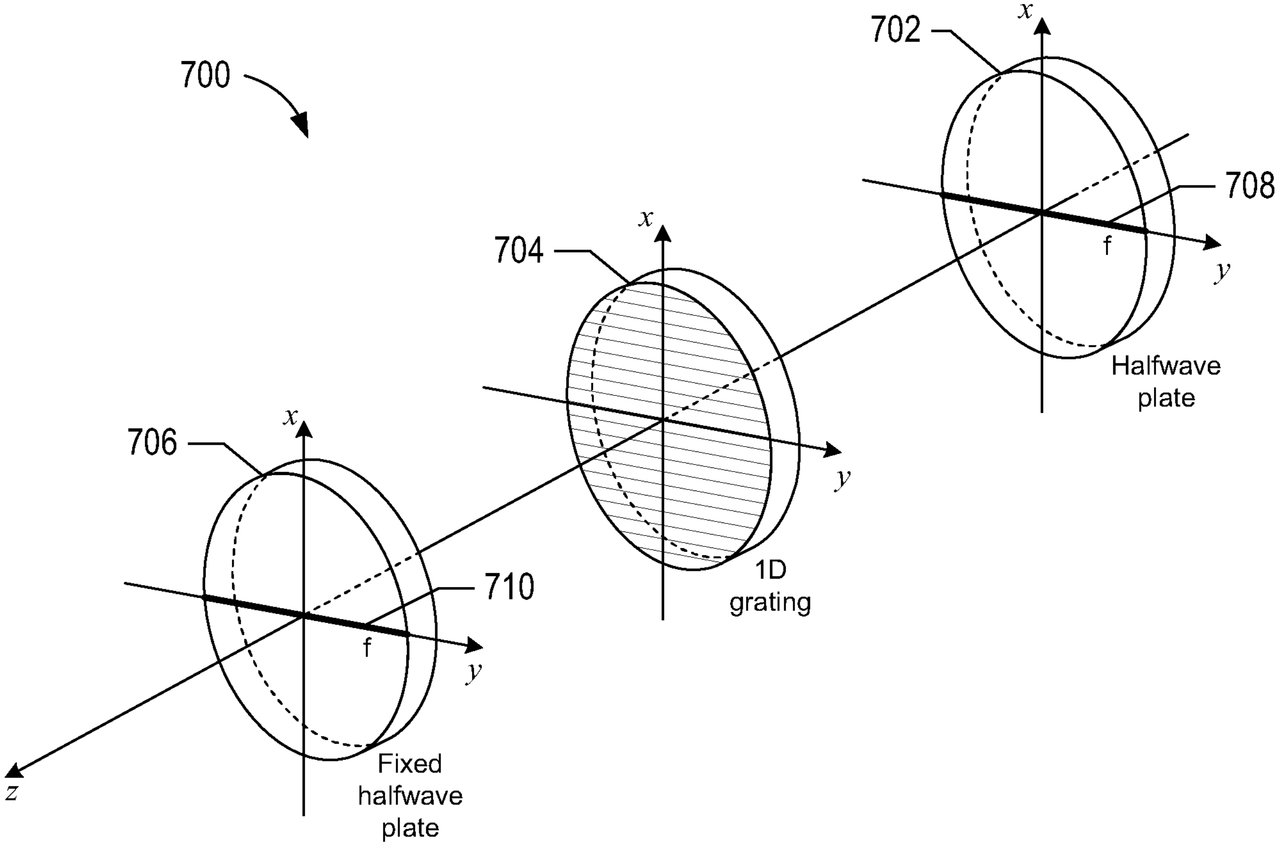
700
702
x
708
y
f
Halfwave plate
704
x
y
1D grating
706
x
710
f
y
z
Fixed halfwave plate

FIG. 7

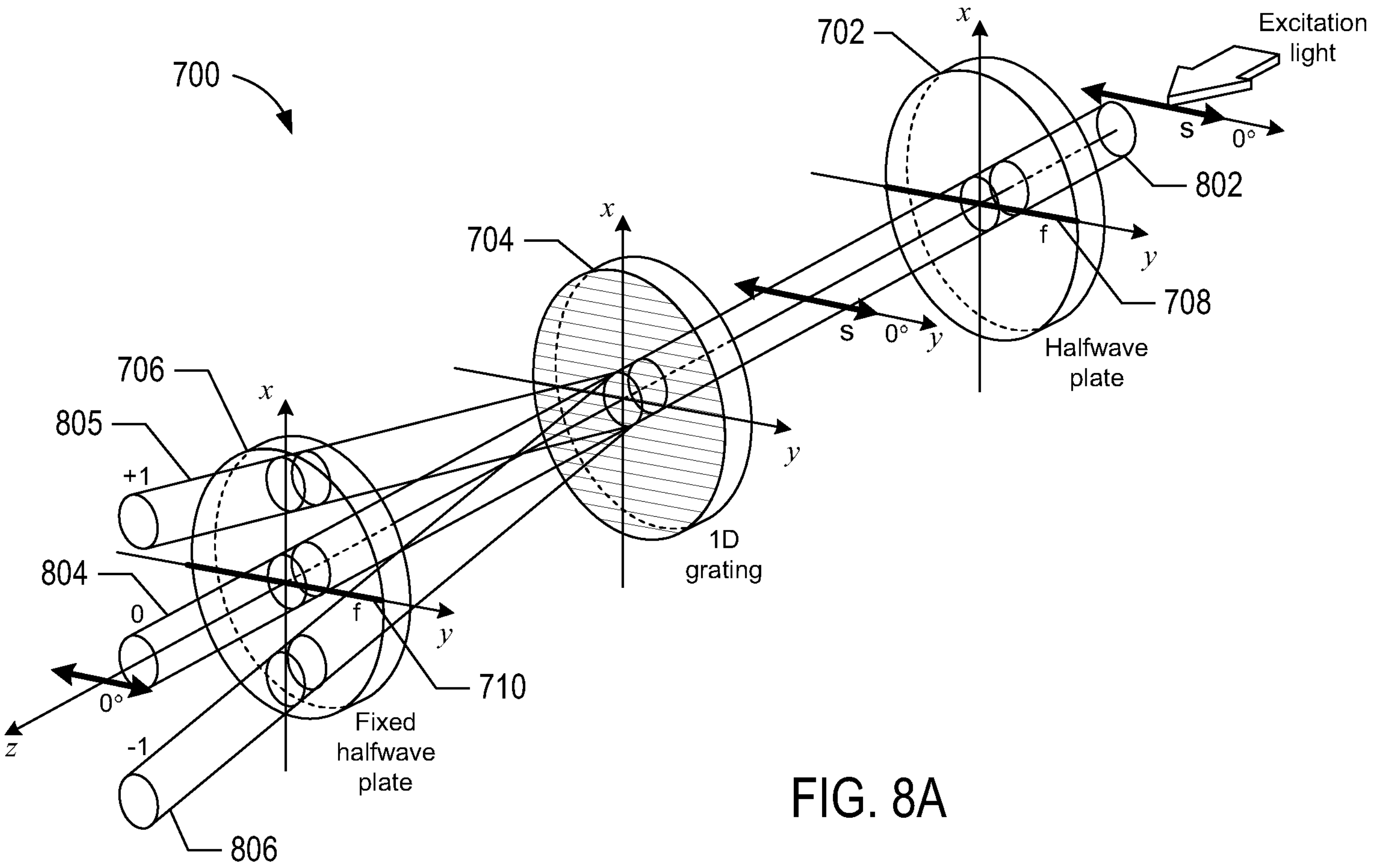
700
702
x
Excitation light
s
0°
802
f
y
708
Halfwave plate
704
x
s
0°
y
y
1D grating
706
x
805
+1
804
0
f
y
710
0°
z
-1
Fixed halfwave plate
806

FIG. 8A

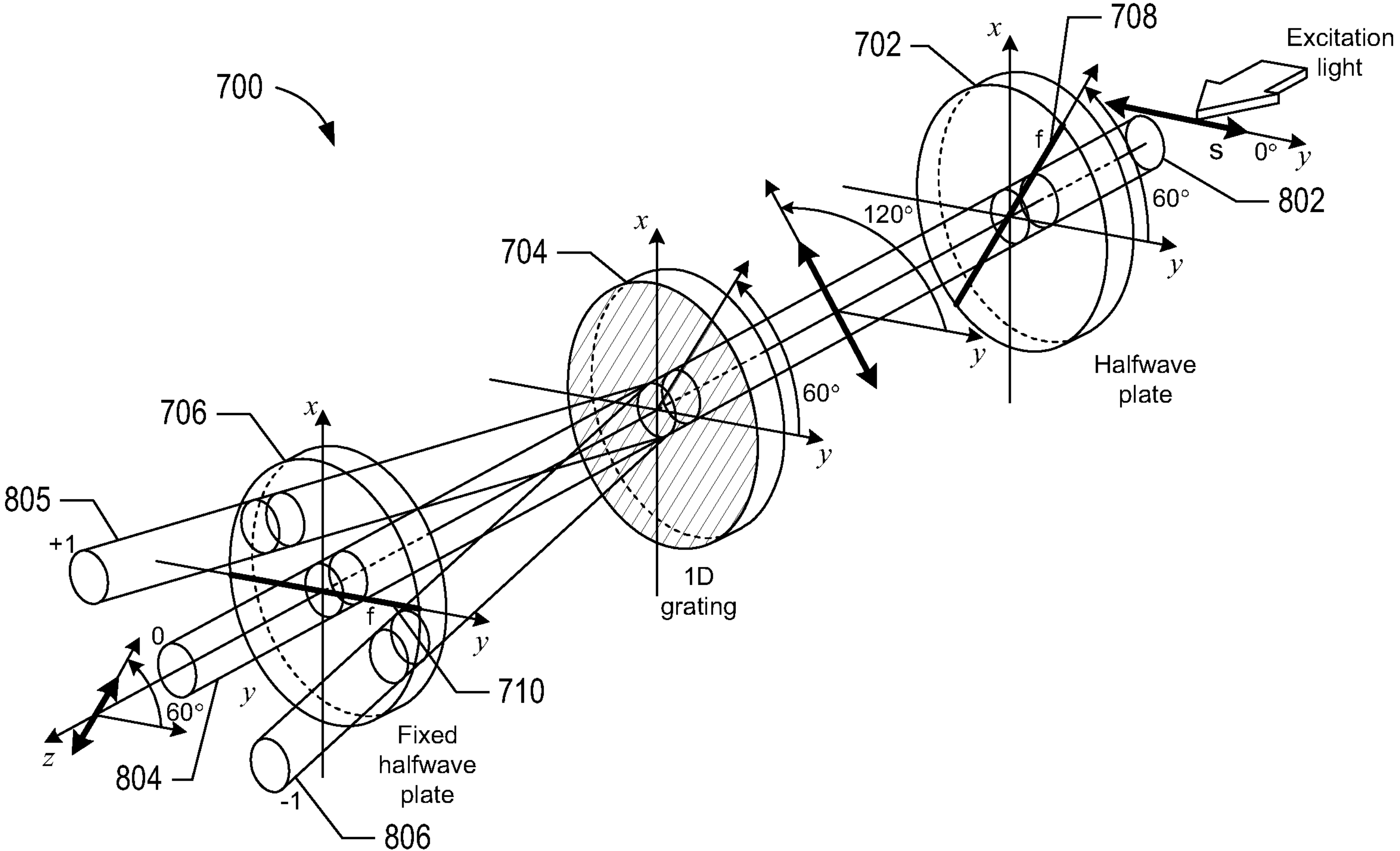
700
702
708
Excitation light
x
s
0°
y
802
60°
y
120°
y
Halfwave plate
704
x
60°
y
1D grating
706
x
805
+1
f
y
0
710
y
60°
z
804
-1
Fixed halfwave plate
806
f

FIG. 8B

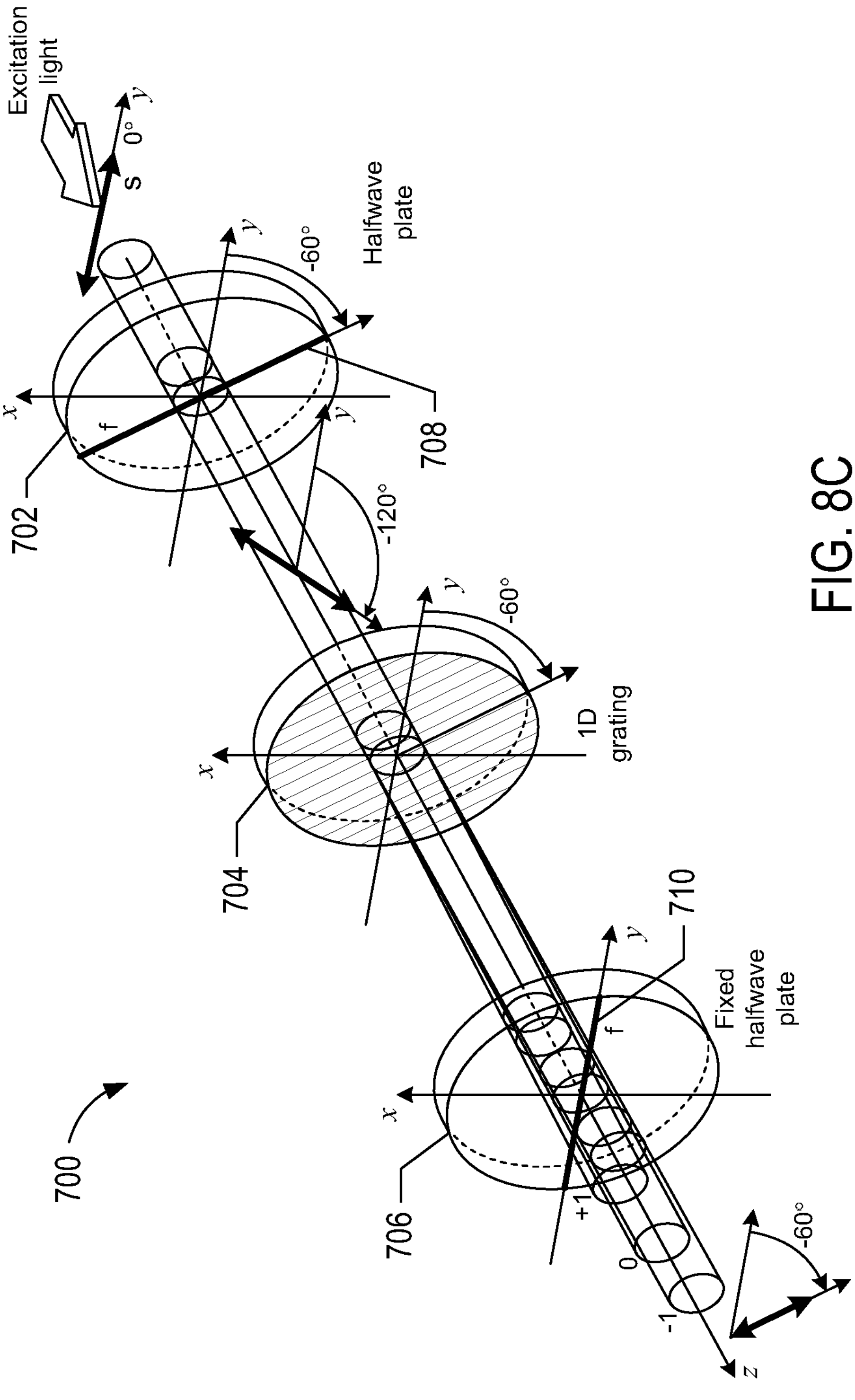
Excitation light
s
0°
y
Halfwave plate
y
-60°
x
f
y
702
708
-120°
y
-60°
x
1D grating
704
y
710
Fixed halfwave plate
x
f
700
706
+1
0
-1
z
-60°

FIG. 8C

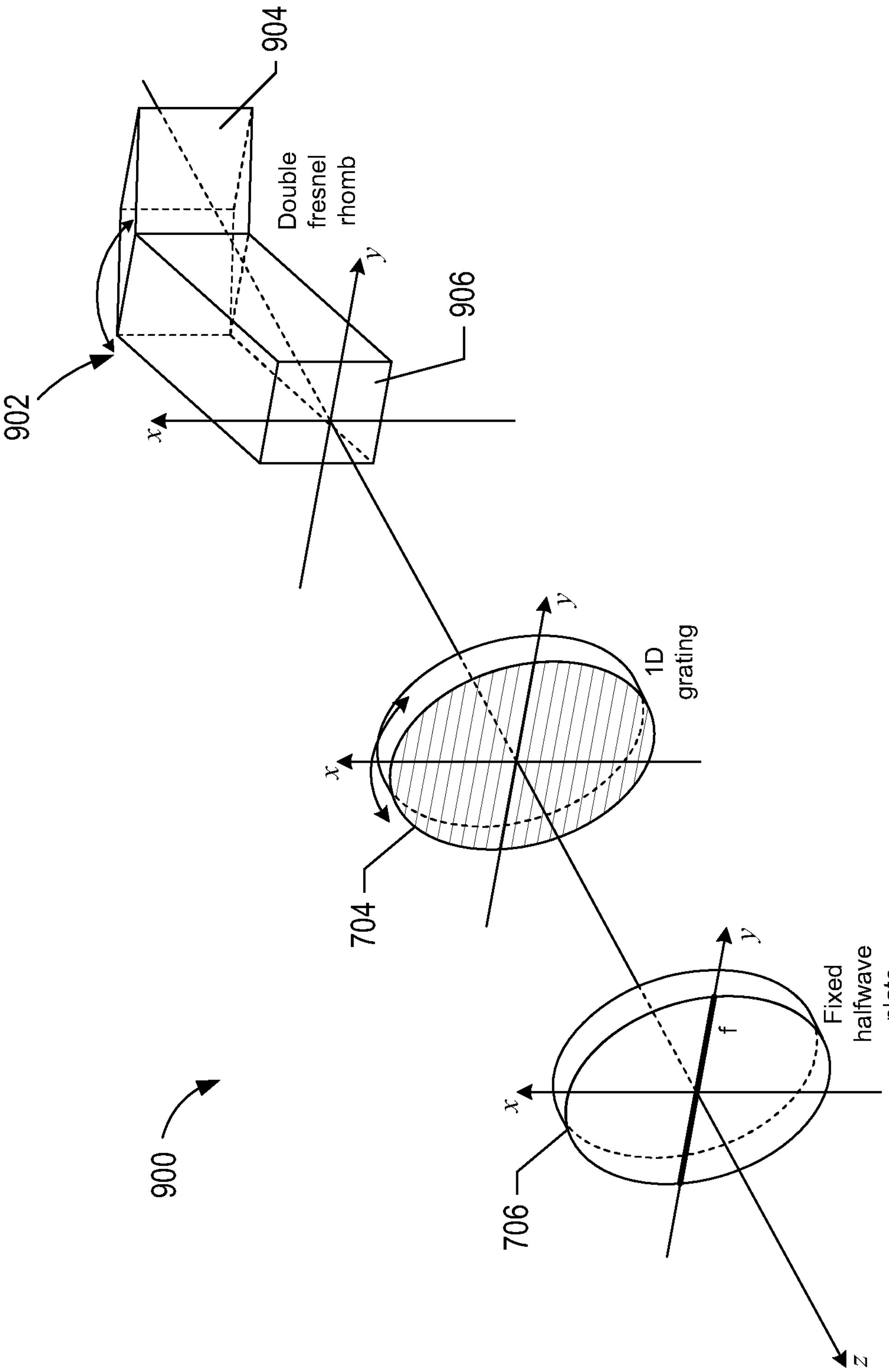
900
902
904
Double fresnel rhomb
906
x
y
704
1D grating
x
y
706
Fixed halfwave plate
f
x
y
z

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050383

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet

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)

IPC: G01N, G02B

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

SE, DK, FI, NO classes as above

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

EPO-Internal, PAJ, WPI data, COMPENDEX, EMBASE, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	US 20120008197 A1 (BORCK SEBASTIAN ET AL), 12 January 2012 (2012-01-12); paragraphs [0009]-[0010], [0022], [0052]-[0054]; figure 4 --	1-19
X	US 20090161203 A1 (KEMPE MICHAEL ET AL), 25 June 2009 (2009-06-25); paragraph [0037] --	1, 2, 7, 8
P, A	WO 2012049831 A1 (NIPPON KOGAKU KK ET AL), 19 April 2012 (2012-04-19); figure 1 --	1-19
A	WO 2011072175 A2 (APPLIED PREC INC ET AL), 16 June 2011 (2011-06-16); abstract -- --------	1-19

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

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"O" document referring to an oral disclosure, use, exhibition or other means	
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Date of the actual completion of the international search 10-09-2012	Date of mailing of the international search report 12-09-2012
Name and mailing address of the ISA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Erik Westin Telephone No. + 46 8 782 25 00

Continuation of: second sheet

International Patent Classification (IPC)

G02B 21/00 (2006.01)
G01N 21/64 (2006.01)
G02B 21/06 (2006.01)
G02B 27/28 (2006.01)

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Cited literature, if any, will be enclosed in paper form.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SE2012/050383

US	20120008197	A1	12/01/2012	DE	102010026205	A1	12/01/2012
US	20090161203	A1	25/06/2009	US	20120019647	A1	26/01/2012
WO	2012049831	A1	19/04/2012	NONE			
WO	2011072175	A2	16/06/2011	US	20110194175	A1	11/08/2011