Lightsheet Microscopy with Rotational-Shear Interferometry

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Field of the Invention

[0001] The present invention relates generally to lightsheet microscopy, and more particularly but not exclusively to rotational-shear-interferometer lightsheet microscopes.

Background of the Invention

Lightsheet Microscopy

[0002] Lightsheet microscopy is a technique for imaging a sample in all three spatial dimensions ("3-D"), in which a "sheet" of light illuminates one slice at a time of the sample under study. This is illustrated in Figure 1. (Lightsheet microscopy is sometimes also referred to as "selective plane illumination microscopy.") A "detection microscope" records a two-dimensional ("2-D") image of the region illuminated by the lightsheet. The lightsheet may be scanned step-by-step through the sample, or the sample may be scanned step-by-step through the lightsheet. A 2-D image is recorded at each step. Eventually the entire object may be illuminated and imaged, and the 2-D images fused together with software to make a 3-D image of the sample.

[0003] The 3-D imaging of fluorescent labels in a biological specimen is an example of a common application of lightsheet microscopy. The illumination light may serve to activate the fluorophores which fluoresce in response. The detection microscope may then capture the light emitted by the fluorophores and form a 2-D image of the locations of the fluorescent labels in response to the illumination by the lightsheet. As the lightsheet and/or sample is scanned, a 2-D image is captured at each scan step. A 3-D image may be assembled from the 2-D images, showing the distribution of fluorescent labels within the specimen.

[0004] Many current lightsheet microscopes suffer from at least one of the following difficulties.

Exemplary Difficulty #1 – Exacting Alignment

[0005] A lightsheet microscope requires alignment between (1) the location of the lightsheet and (2) the region-of-focus of the detection microscope. This alignment requirement is illustrated in Figures 2 and 3. The alignment is required largely for system focus. Without the alignment the 2-D images recorded by the detection microscope will be blurred, and blurring is undesirable.

[0006] With many current lightsheet microscopes this alignment is exacting. Significant effort and cost are needed to meet the alignment requirement. The "z-offset" and the "tilt-offset" both need to be minimized to a value that is exacting to achieve. As used herein the terms "z-alignment" and the "tilt-alignment" refer to the alignment of such offsets, as defined below under "Definitions." The exacting nature of these alignments has negative impacts at each stage in the life of the lightsheet microscope. The need for exacting z-alignment and tilt-alignment can complicate the design, manufacture, and/or use of many current lightsheet microscopes.

[0007] For example, the choice of construction materials is constrained to materials able to maintain shape and dimension against even small thermal and mechanical disturbances. These constraints impede the system design. Further, the system designer may be pushed harder to make tradeoffs against other system parameters and thus reduce overall system performance. For example, these constraints may push the system designer to decrease the numerical aperture of the detection microscope in order to increase the depth-of-field of the detection microscope and thus allow for easier system alignment. A decrease in the numerical aperture of a microscope degrades the lateral spatial resolution of the microscope, which is undesirable. Further, exacting alignment requirements may force the system designer to incorporate alignment mechanisms (tip/tilt controls, etc.) with a finer adjustment capability. This complexity may increase system cost and may impede the system designer.

[0008] Furthermore, the exacting alignment requirements make system manufacture more difficult and more expensive. More effort may be required to achieve the alignment requirements. And greater cost may be required since the construction material choices are constrained and since more system complexity may be required.

[0009] Even after the system is manufactured, the exacting alignment requirements may negatively impact the use of the system in the field. The user generally may

need to perform a final alignment of the system in the moments before each measurement is performed. More effort may be required if the alignment is more exacting. Related to this is a difficulty with many current lightsheet microscopes described by Huisken in US patent application publication 2011/0115895, the entire contents of which application are incorporated herein by reference: typically, "the [lightsheet] is aligned before the experiment to illuminate the focal plane of the detection lens. This alignment is not changed for individual samples. However, samples differ tremendously in their optical properties. Refraction at the medium-sample interface will divert the [lightsheet] away from the focal plane and result in a blurry image." This reduces the variety of samples that can be imaged without re-alignment. Furthermore, alignment generally must be maintained throughout the duration of the measurement. The exacting nature of the alignment requirements may make this more difficult. The difficulty is more acute for longer measurements since the alignment must be maintained for a longer time.

Exemplary Difficulty #2 – Limited Lateral Spatial Resolution

[0010] Another difficulty with many current lightsheet microscopes is the limitation on the lateral spatial resolution that can be achieved by the detection microscope. Indeed, many current lightsheet microscopes use one or more conventional microscopes for the detection microscope. The lateral spatial resolution of a conventional microscope is limited in a known way. This is a limit to image quality.

[0011] Thus, there is a need in the art for improved lightsheet microscopes that may address, for example, one or more of the above-noted difficulties or additional difficulties.

Summary of the Invention

[0012] In one of its aspects the present invention provides a RSI lightsheet microscope that combines lightsheet illumination and rotational-shear interferometry.

[0013] As used herein, "2-D" stands for two-dimensional; "3-D" stands for three-dimensional; "MTF" Stands for modulation transfer function; and, "RSI" stands for rotational-shear interferometer.

Definitions

[0014] As used herein the following terms have the following meanings.

[0015] The term "conventional imager" refers to an imager that forms a *direct* image on a detector. The term "conventional microscope" refers to a microscope that forms a *direct* image on a detector. An RSI imager is not a conventional imager since the data recorded by the detector needs to be processed to infer an image. Likewise, an RSI microscope is not a conventional microscope.

[0016] The term "lightsheet microscope" refers to a system that includes *both* lightsheet illumination and a detection microscope. The terms "conventional microscope" and "RSI microscope" each refer *only* to the detection microscope, not the lightsheet illumination system.

[0017] The "region-of-focus" of an imaging system refers to the 3-D region over which an object point will appear "in focus" in the 2-D image. In Figures 1, 2, 3, 5, 6, 9A, 9B, 9C, and 9D, a pair of dashed lines marks the boundaries of the "region-of-focus" of the detection microscope. For example, in Figure 1 the region-of-focus is labeled **103**. An object point located between the pair of dashed lines will appear in focus in the 2-D image. An object point located outside the region-of-focus will appear blurred in the 2-D image.

[0018] The "depth-of-field" of an imaging system refers to the distance over which the "region-of-focus" extends in the "z" direction. In Figures 1, 2, 3, 5, 6, 9A, 9B, 9C, and 9D the "depth-of-field" is the distance between the two dashed lines in the "z" direction.

[0019] The "plane-of-mid-focus" refers to the plane within the region-of-focus halfway between the two ends of the region-of-focus in the "z" direction. For example, in Figure 1 the "plane-of-mid-focus" is halfway between the two dashed lines that mark region-of-focus **103**.

[0020] In a system with "field curvature" aberration, the mid-focus surface may be curved rather than planar. For simplicity of discussion, systems discussed here are approximated to have no "field curvature." The same concepts apply when "field curvature" is present.

[0021] "Tilt-offset" refers to the angle between (1) the plane of the lightsheet and (2) the plane-of-mid-focus of the detection microscope. This is illustrated in Figure 2. For example, in Figure 2 the "tilt-offset" is labeled **204** and has a value of 22

degrees. This value was chosen for clarity and ease of illustration. In Figures 1, 3, 5, 9A, 9B, 9C, and 9D the "tilt-offset" is zero.

[0022] "Tilt-alignment" refers to the alignment that minimizes the "tilt-offset." The "tilt-alignment" can involve adjustment of (1) the tilt angle of the lightsheet, (2) the tilt angle of the region-of-focus of the detection microscope, or (3) a combination of (1) and (2).

[0023] "z-offset" refers to the distance in the "z" direction between (1) the plane of the lightsheet (the plane through the center of the lightsheet) and (2) the plane-of-mid-focus of the detection microscope after correcting for "tilt-offset." This is illustrated in Figure 3. In Figure 1 and Figure 2 the "z-offset" is zero. In Figure 3 the "z-offset" is non-zero.

[0024] "z-alignment" refers to the alignment that minimizes the "z-offset." The "z-alignment" can involve adjustment of (1) the location of the lightsheet, (2) the location of the region-of-focus of the detection microscope, or (3) a combination of (1) and (2).

Brief Description of the Drawings

[0025] The foregoing summary and the following detailed description of exemplary embodiments of the present invention may be further understood when read in conjunction with the appended drawings, in which:

[0026] Figure 1 schematically illustrates the concept of lightsheet microscopy;

[0027] Figure 2 schematically illustrates the meaning of the terms tilt-offset and tilt-alignment;

[0028] Figure 3 schematically illustrates the meaning of the terms z-offset and z-alignment;

[0029] Figure 4 schematically illustrates a lightsheet microscope with a rotational-shear interferometer in accordance with the present invention;

[0030] Figure 5 schematically illustrates why the z-alignment is easier to perform when the depth-of-field of the detection microscope is large;

[0031] Figure 6 schematically illustrates why the tilt-alignment is easier to perform when the depth-of-field of the detection microscope is large;

[0032] Figure 7 schematically illustrates the light path in one embodiment of a rotational-shear interferometer;

[0033] Figure 8 schematically illustrates one method of operation;

[0034] Figures 9A, 9B, 9C, and 9D further schematically illustrate a method of operation; and

[0035] Figure 10 schematically illustrates an alternate embodiment involving a lightsheet microscope with two rotational-shear interferometers in accordance with the present invention.

Detailed Description

[0036] Turning now to the figures, Figure 1 schematically illustrates the concept of lightsheet microscopy. Figure 1 illustrates part of a lightsheet microscope representing a snapshot at one scan step of a sample **100** under study. Sample **100** could be a biological specimen, for example. Sample **100** is three-dimensional and extends in the "+/- x", "+/- y", and "+/- z" directions. (Mutually orthogonal coordinate axes indicate the "x", "y", and "z" directions, with each arrow on each coordinate axis pointing in the positive direction along the axis. For example, the "+y" direction is to the right on the page.) For simplicity of illustration, only a two-dimensional slice of sample **100** is drawn.

[0037] Lightsheet **101** enters sample **100** in the direction indicated by arrow **102** and represented by a pair of thick lines. The lightsheet **101** is parallel to the x-y plane and has a slight curvature, customary of the behavior of a Gaussian beam. A Gaussian beam is one form of illumination used to generate a lightsheet. The most-narrow part of lightsheet **101** is located near the center of sample **100**. Lightsheet **101** expands slightly in the "+/- z" direction as there is an increase in the distance from the most-narrow part of lightsheet **101**. This comports with the behavior of a Gaussian beam. The detection microscope is not shown in Figure 1. Light from sample **100** travels in the "+z" direction to a detection microscope. A region-of-focus **103** is centered in the "z" direction on lightsheet **101**. Figure 1 is meant to be compared directly to Figures 2, 3, 5, and 6. These Figures are all drawn on the same scale, each of which includes a region-of-focus. The depth-of-field is the same in Figures 1–3.

[0038] Figure 2 schematically illustrates the meaning of the terms tilt-offset and tilt-alignment. Like Figure 1, Figure 2 illustrates part of a lightsheet microscope representing a snapshot at one scan step of a sample **200** under study. Light from sample **200** travels in the "+z" direction to the detection microscope. The detection microscope is not shown. The region-of-focus is labeled **203**. A difference between Figure 2 and Figure 1 is that in Figure 2 the lightsheet **201** is tilted. The lightsheet **201** is tilted about the "x" axis, and arrow **202** indicates the direction the lightsheet **201** enters sample **200**.

[0039] In Figure 2 there is a sufficiently-large tilt-offset **204** between lightsheet **201** and region-of-focus **203** as to produce blur in the recorded image. Parts of the illuminated region of sample **200** are outside region-of-focus **203**. Light from points outside the region-of-focus of the detection microscope contributes to blur in the recorded image. To avoid blur in the recorded image, only a small tilt-offset **204** is allowed. The region of the sample illuminated by the lightsheet **201** (within the field-of-view of interest) should fit completely within the region-of-focus of the detection microscope. This alignment requirement is the tilt-alignment. The system illustrated in Figure 2 does not have proper tilt-alignment, because the tilt-offset **204** is too large.

[0040] Figure 3 schematically illustrates the meaning of the terms z-offset and z-alignment. Like Figures 1 and 2, Figure 3 illustrates part of a lightsheet microscope representing a snapshot at one scan step of a sample **300** under study. Light from sample **300** travels in the "+z" direction to the detection microscope. The detection microscope is not shown. The region-of-focus is labeled **303**. A difference between Figure 3 and Figure 1 is that in Figure 3 the region-of-focus is offset in the "z" direction from lightsheet **301**. Arrow **302** indicates the direction the lightsheet **301** enters sample **300**. In Figure 3 there is a sufficiently-large z-offset **304** between lightsheet **301** and region-of-focus **303** as to produce blur in the recorded image. The illuminated region of sample **300** is outside region-of-focus **303**. Light from points outside the region-of-focus **303** of the detection microscope produces blur in the recorded image.

[0041] To avoid blur in the recorded image, only a small z-offset **304** is allowed. The region of the sample **300** illuminated by the lightsheet **301** (within the field-of-

view of interest) should fit completely within the region-of-focus **303** of the detection microscope. This alignment requirement is the z-alignment. The system illustrated in Figure 3 does not have proper z-alignment, because the z-offset **304** is too large.

[0042] Figure 4 schematically illustrates a RSI (rotational shear interferometer) lightsheet microscope **450** in accordance with the present invention. The RSI lightsheet microscope **450** includes an optical source/optics **401** that, in cooperation with an objective **402,** generates a lightsheet used to illuminate a sample **400**. Specifically, the objective **402**, which may be a conventional microscope objective, is disposed between the optical source/optics **401** and sample **400** to create and deliver the lightsheet to the sample **400**. Light from sample **400** is collected by a collection objective **403**, which may also be a conventional microscope objective, and is delivered to an RSI **404**. The objectives **402, 403** do not need be conventional microscope objectives; other suitable optical elements for creating the lightsheet and collecting light from the sample **400**, respectively, may be used. As seen in Figures 5 and 6, the RSI lightsheet microscope **450** provides enhanced performance with regard to z-alignment and tilt-alignment, due, in part to the increased depth-of-field of the RSI **404**. In particular, Figure 5 schematically illustrates why the z-alignment is easier to perform when the depth-of-field of the detection microscope is large.

[0043] *Operation*

[0044] There are several manners in which the embodiment **450** can be operated. Some examples are as follows. This list is not meant to be limiting.

[0045] *Exemplary Manner of Operation #1*

[0046] The lightsheet and the RSI microscope are both held fixed with no adjustment. The sample is translated (and possibly rotated) in steps through the lightsheet. At each step the RSI records a snapshot. This process is repeated until the entire sample has been imaged.

[0047] *Exemplary Manner of Operation #2*

[0048] In this scenario the depth-of-field of the RSI microscope is large enough to encompass the entire sample. The system is set up so the sample is completely contained within the depth-of-field. The lightsheet is scanned through the entire

sample. At each scan step the RSI microscope records a snapshot. No intermediate refocusing of the RSI microscope is required.

[0049] *Exemplary Manner of Operation #3*

[0050] In this scenario, the depth-of-field of the RSI microscope is not large enough to encompass the entire sample. The sample is held fixed in location and orientation throughout the measurement. The lightsheet is scanned through the sample in steps. The RSI microscope must be refocused one or more times as the lightsheet is scanned.

[0051] A flowchart is drawn in Figure 8. The steps outlined in the flowchart are further illustrated in Figures 9A, 9B, 9C, and 9D.

[0052] The first step is step **800**. The RSI focus is adjusted so the region-of-focus is near one end of the sample under study. This is further illustrated in Figure 9A. Sample **900** is the sample under study. Region-of-focus **901** has been located near one end of sample **900**.

[0053] The next step is step **801**. The lightsheet is placed at its initial location within the sample. This corresponds to lightsheet **902**. Arrow **903** illustrates the direction lightsheet **902** enters sample **900**. Lightsheet **902** is not right at the edge of region-of-focus **901**. Instead lightsheet **902** is separated from the edge of region-of-focus **901** by a distance labeled **908**. There is a tradeoff a user makes in choosing a value for distance **908**. A small value for distance **908** means more of the sample can be scanned before the RSI microscope must be refocused. A large value for distance **908** eases the z-alignment and tilt-alignment of the system.

[0054] The next step is step **802**. The RSI microscope records a snapshot of the light from the sample.

[0055] The next step is step **803**. Step **803** is a yes/no branch. If the lightsheet has been scanned through the intended area of the region-of-focus, the "yes" branch is followed and step **805** is next. Otherwise the "no" branch is followed and step **804** is next.

[0056] Lightsheet **902** is not at the end of the intended area of region-of-focus **901**, so the "no" branch is followed to step **804**. In step **804** the lightsheet location is stepped. Then as indicated with the arrow, the next step is **802**, where a snapshot is

again recorded with the RSI. This loop is repeated until the lightsheet location is at the end of the intended area of illumination of region-of-focus **901**. Lightsheet **906** indicates the last intended location for the lightsheet. Lightsheet **906** is a distance **909** from the edge of region-of-focus **901**. This is a buffer. The tradeoff is the same as was discussed in connection with buffer **908**.

[0057] The lightsheet is stepped through a number of locations from location **902** to location **906**. Location **904** is in the middle. Ellipses indicate that additional steps are taken between the lightsheet locations that are drawn. Location **904** is an intermediate location for the lightsheet during the scan. Arrow **903** shows the direction lightsheet **902** enters sample **900**. Arrow **905** shows the direction lightsheet **904** enters sample **900**. Arrow **907** shows the direction lightsheet **906** enters sample **900**.

[0058] When the lightsheet is at location **906** and step **803** is reached, the "yes" branch is followed to step **805**.

[0059] Step **805** is another yes/no branch. If the entire sample has been imaged then the "yes" branch is followed to step **807**, which is the close of the flowchart. Otherwise the "no" branch is followed to step **806**.

[0060] In step **806** the location of the RSI region-of-focus is stepped. Compare Figure 9A to Figures 9B, 9C, and 9D. In going from one of these Figures to the next, the lightsheet region-of-focus is stepped through the sample. This involves moving from step **806** to step **802** as indicated in Figure 8, and repeating the loop until the "no" branch is followed from step **805** to step **807**.

[0061] At each location of the lightsheet region of focus, the lightsheet is scanned through as before in connection with Figure 9A.

[0062] In Figure 9B lightsheets **911**, **913**, and **915** travel in the directions indicated by arrows **912**, **914**, and **916** respectively. Markers **917** and **918** indicate the length of each buffer region. The region-of-focus is labeled **910**. The sample is again labeled **900**.

[0063] In Figure 9C lightsheets **920**, **922**, and **924** travel in the directions indicated by arrows **921**, **923**, and **925** respectively. Markers **926** and **927** indicate the length

of each buffer region. The region-of-focus is labeled **919**. The sample is again labeled **900**.

[0064] In Figure 9D lightsheets **929**, **931**, and **933** travel in the directions indicated by arrows **930**, **932**, and **934** respectively. Markers **935** and **936** indicate the length of each buffer region. The region-of-focus is labeled **928**. The sample is again labeled **900**.

[0065] As indicated by the flowchart in Figure 8, the last location of the lightsheet within one region-of-focus is the same as the first location of the lightsheet within the next region-of-focus. This provides data that assists with co-registration of the 2-D images recorded from different regions-of-focus.

[0066] Figure 5 schematically illustrates the z-alignment performance of the RSI lightsheet microscope **450** by showing a snapshot at one scan step of a sample **500** under study. Here a lightsheet **501** illuminates sample **500**, and arrow **502** indicates the direction lightsheet **501** enters sample **500**, with light from sample **500** traveling in the +z direction to the detection microscope, *i.e.*, the RSI **404** and collection objective **403**. The magnitude of the z-offset in Figure 5 is the same as magnitude of z-offset **304** in Figure 3. A difference between Figure 5 and Figure 3 is that in Figure 5 the detection microscope has a larger depth-of-field. The depth-of-field for region-of-focus **503** in Figure 5 is larger (by a factor of approximately four) than the depth-of-field for region-of-focus **303** in Figure 3. Figure 5 and Figure 3 are drawn on the same scale as each other. The factor-of-four difference is only an example used for illustrative purposes. Other values are possible.

[0067] In Figure 5 the system is in focus even though the z-offset is not zero. The system has proper z-alignment. By comparison, the system in Figure 3 is not in focus even though the z-offset is the same in Figures 3 and 5. The large depth-of-field in Figure 5 is what allows the system to be in z-alignment despite the non-zero z-offset. In Figure 5 a larger z-offset would be needed to place the system out of alignment. This illustrates why the z-alignment is easier to perform when the depth-of-field of the detection microscope is large.

[0068] Figure 6 schematically illustrates the tilt-alignment performance of the RSI lightsheet microscope **450** by showing a snapshot at one scan step of the sample **600** under study. In particular, Figure 6 illustrates why the tilt-alignment is easier to

perform when the depth-of-field of the detection microscope is large. A lightsheet **601** illuminates sample **600**, and arrow **602** indicates the direction lightsheet **601** enters sample **600**, with light from sample **600** traveling in the +z direction to the detection microscope. The magnitude of the tilt-offset in Figure 6 is the same as the magnitude of the tilt-offset **204** in Figure 2. A difference between Figure 6 and Figure 2 is that in Figure 6 the detection microscope has a larger depth-of-field. The depth-of-field for region-of-focus **603** in Figure 6 is larger (by a factor of approximately four) than the depth-of-field for region-of-focus **603** in Figure 2. Figure 6 and Figure 2 are drawn on the same scale as each other. The factor-of-four difference is only an example used for illustrative purposes. Other values are possible.

[0069] In Figure 6 the system is in focus even though the tilt-offset is not zero. The system has proper tilt-alignment. By comparison the system in Figure 2 is not in focus even though the tilt-offset is the same in Figures 2 and 6. The large depth-of-field in Figure 6 is what allows the system to be in tilt-alignment despite the non-zero tilt-offset. In Figure 6 a larger tilt-offset would be needed to place the system out of alignment. This illustrates why the tilt-alignment is easier to perform when the depth-of-field of the detection microscope detection microscope is large. Thus, Figures 5 and 6 illustrate the effects of having a relatively larger depth-of-field provided by the RSI **404** as contrasted with the relatively smaller depth-of-field illustrated in Figures 1 – 3 associated with a conventional microscope, such as exists on many current lightsheet microscopes. That is, the depth-of-field illustrated in Figure 1 – 3 is the depth-of-field of a *conventional* microscope, whereas the depth-of-field illustrated in Figures 5 and 6 is the depth-of-field of an *RSI* microscope **450** in accordance with the present invention.

Rotational-Shear Interferometer

[0070] Turning now to the rotational-shear interferometer **404** more specifically, the RSI **404** is an instrument in which light entering through an aperture is split into two beams. The two beams are recombined so as to produce interference fringes. The fringes can be analyzed to infer an image of the scene in front of the RSI **404**. (As used herein, when an RSI is used in this manner, it is referred to as an RSI imager, and when an RSI is used more-specifically in a microscope configuration, it is

referred to as an RSI microscope.) The angle of rotational-shear can be set to different values, depending on the application. When the angle of rotational-shear is 180 degrees, there term "180-degree RSI imager" is used herein.

[0071] The imaging performance of an RSI microscope may compared to the imaging performance of a conventional microscope in the cases where the following two conditions are met. (1) The entrance pupil of each microscope is the same distance from the object being imaged. (2) The sizes of the entrance pupils of the two microscopes are the same as each other. Under these two conditions, the following two comparisons can be made.

(a) Long depth-of-field.

[0072] The depth-of-field of the RSI microscope is long compared to the depth-of-field of the conventional microscope.

[0073] The reason the RSI microscope has a long depth-of-field is as follows. Consider an object point located front of the RSI microscope. The object is on the axis of the RSI microscope. Light from the object point generates two wavefronts incident on the RSI detector. Now move the object point along the axis of the RSI microscope to a new location a short distance away. There is a change in the curvature of the two wavefronts incident on the RSI detector. The magnitude of the change-in-curvature is the same for both wavefronts. The change-in-curvature is common-mode. Interferometers are generally insensitive to common-mode changes. The fringe pattern recorded by the RSI detector does not change much with the movement of the object point along the imager's axis. For this reason, the RSI microscope has a long depth-of-field.

(b) Superior lateral spatial resolution.

[0074] Under the scenario where spatially-incoherent light is used to image the scene, a 180-degree RSI imager is characterized by a modulation transfer function (MTF) superior to that of a conventional imager. The MTF is superior by up to a factor of two, as measured by the area under the MTF curve. The MTF is a measure of the lateral spatial resolution of the imaging system.

[0075] The image generated by an RSI imager is a *conical* projection of the 3-D scene in front of the RSI. The vertex of the cone is the center of the RSI imager's entrance pupil.

RSI layout

[0076] Figure 7 illustrates a layout for a simple version of an RSI microscope, including an object point from the sample under study, and including a model for the objective lens. The system illustrated in Figure 7 is drawn as 2-dimensional. Most current RSIs are 3-dimensional. Figure 7 is limited to 2 dimensions for simplicity of illustration. The figure provides the information needed without the complication of 3-dimensional drawings.

[0077] The lenses illustrated in Figure 7 are illustrated as "thin lenses," as is common in the field of optics.

[0078] Object point **700** emits light towards the objective, such as objective **403**. In the thin-lens model illustrated in Figure 7, the objective (which typically has many optical surfaces internally) is represented by a single thin lens **701**. Lens **701** collimates the light. The light travels to aperture **702**, commonly referred to in the field of optics as the "system stop." Aperture **702** truncates the beam. The beam propagates to thin-lenses **703** and **704**. Optics **703** and **704** work together to image aperture **702** to detectors **709** and **710**. After leaving lens **704** the light propagates to beamsplitter **705**. One beam travels to fold mirrors **706** and **707**, then to beamsplitter **708**. The other beam travels to fold mirrors **711**, **712**, and **713**, then to beamsplitter **708**. Two beams are incident on each detector **709** and **710**. The two beams respond in a counter-tilt fashion to movement of object point **700** within the "x-y" plane. The counter-tilt is due to the fact that one beam experiences an odd number of reflections while the other beam experiences an even number of reflections.

[0079] As illustrated in Figure 7, an object point at location **700** generates wavefronts on detectors **709** and **710** that are flat. When the object point is moved to a different location along the z-axis, the wavefronts incident on detectors **709** and **710** are no longer flat. I refer to the RSI as being at "best focus" when the wavefronts incident on detectors **709** and **710** are flat. There are various ways to adjust the RSI focus. One way is to adjust the locations of lenses **703** and **704**, possibly including the separation between the two lenses, in the "+/- z" direction.

Advantages

[0080] As demonstrated above, the RSI lightsheet microscope **450** provides multiple advantages. For example, compared to many current lightsheet microscopes, the tilt-alignment and the z-alignment are less exacting. This mitigates one or more of the negative impacts listed above under Difficulty #1. The reason the tilt-alignment and the z-alignment are less exacting is as follows. The depth-of-field of the detection microscope of the exemplary RSI lightsheet microscope **450** is larger than the depth-of-field of the detection microscope in many current lightsheet microscopes. A larger depth-of-field makes it easier to perform the tilt-alignment and z-alignment. In addition, compared to the detection microscope in many current lightsheet microscopes (a conventional microscope), the lateral spatial resolution of the detection microscope of the exemplary RSI lightsheet microscope **450** is superior (as measured by the area under the MTF curve) when using spatially-incoherent light, which mitigates Difficulty #2 noted above.

[0081] In addition to the particular exemplary RSI lightsheet microscope **450** disclosed above, further variations are included within the scope of the present invention. For example, the lightsheet microscope **450** may include more than one lightsheet source, such as counter-propagating, co-planar lightsheet illumination of a sample as disclosed for example in US patent application publication 2011/0115895. In addition, the RSI lightsheet microscope **450** can use more than one detection microscope. For example, a second detection microscope may view the sample from a perspective 180 degrees away from the first detection microscope. This is illustrated in Figure 10. Sample **1000** is illuminated by the lightsheet generated by **1001** and **1002** working in concert. Objective **1003** presents light from the sample to RSI **1004**. Objective **1005** presents light from the sample to RSI **1006**. Reference **1050** refers to the entire system. More than two detection microscopes may also be used. Furthermore, a microscope objective may be used for both transmission of light to the sample and receipt of light from the sample.

[0082] Still further, adaptive optics may be incorporated into RSI lightsheet microscopes in accordance with the present invention. One use of adaptive optics is to compensate for the otherwise-detrimental light-scattering properties of the sample.

Additionally, the RSI **404** may be constructed and used in a number of configurations, such as a Michelson or Mach-Zehnder configuration.

[0083] Moreover, the rotational-shear angle of the RSI can be set to different values. There are different ways to set the rotational-shear angle to a given value. For example consider the case of a 180-degree rotational-shear angle. This corresponds to a counter-tilt of the two beams incident on the RSI detector. One way to produce a counter-tilt is to use an odd number of reflections in one arm of the interferometer and an even number of reflections in the other arm. A different way to produce a counter-tilt is to send the light in one arm of the interferometer through an intermediate focus within the arm.

[0084] One can also adjust the angle at which the two beams are incident on the detector. For a point at the center of the field-of-view, the two resulting beams can be incident on the detector at normal incidence or at some different angle (e.g., +/- 3 degrees). If the two beams are incident at normal incidence, there will be ambiguity (the twin image problem). If the angle-of-incidence of each beam corresponding to the center of the field-of-view is large enough, the twin image problem is avoided.

[0085] Still further, the RSI may be used in a modified form known as a quadrature-phase interferometer.

[0086] The RSI **404** may also use fringe-scanning to obtain a time series of exposures with different phase differences between the two arms of the interferometer. The RSI **404** may be configured to compensate or correct for differences in the polarization response of the two arms of the interferometer, for example by the addition of phase plates. The RSI **404** may further be configured to achromatize the fringe pattern to increase the spectral bandwidth of the RSI **404**. The RSI **404** may use mirrors that may or may not contain a roofline through the middle of the mirror, and may optionally include a prism to steer light.

[0087] Different types of beamsplitters may also be used within the RSI **404**, such as cube or pellicle beam splitters, or even a glass plate that reflects off one of its external surfaces. There are also different ways to convert the fringe pattern recorded on the RSI detector into an image. One method is to Fourier-transform the fringe pattern, and a second method is to fit the fringe pattern with a set of orthogonal functions. In the case of a sparse image, a procedure exists to convert the

fringe pattern recorded on the RSI detector into an image with spectral information for each point in the image.

[0088] Sometimes the lightsheet activates quantum dots rather than fluorophores. Sometimes it is scattered lightsheet-light from small particles like beads that is used for the imaging.

[0089] An RSI lightsheet microscope **450** in accordance with the present invention may be used in conjunction with a technique like Photo-Activated Localization Microscopy (PALM) or Stochastic Optical Reconstruction Microscopy (STORM). PALM and STORM are used for imaging on a spatial scale smaller than the wavelength of light. An RSI lightsheet microscope **450** used in accordance with the present invention may be configured for two-photon lightsheet microscopy.

[0090] Different techniques can be used to generate the lightsheet, such as a cylindrical lens or the rapid scanning of an axial beam. Different beams can be used in the lightsheet, such as Gaussian beams or Bessel beams.

[0091] There are other techniques for stepping the lightsheet through the sample (or the sample through the lightsheet). For example the stepping may skip regions of the sample known to be empty of interesting targets.

[0092] These and other advantages of the present invention will be apparent to those skilled in the art from the foregoing specification. Accordingly, it will be recognized by those skilled in the art that changes or modifications may be made to the above-described embodiments without departing from the broad inventive concepts of the invention. It should therefore be understood that this invention is not limited to the particular embodiments described herein, but is intended to include all changes and modifications that are within the scope and spirit of the invention as set forth in the claims.

Claims

What is claimed is:

1. A method for lightsheet imaging of a sample, comprising:
   providing a rotational-shear interferometer;
   setting a region-of-focus of the rotational-shear interferometer at an initial location relative to the sample;
   positioning a lightsheet at an initial location within the sample; and
   recording light emitted from the sample.

2. The method according to claim 1, wherein the step of positioning the lightsheet comprises positioning the lightsheet within the region-of-focus.

3. The method according to any one of the preceding claims, comprising moving the location of the lightsheet relative to the sample and then recording light emitted from the sample at the new location.

4. The method according to claim 3, where the step of moving the location of the lightsheet relative to the sample comprising moving the sample.

5. The method according to claim 3, where the step of moving the location of the lightsheet relative to the sample comprising moving the lightsheet.

6. The method according to any one of the preceding claims, comprising moving the location of the region-of-focus of the rotational-shear interferometer within the sample.

7. The method according to claim 6, wherein the step of moving the location of the region-of-focus comprises adjusting the rotational-shear interferometer focus.

8. An optical imaging system, comprising:
   a source of optical radiation;
   lightsheet optics in optical communication with the source of optical radiation,

the optics configured to receive the optical radiation and configured to generate a lightsheet at a selected location within the optical imaging system;

collection optics in optical communication with the selected location; and

a rotational-shear interferometer in optical communication with the collection optics.

Abstract

Devices and methods for lightsheet microscopy using rotational-shear interferometry are provided. Advantages include improved lateral spatial resolution and easier alignment.

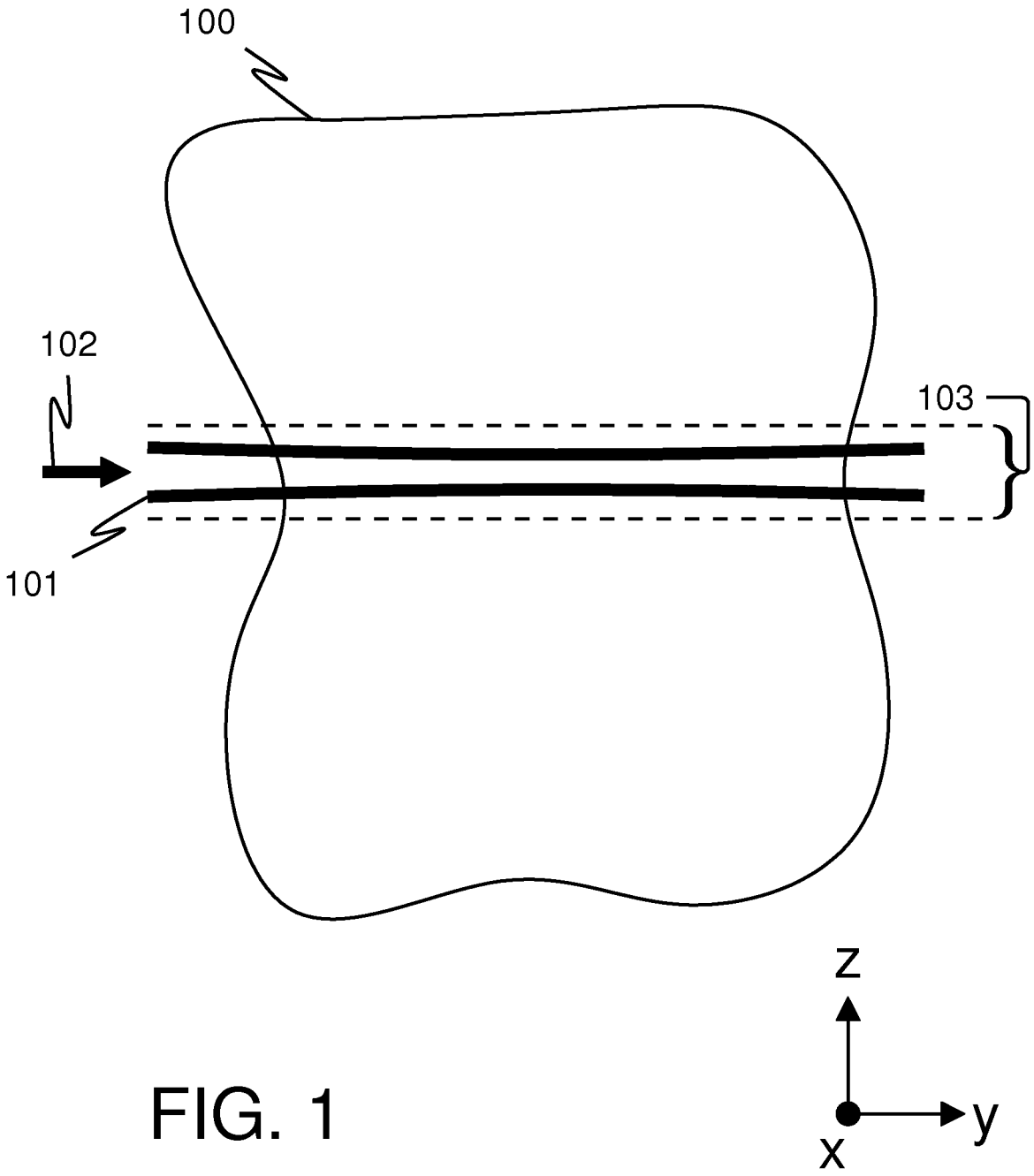


FIG. 1

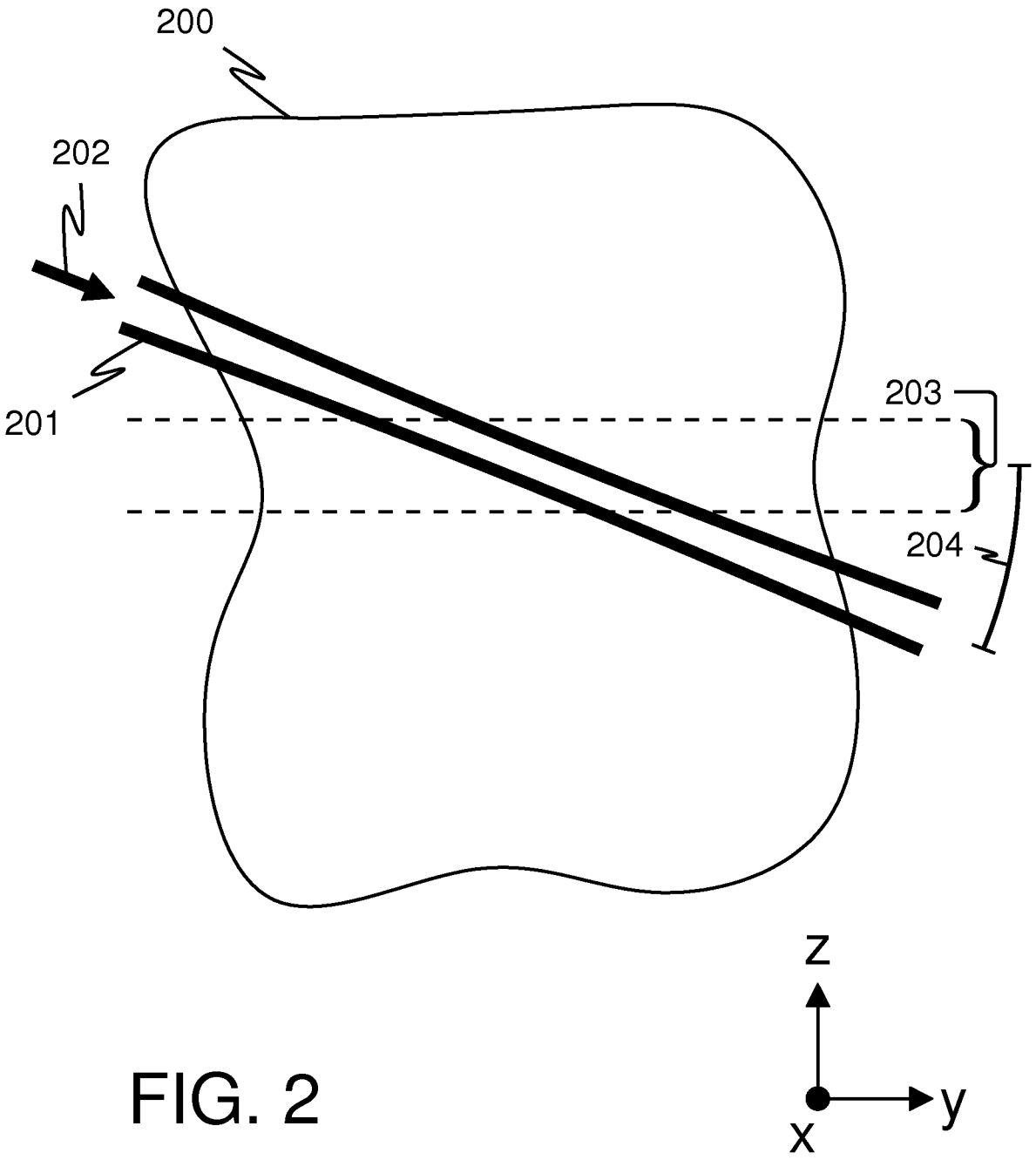


FIG. 2

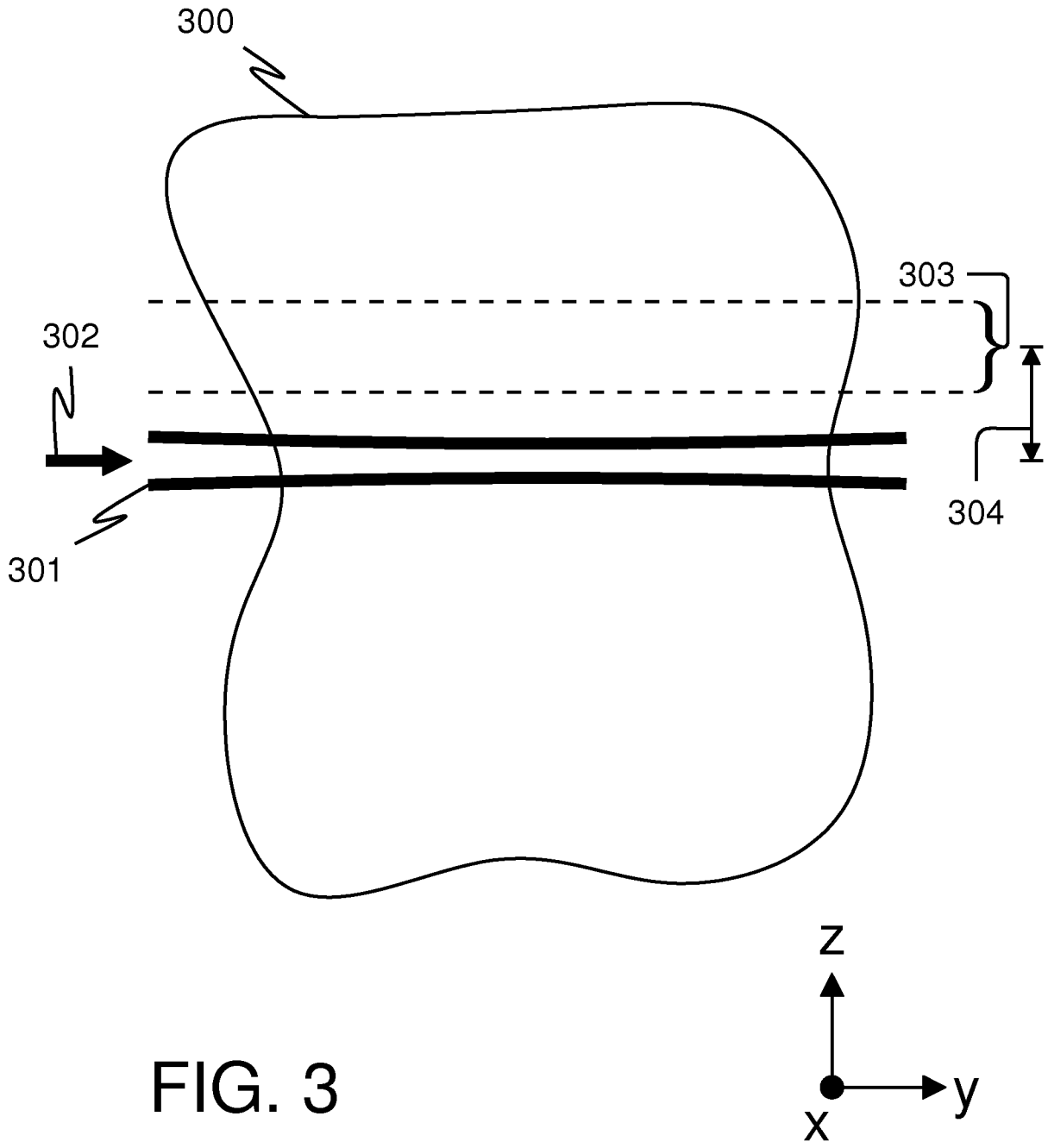


FIG. 3

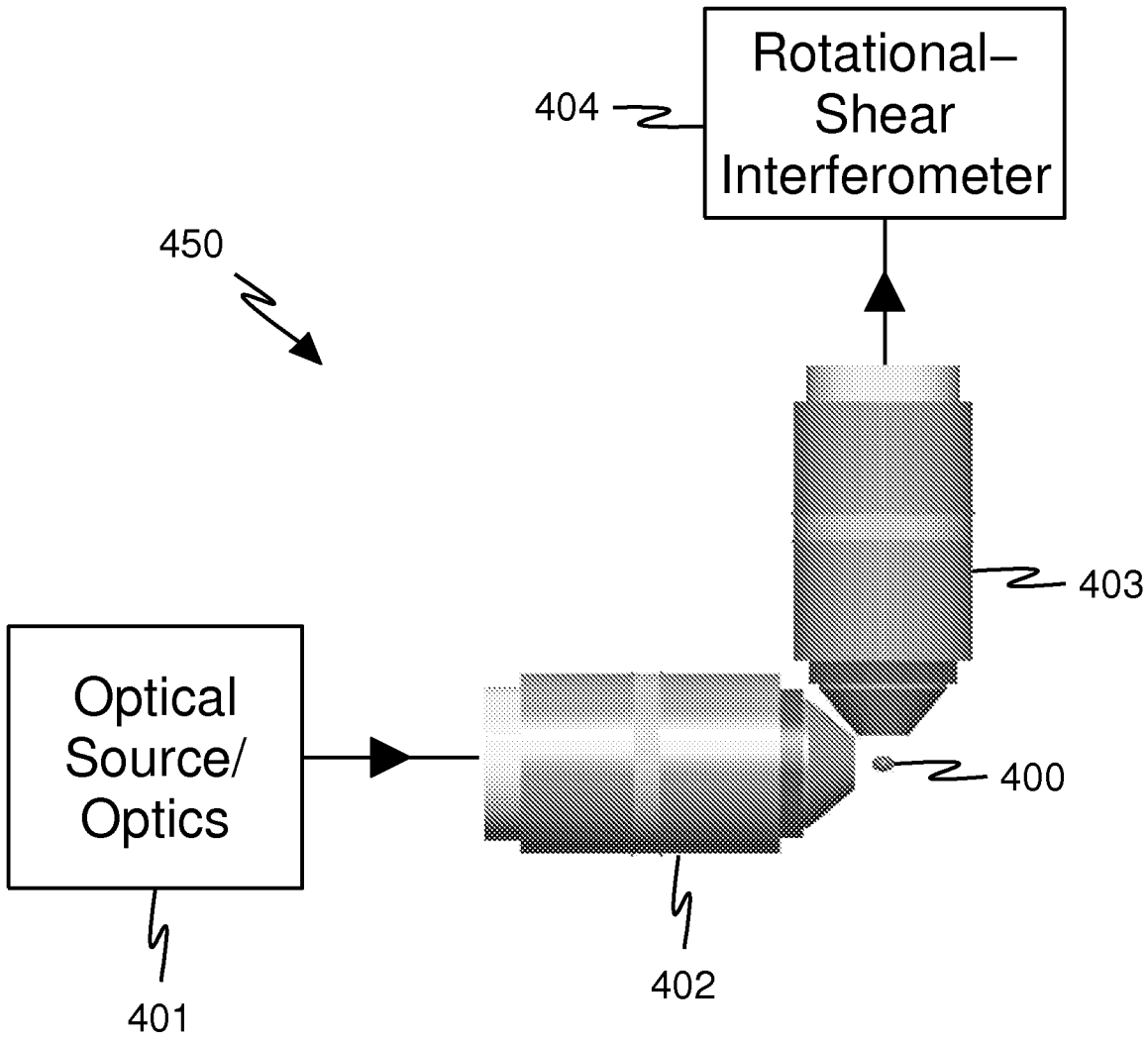
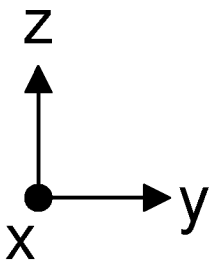

Rotational–
Shear
Interferometer
404
450
403
Optical
Source/
Optics
400
401
402

FIG. 4


z
y
x

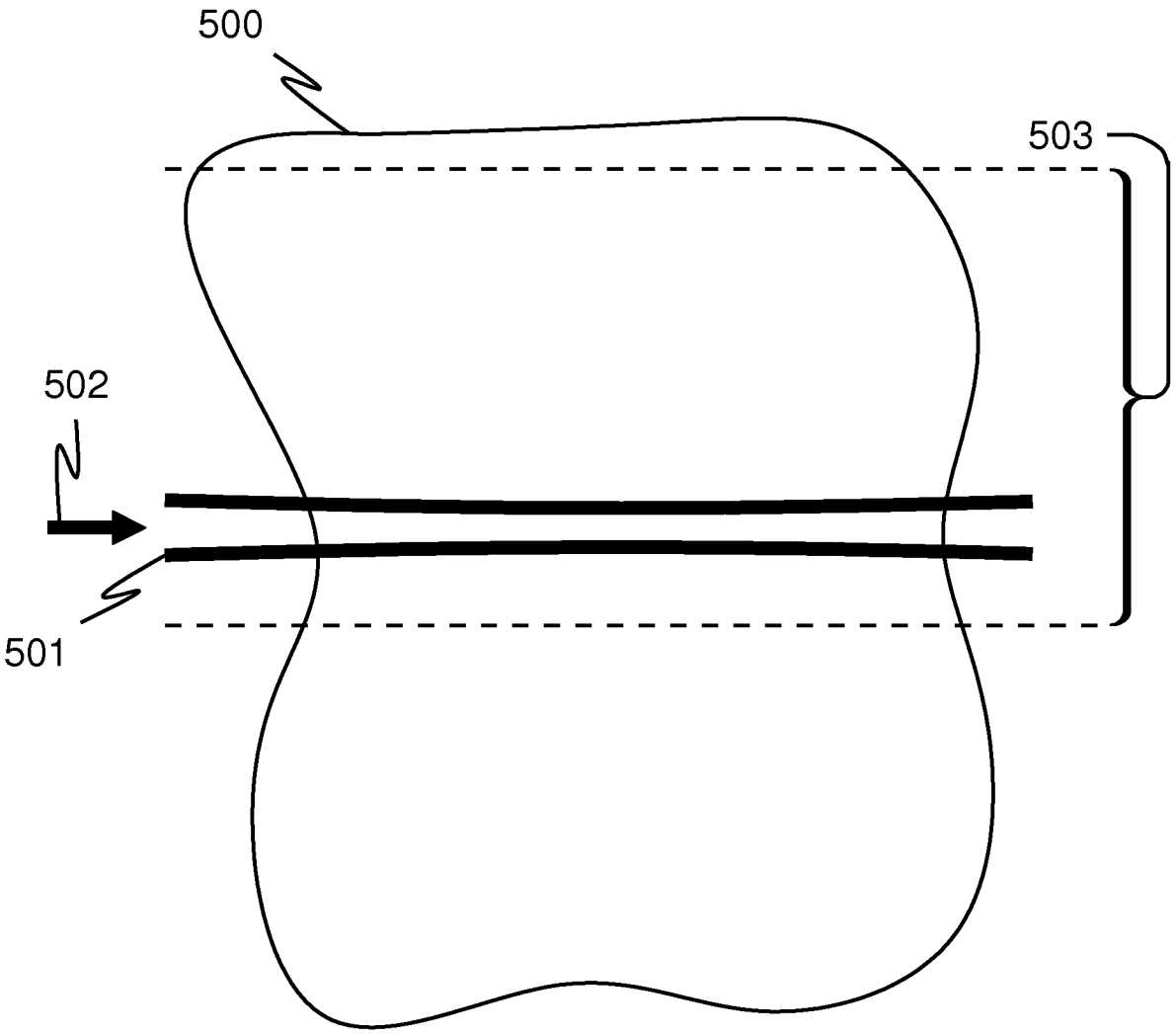
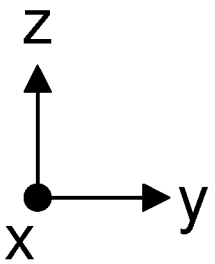


FIG. 5



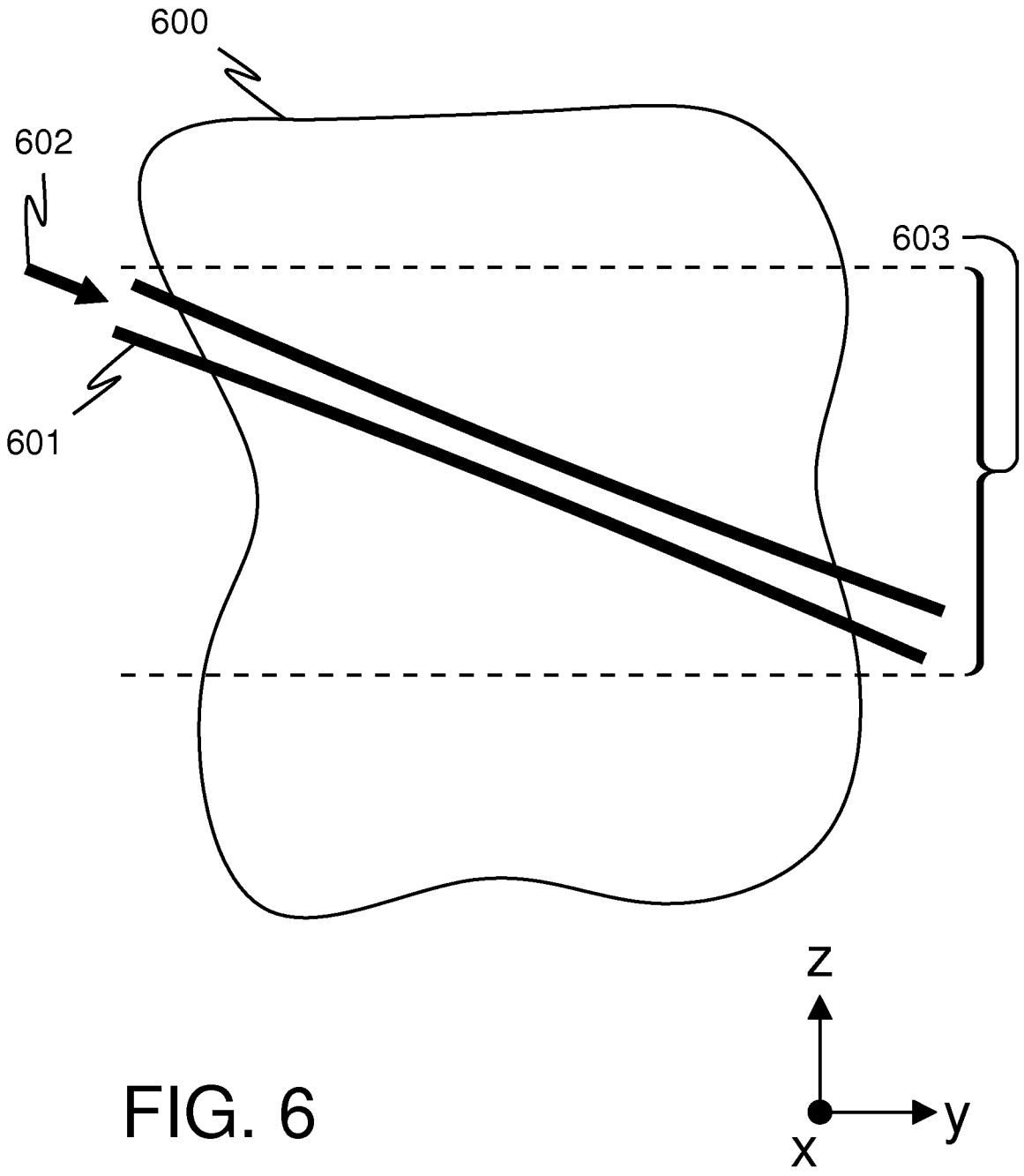


FIG. 6

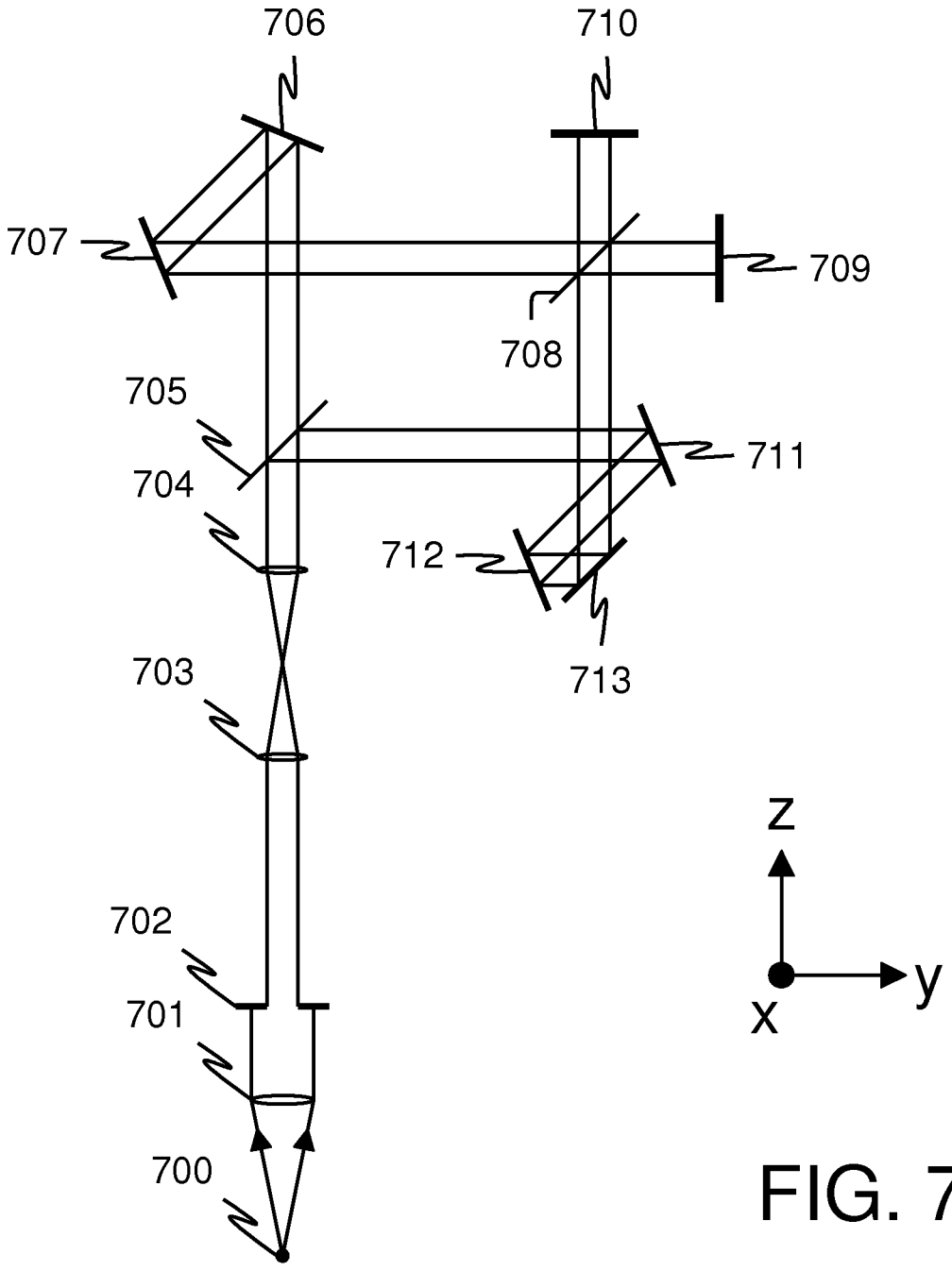


FIG. 7

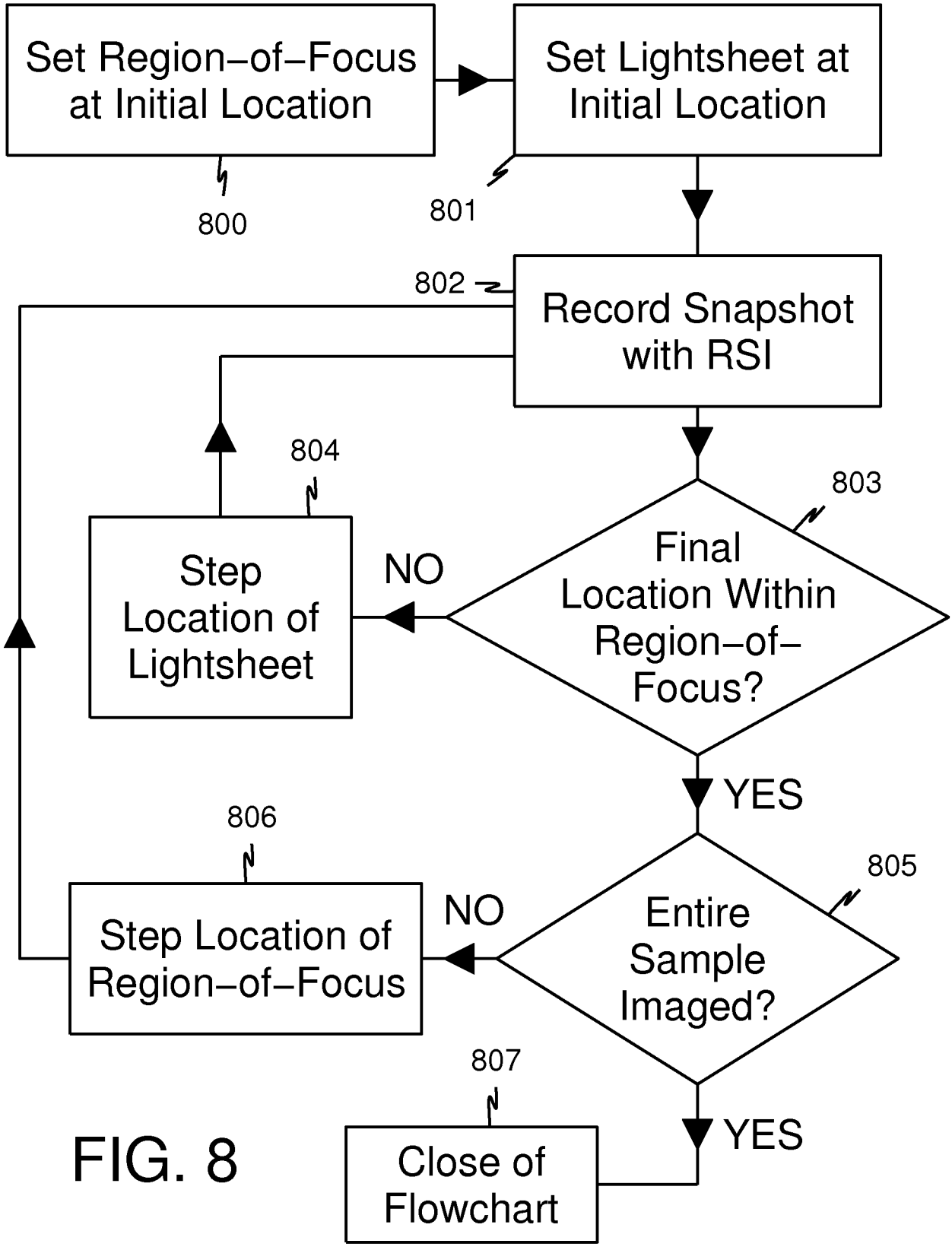

Set Region–of–Focus at Initial Location
800
Set Lightsheet at Initial Location
801
802
Record Snapshot with RSI
804
Step Location of Lightsheet
NO
803
Final Location Within Region–of–Focus?
YES
806
Step Location of Region–of–Focus
NO
805
Entire Sample Imaged?
807
Close of Flowchart
YES

FIG. 8

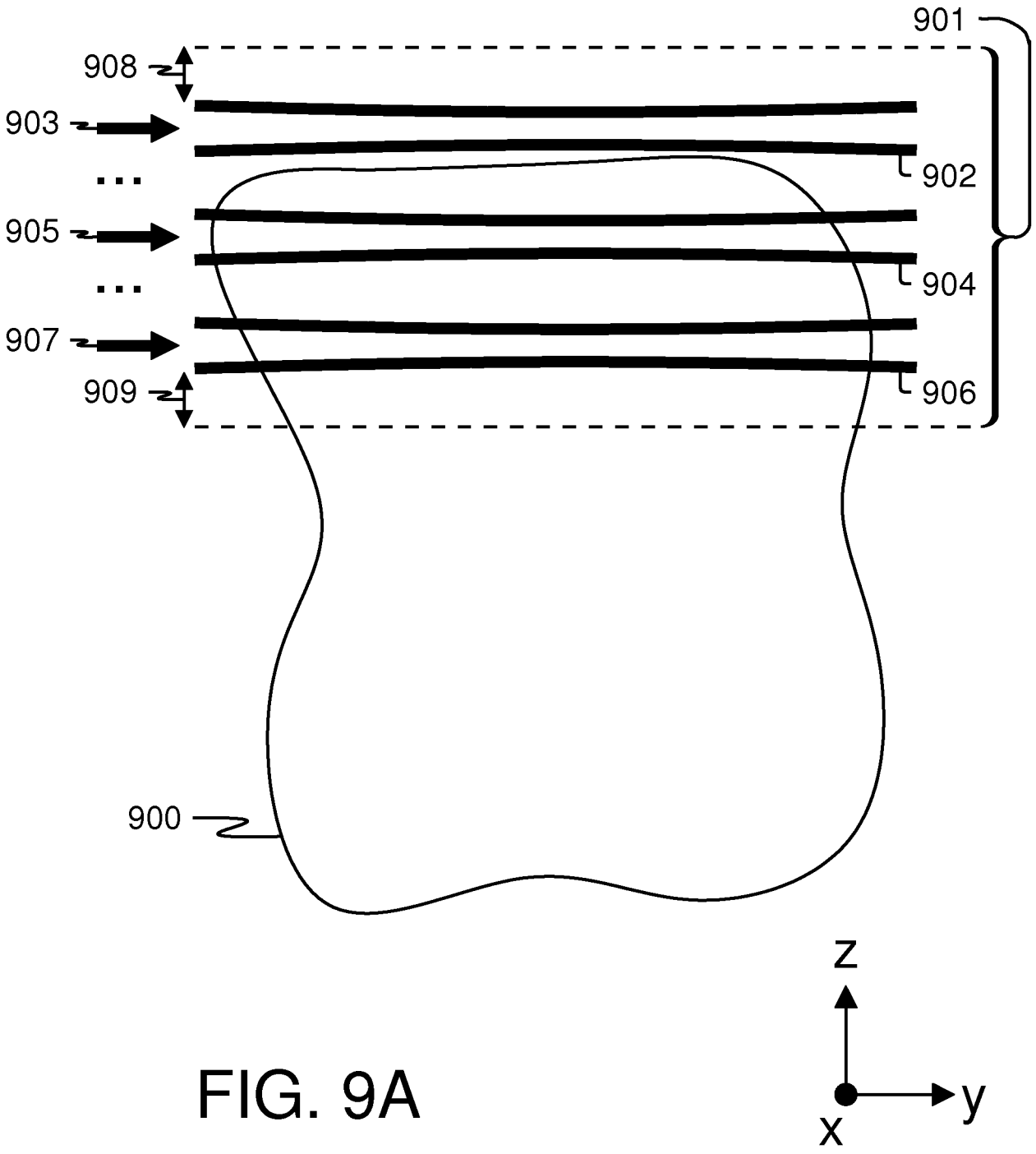


FIG. 9A

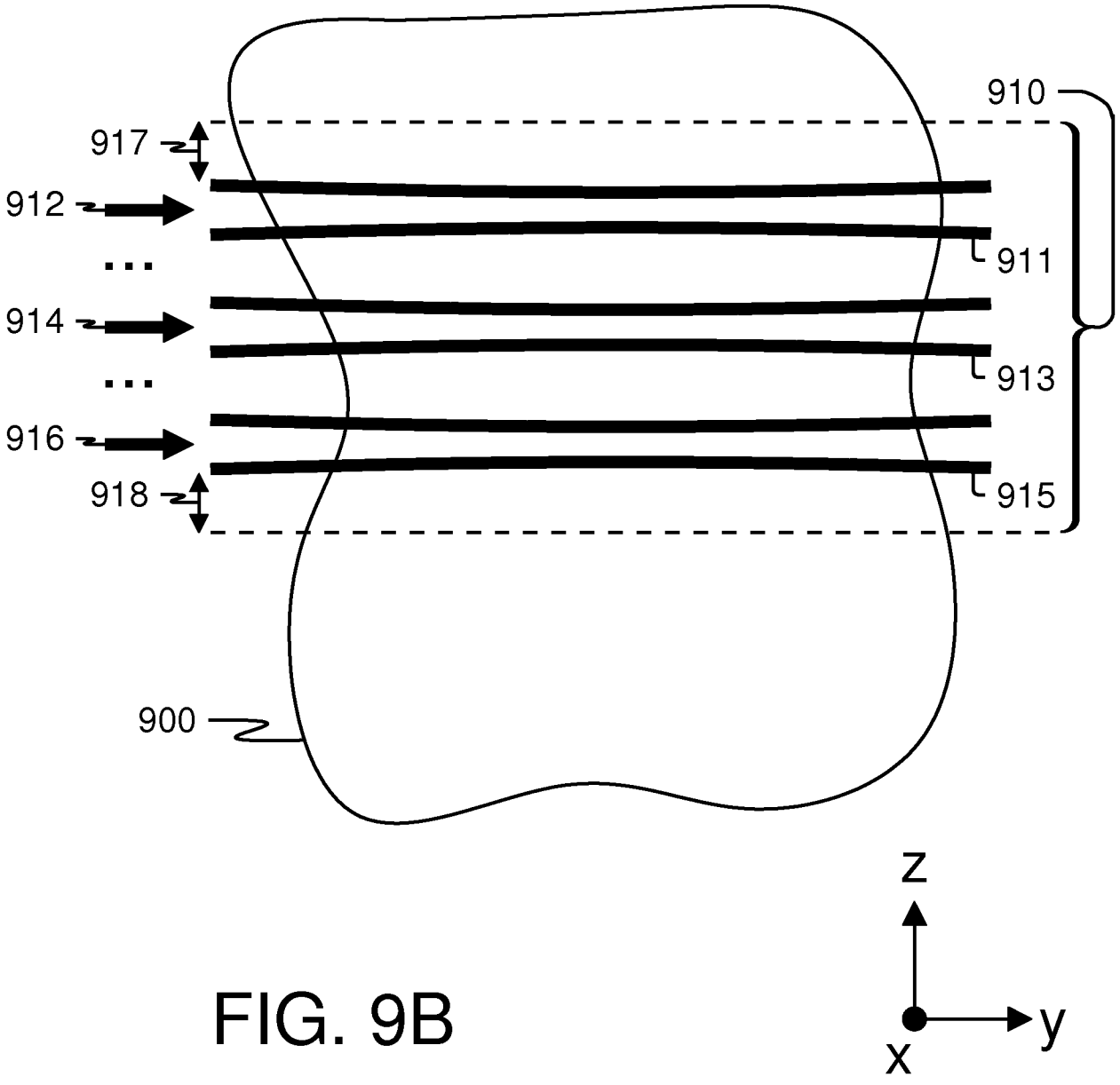


FIG. 9B

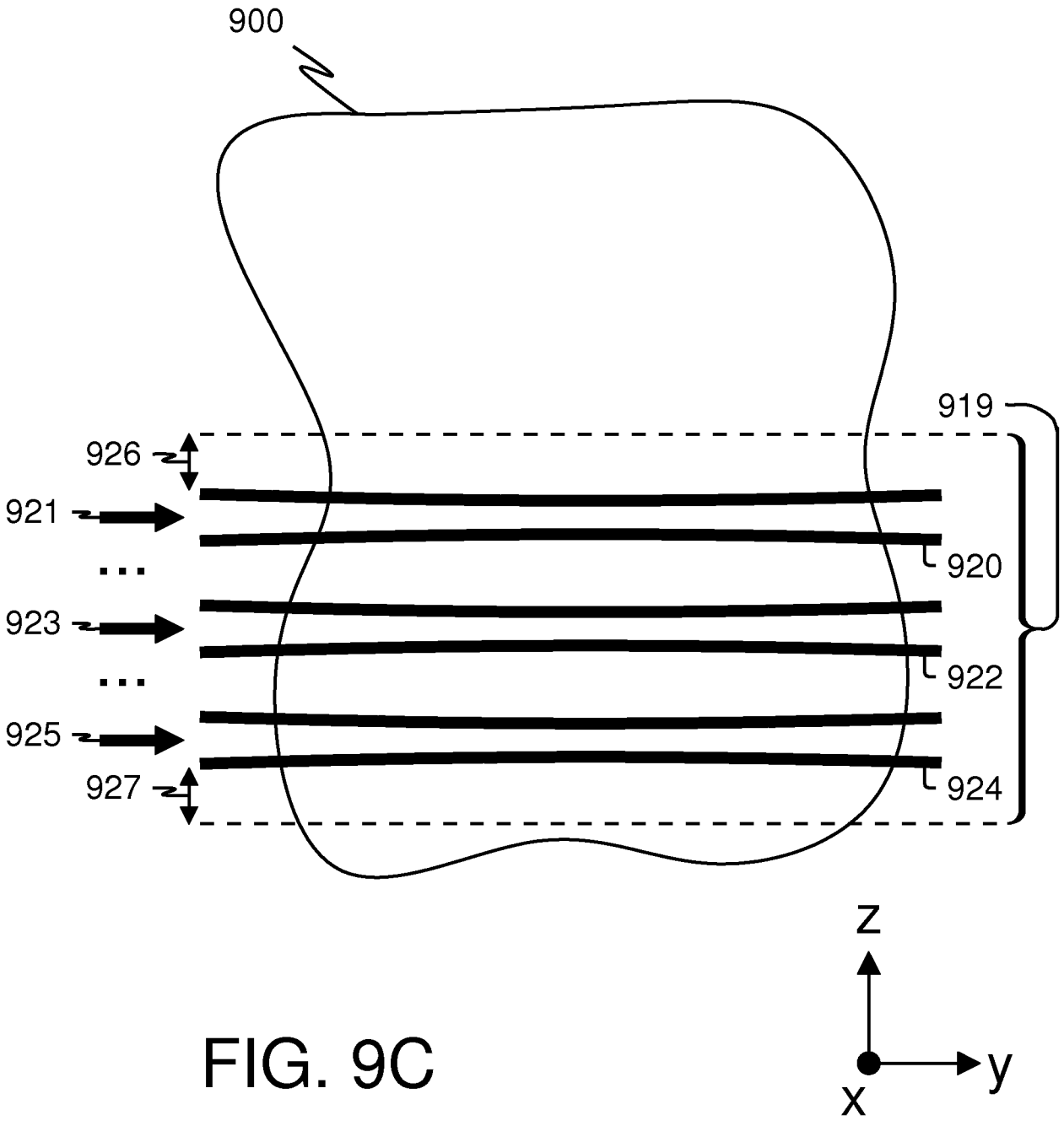


FIG. 9C

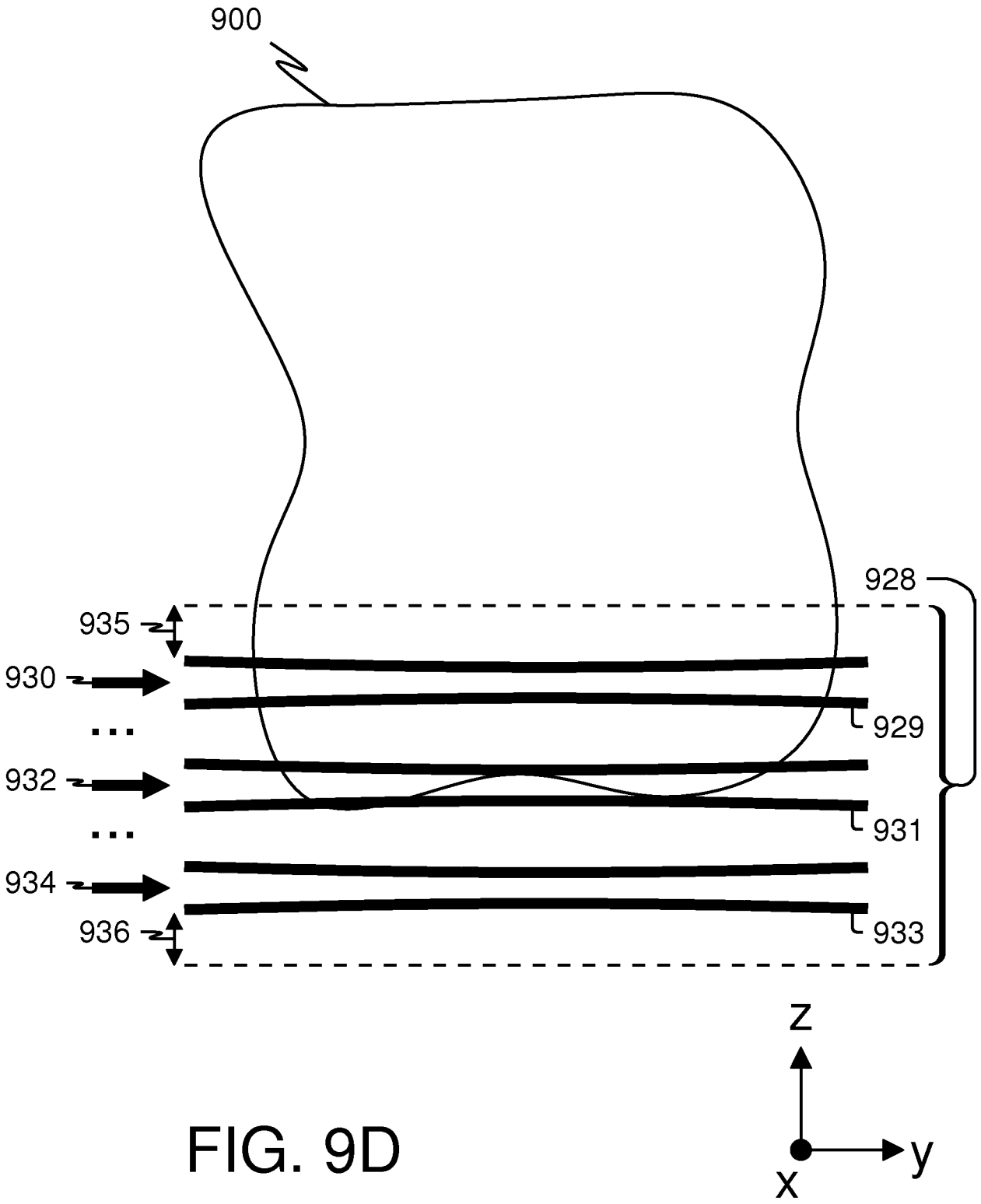


FIG. 9D

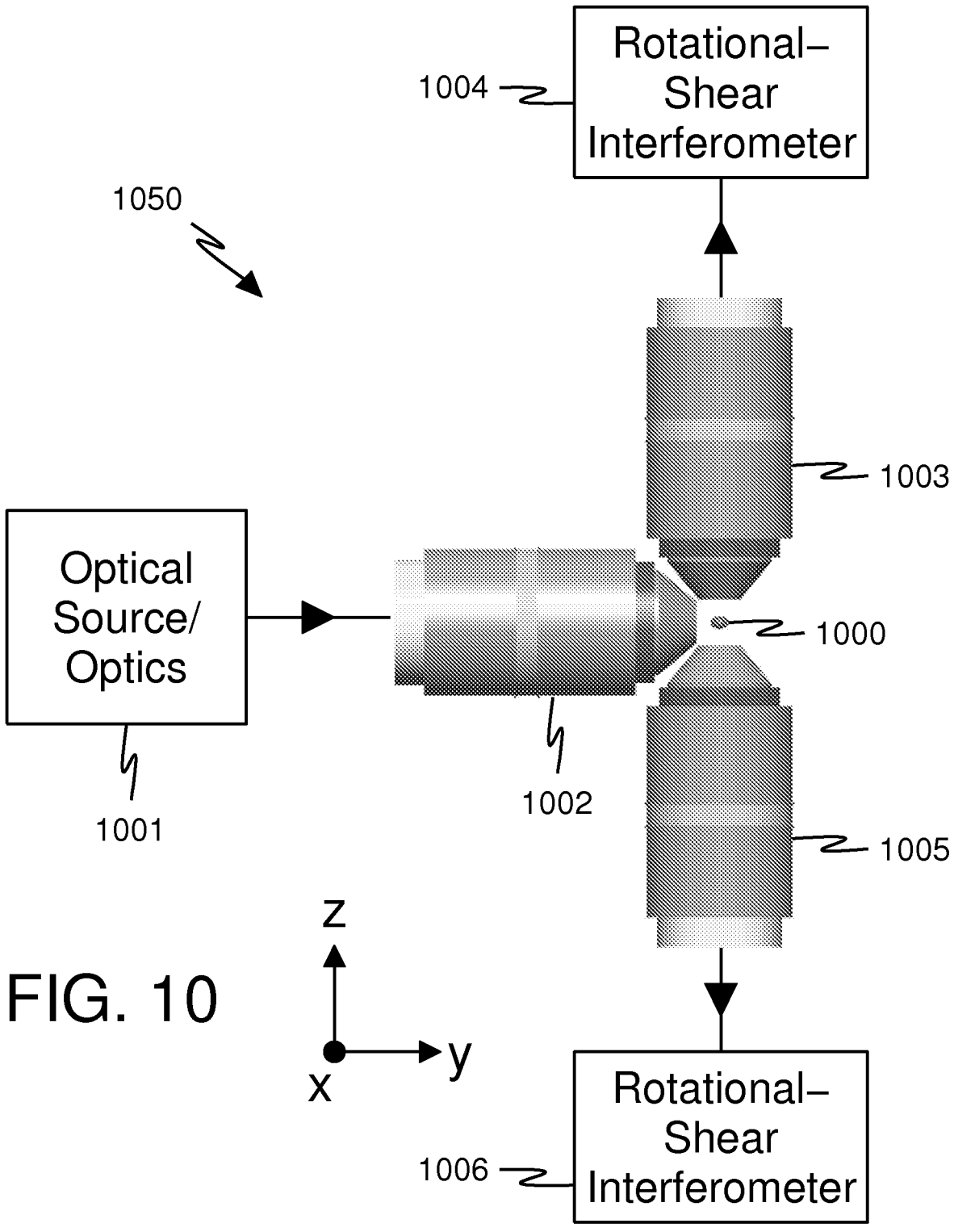

Rotational–
Shear
Interferometer
1004
1050
1003
Optical
Source/
Optics
1000
1001
1002
1005
z
y
x
Rotational–
Shear
Interferometer
1006

FIG. 10