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Fauver et al.

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(45) Date of Patent: Mar. 15, 2011

(54) **FOCAL PLANE TRACKING FOR OPTICAL MICROTOMOGRAPHY**

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(73) Assignees: **University of Washington**, Seattle, WA (US); **Visiongate, Inc.**, Pheonix, AZ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1213 days.

(21) Appl. No.: **11/532,648**

(22) Filed: **Sep. 18, 2006**

(65) **Prior Publication Data**

US 2010/0322494 A1 Dec. 23, 2010

Related U.S. Application Data

(63) Continuation-in-part of application No. 11/203,878, filed on Aug. 15, 2005, now abandoned, which is a continuation-in-part of application No. 10/308,309, filed on Dec. 3, 2002, now Pat. No. 6,944,322, which is a continuation-in-part of application No. 09/927,151, filed on Aug. 10, 2001, now Pat. No. 6,522,775.

(60) Provisional application No. 60/279,244, filed on Mar. 28, 2001.

(51) **Int. Cl.**
G06K 9/00 (2006.01)

(52) **U.S. Cl.** **382/131**; 382/132

(58) **Field of Classification Search** 382/128–132
See application file for complete search history.

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Primary Examiner — Wesley Tucker

(74) *Attorney, Agent, or Firm* — Citadel Patent Law; George A. Leone

(57) **ABSTRACT**

An optical tomography system for imaging an object of interest including a light source for illuminating the object of interest with a plurality of radiation beams. The object of interest is held within an object containing tube such that it is illuminated by the plurality of radiation beams to produce emerging radiation from the object containing tube, a detector array is located to receive the emerging radiation and produce imaging data used by a mechanism for tracking the object of interest.

3 Claims, 21 Drawing Sheets

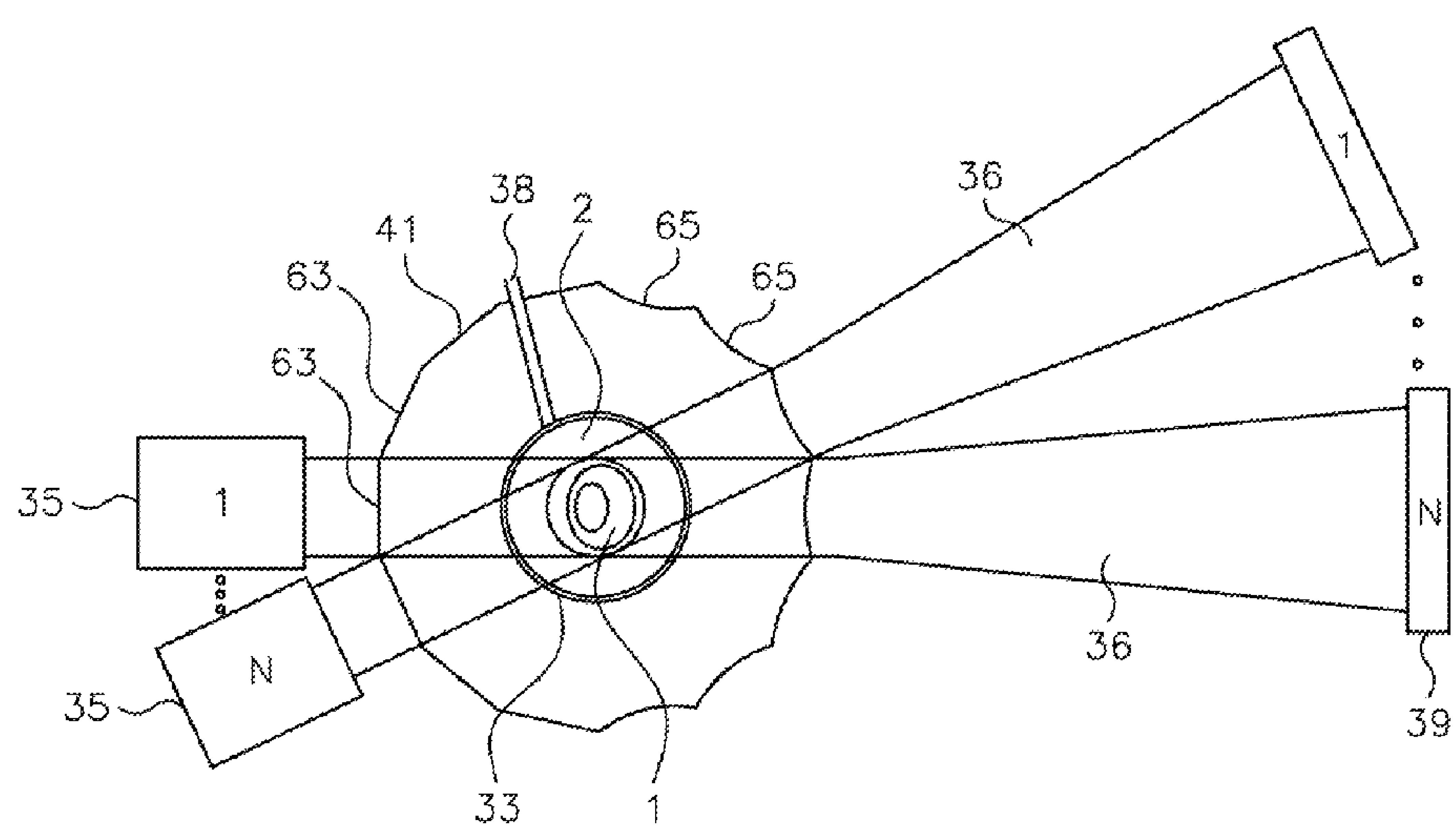

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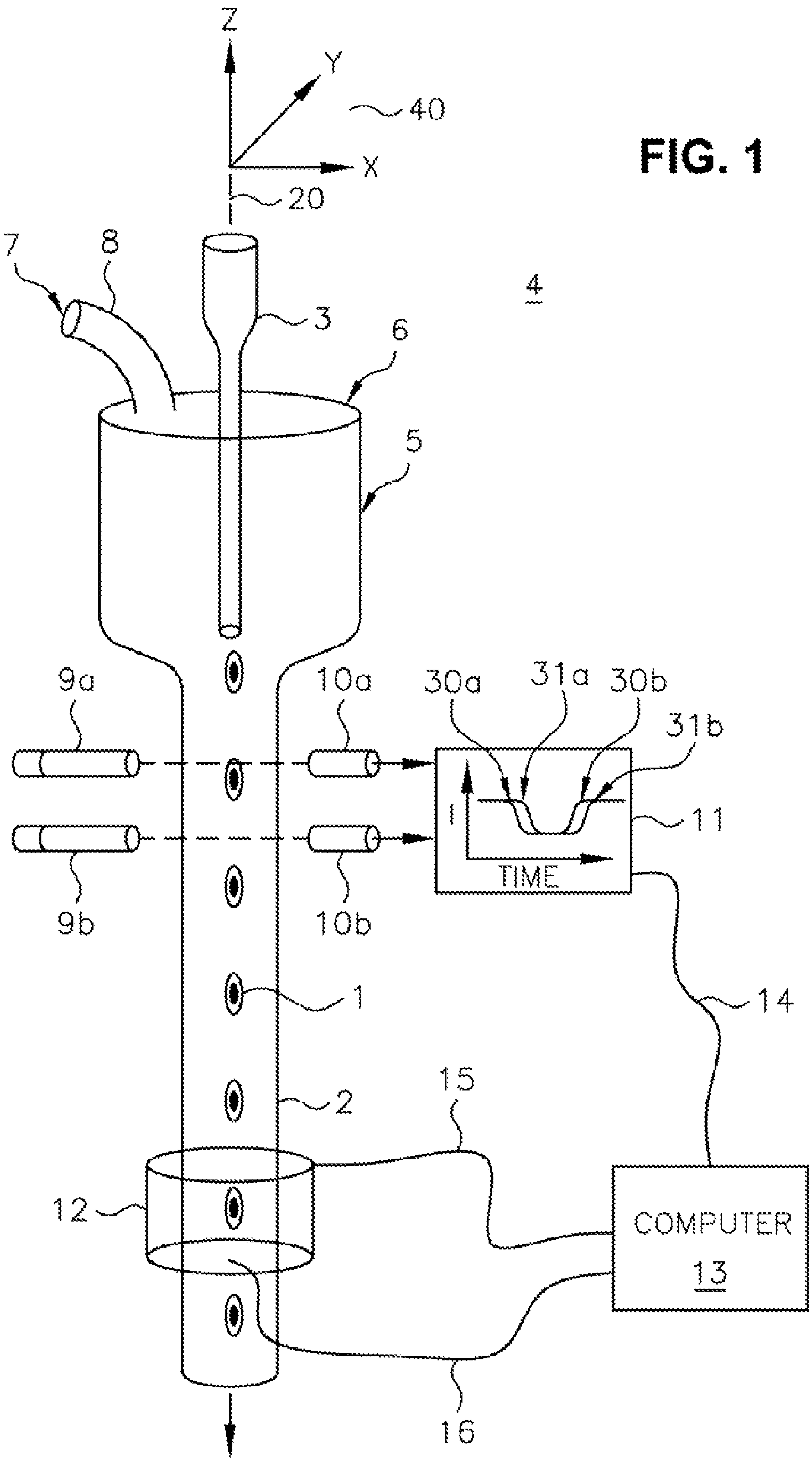
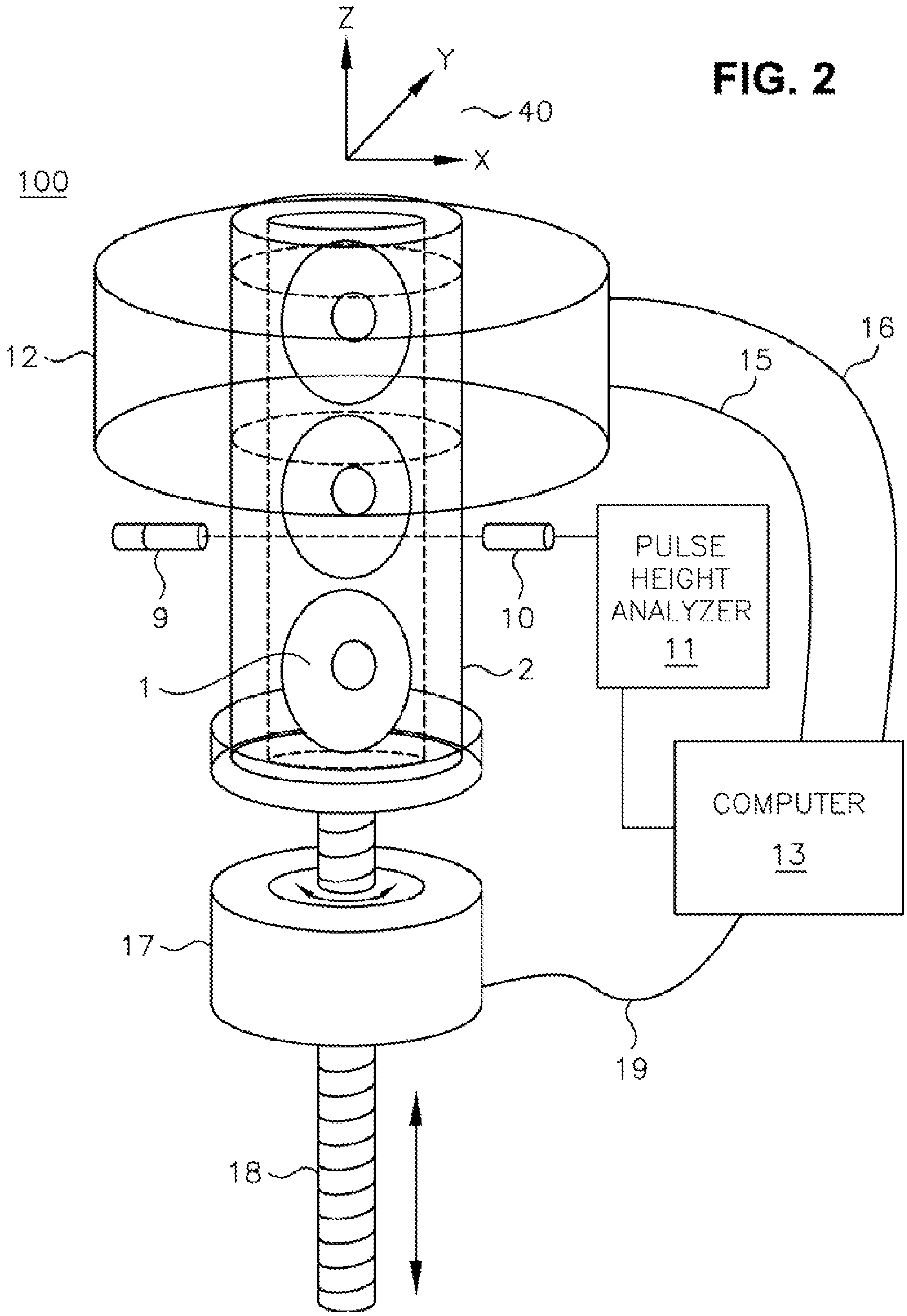
FIG. 1



Z
Y
X
40
20
7
8
3
6
4
5
9a
10a
30a
31a
30b
31b
I
TIME
11
9b
10b
1
14
15
2
12
COMPUTER
13
16

FIG. 2


Z
Y
X
40
100
12
16
15
PULSE HEIGHT ANALYZER 11
9
10
1
2
COMPUTER 13
17
19
18

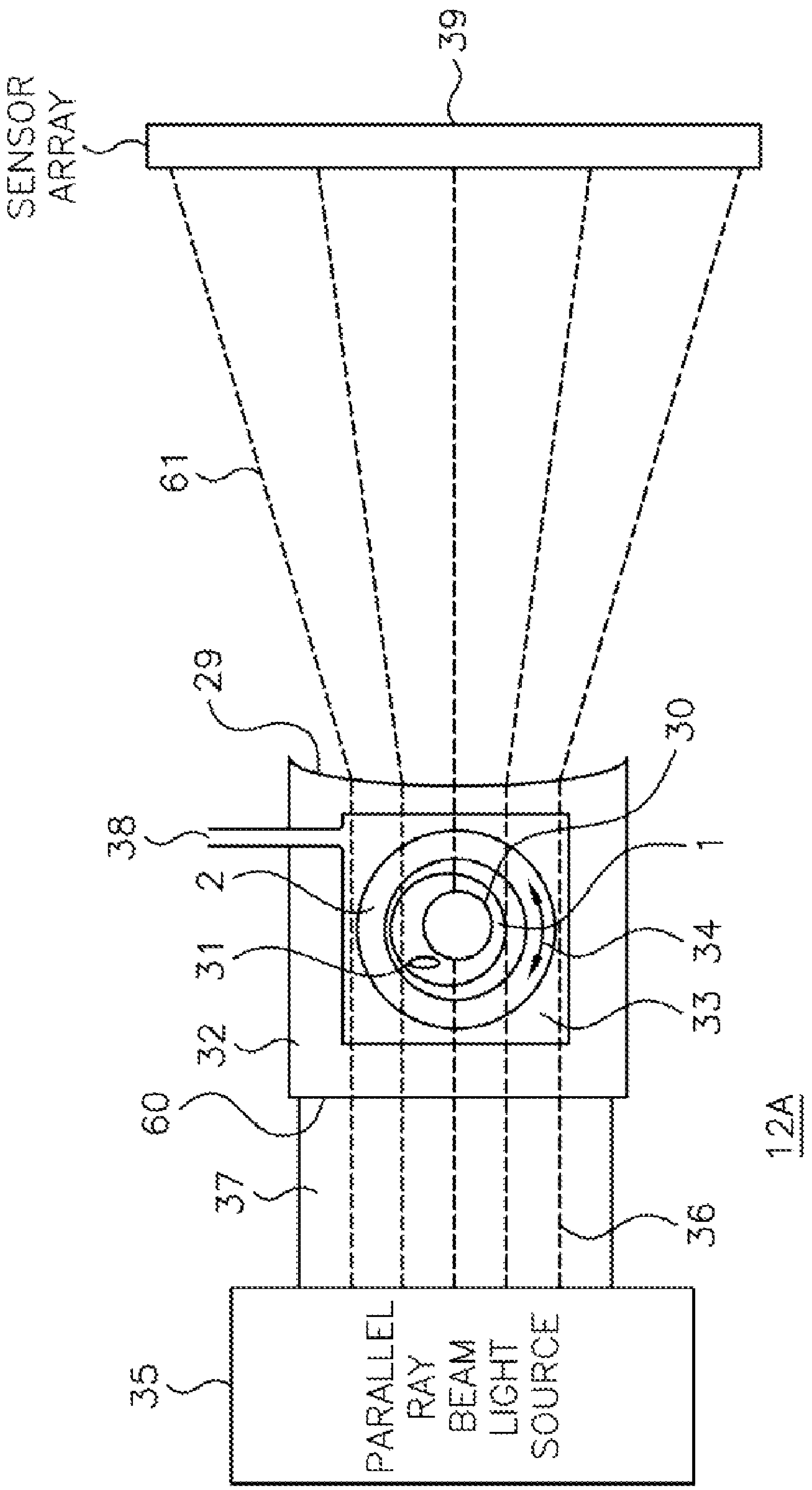

SENSOR ARRAY
39
61
29
38
2
31
32
60
37
30
1
34
33
36
35
PARALLEL RAY BEAM LIGHT SOURCE
12A

FIG. 3

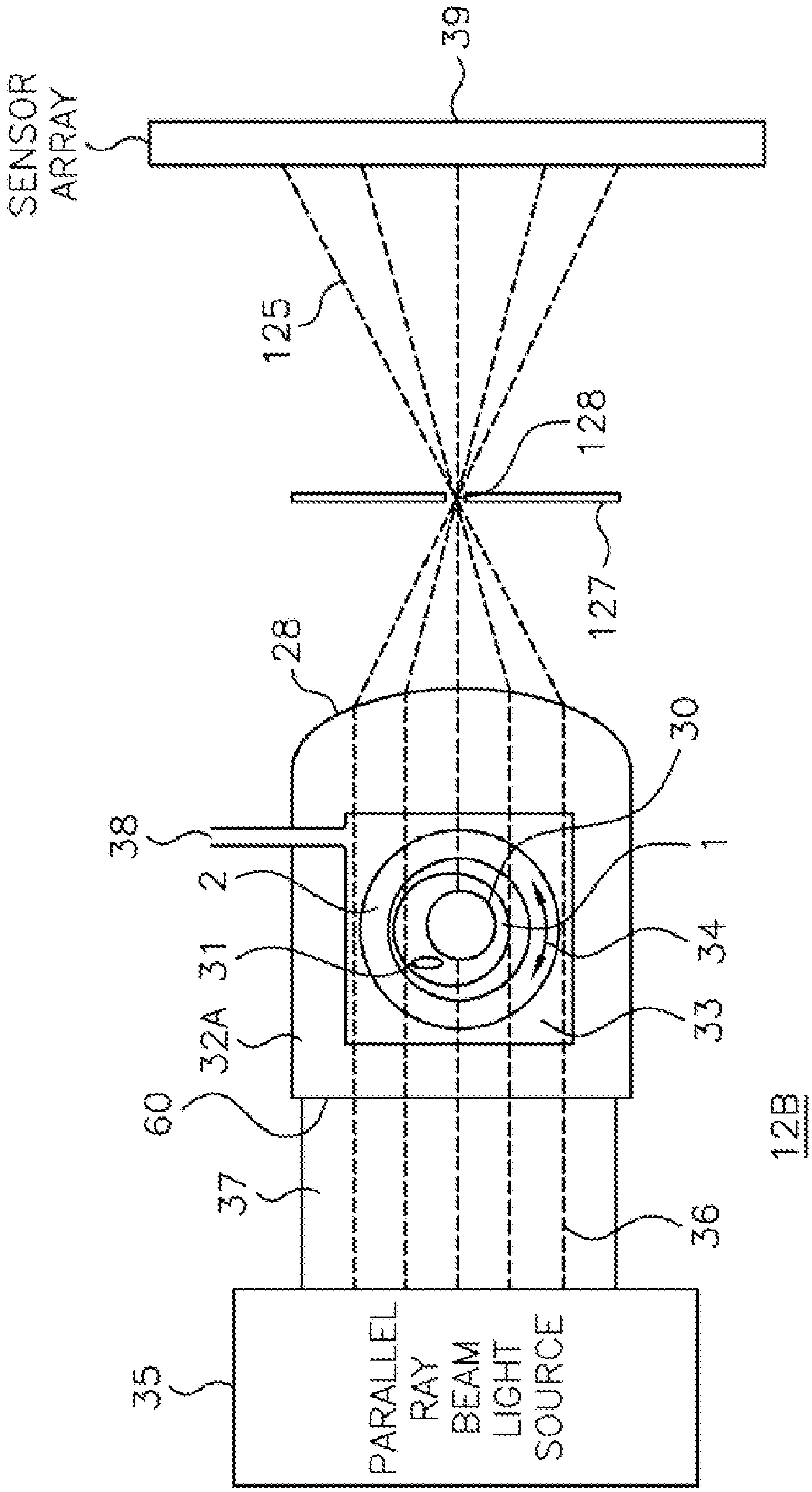
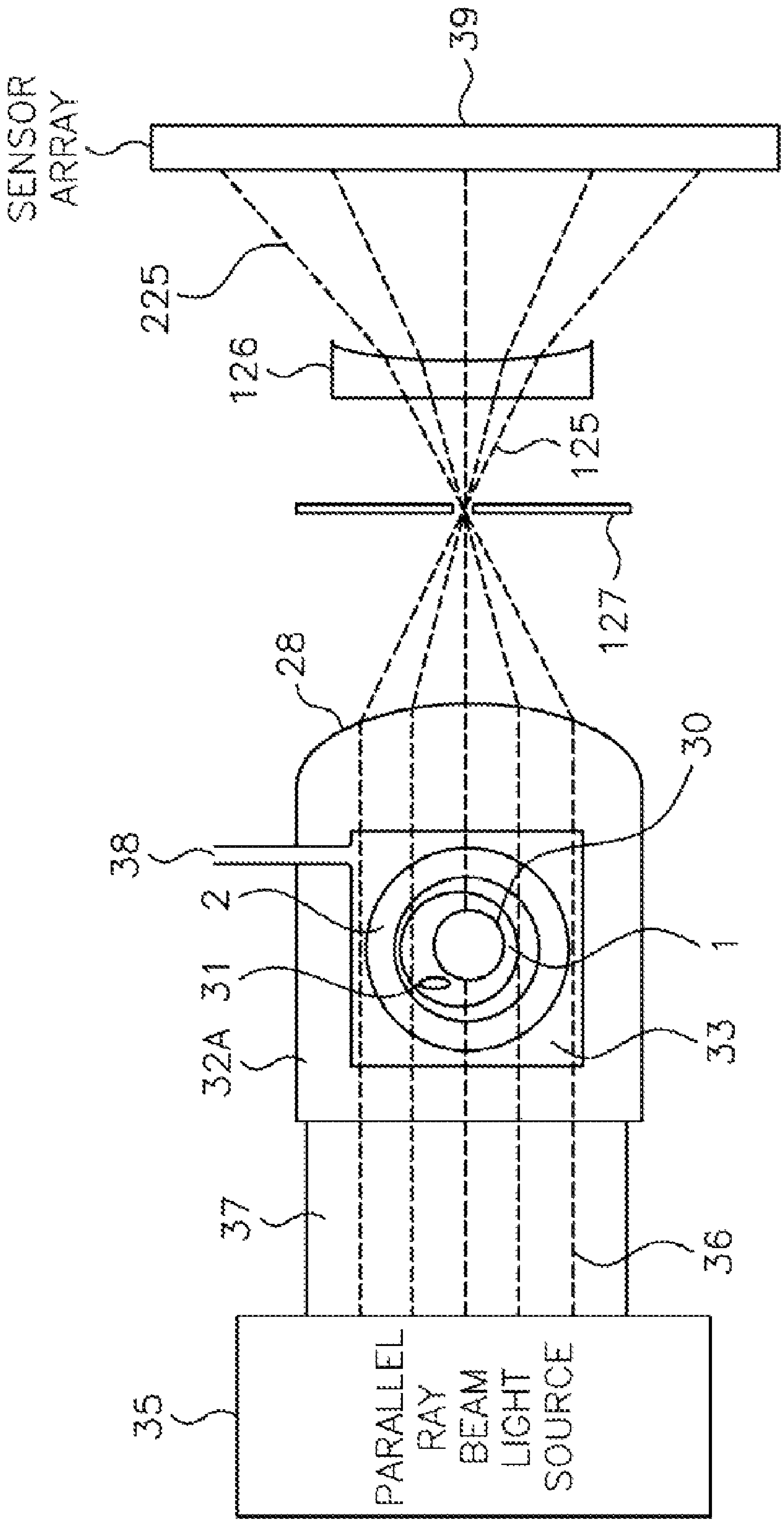

SENSOR ARRAY
39
125
128
127
28
30
38
2
1
31
34
32A
33
60
12B
37
36
35
PARALLEL RAY BEAM LIGHT SOURCE

FIG. 4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225
126
125
127
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2
31
32A
1
33
12D
37
36
35
PARALLEL RAY BEAM LIGHT SOURCE

FIG. 4A

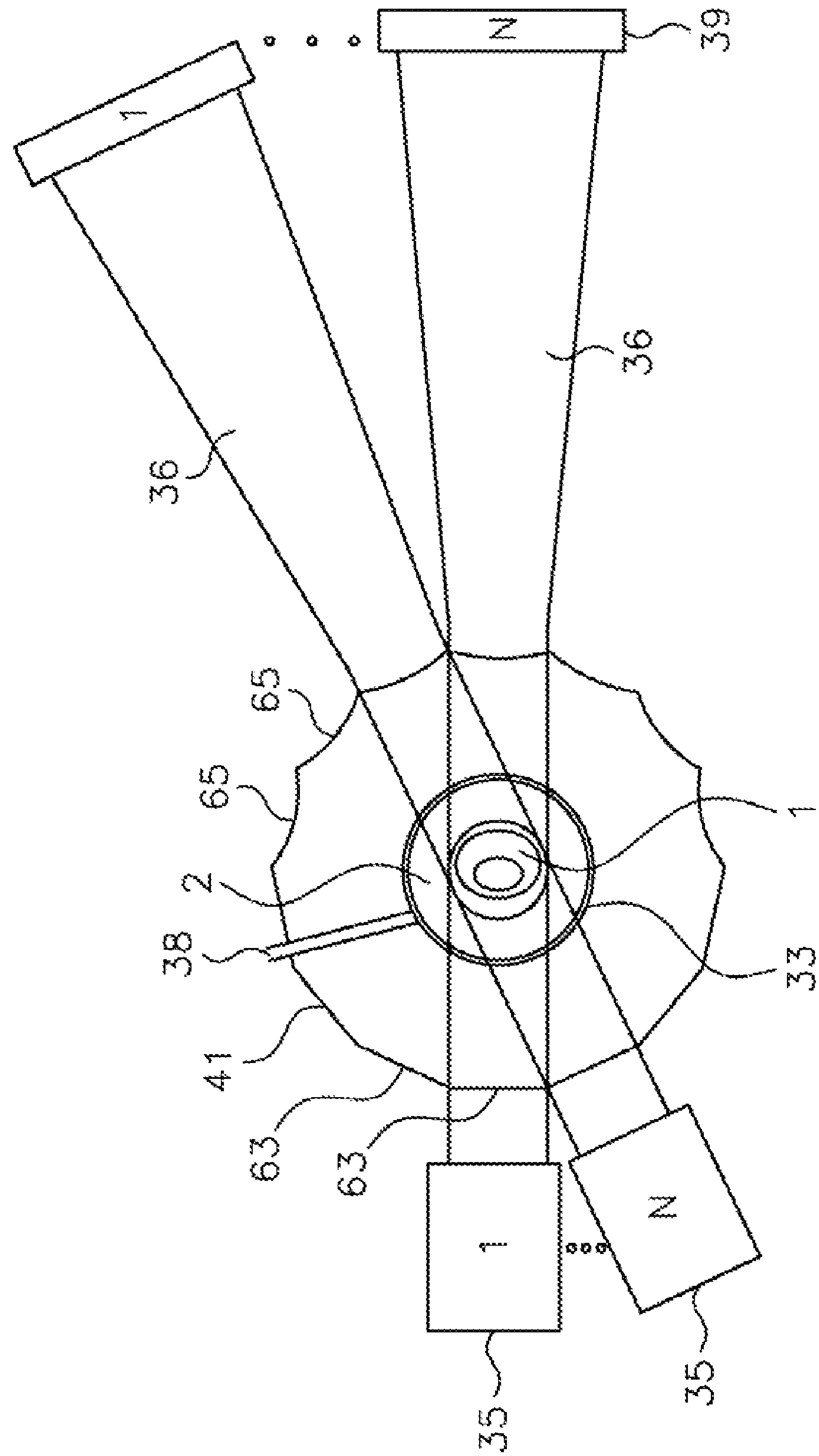


FIG. 5

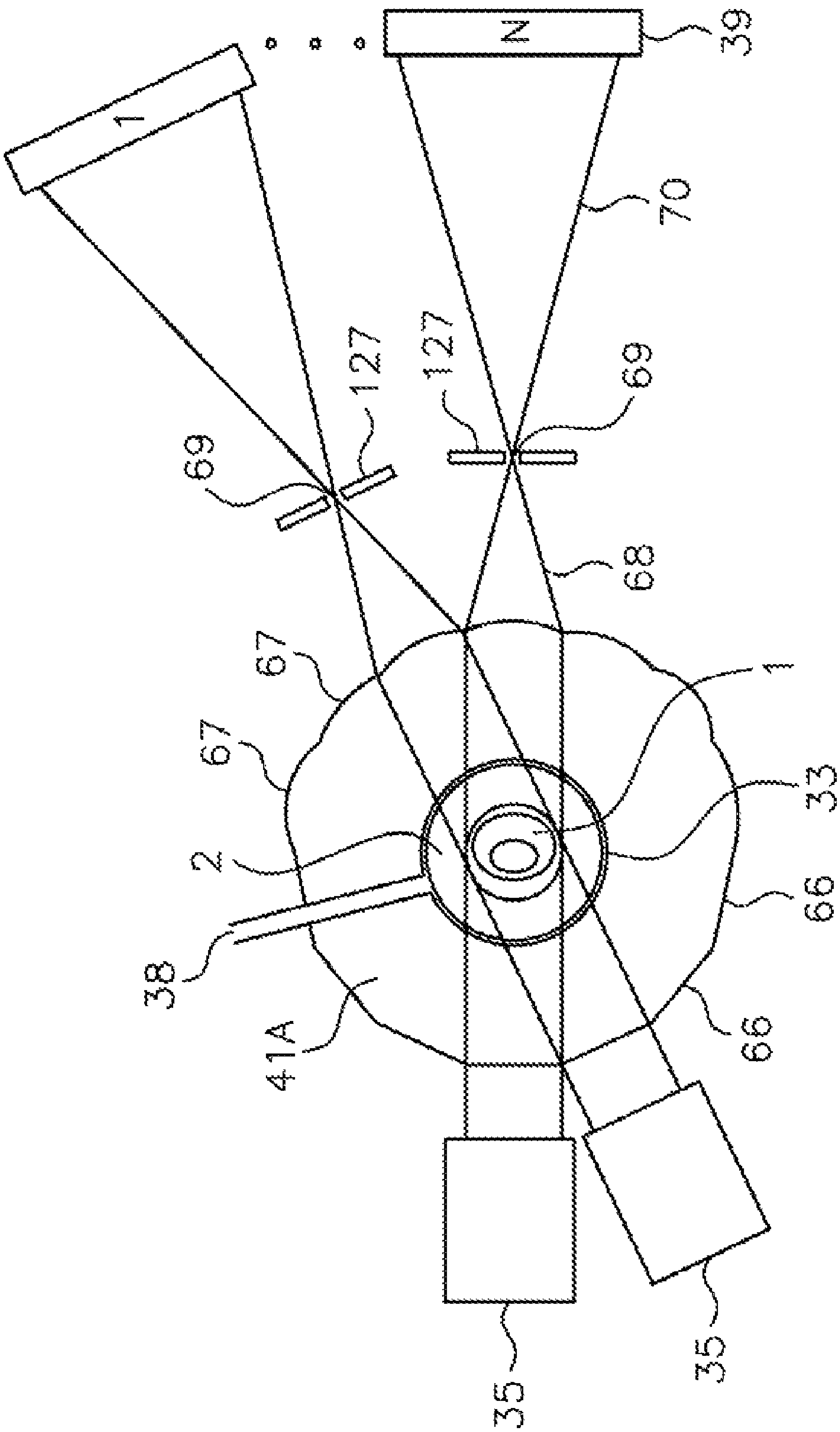


FIG. 5A

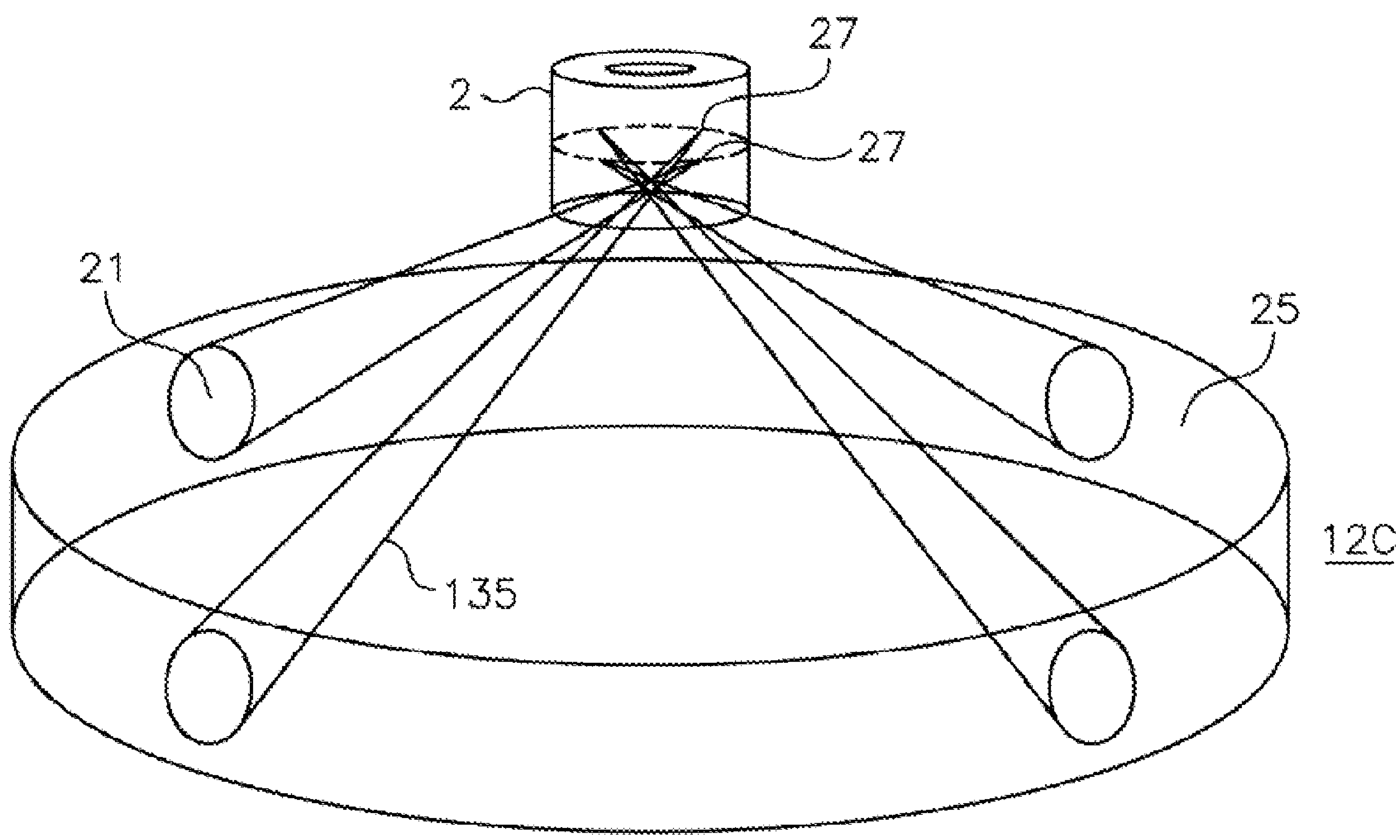
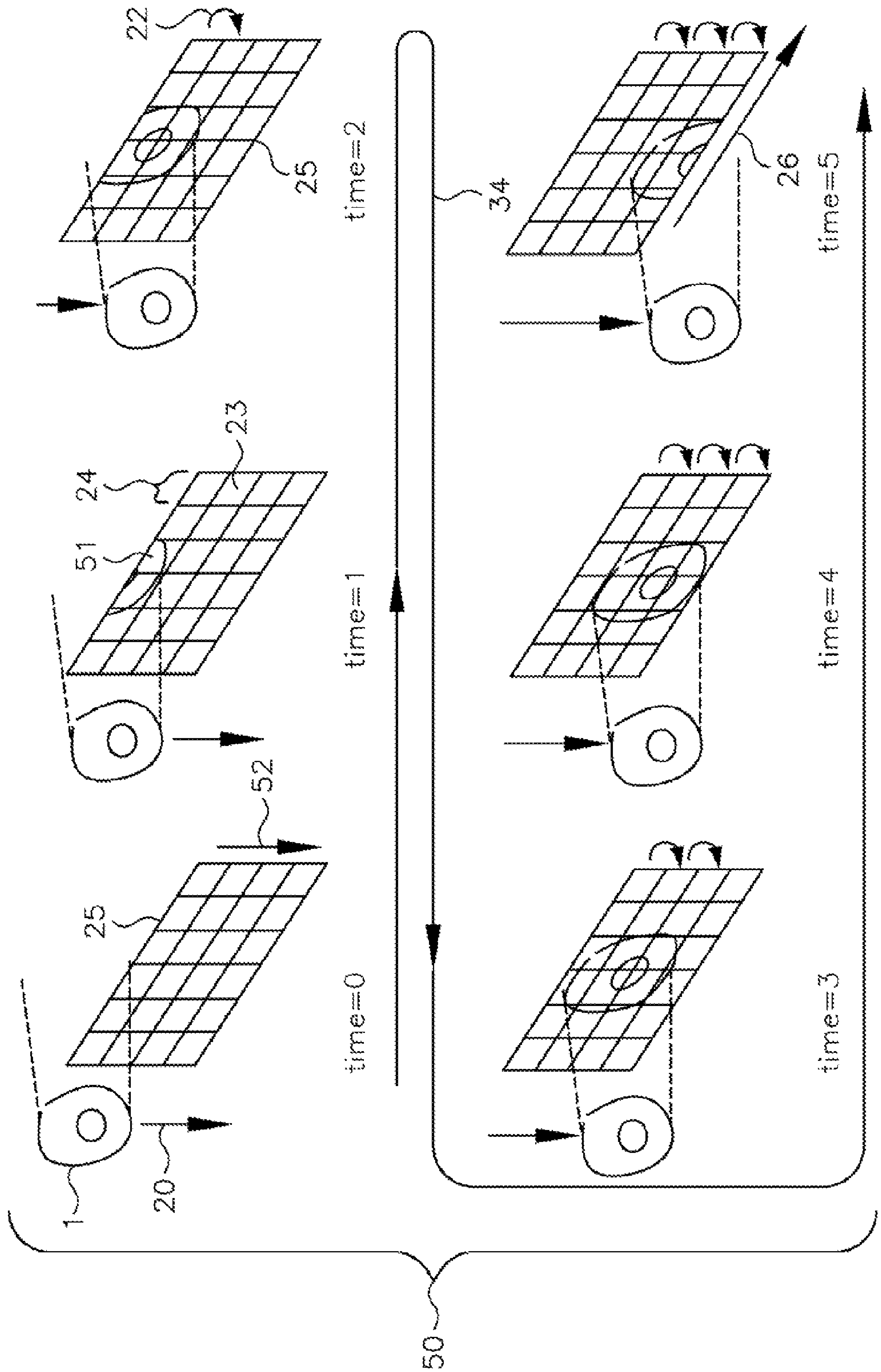


FIG. 6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time=4
time=5
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24
23
22
25
34
26
50

FIG. 7

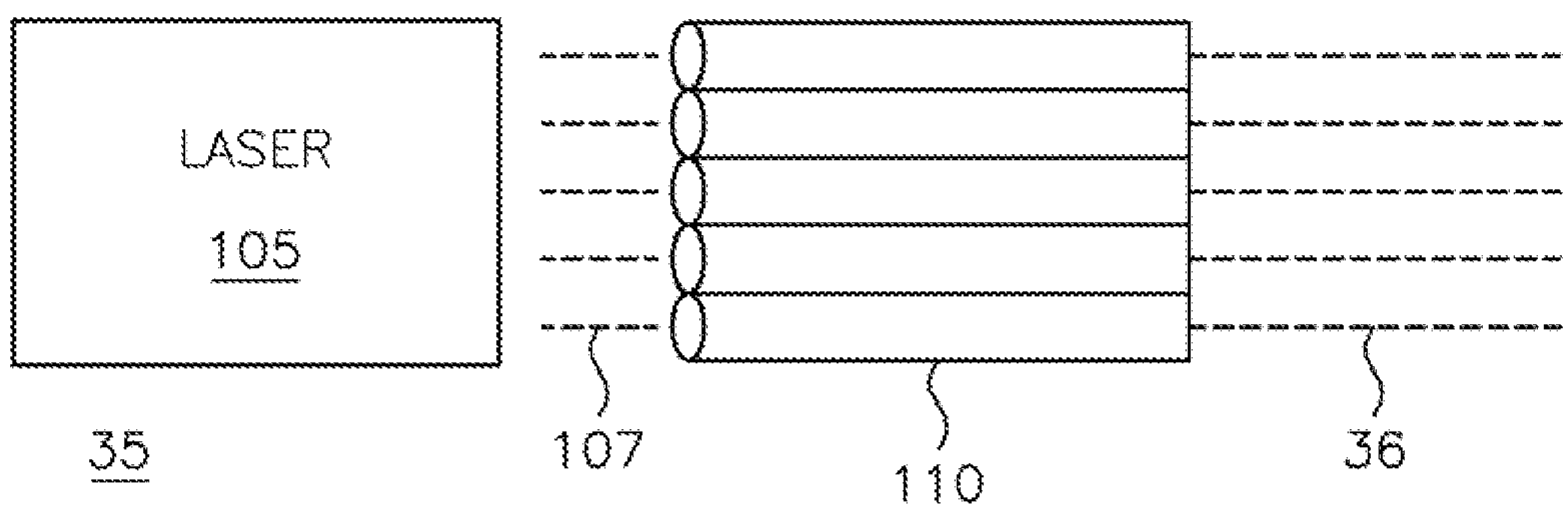
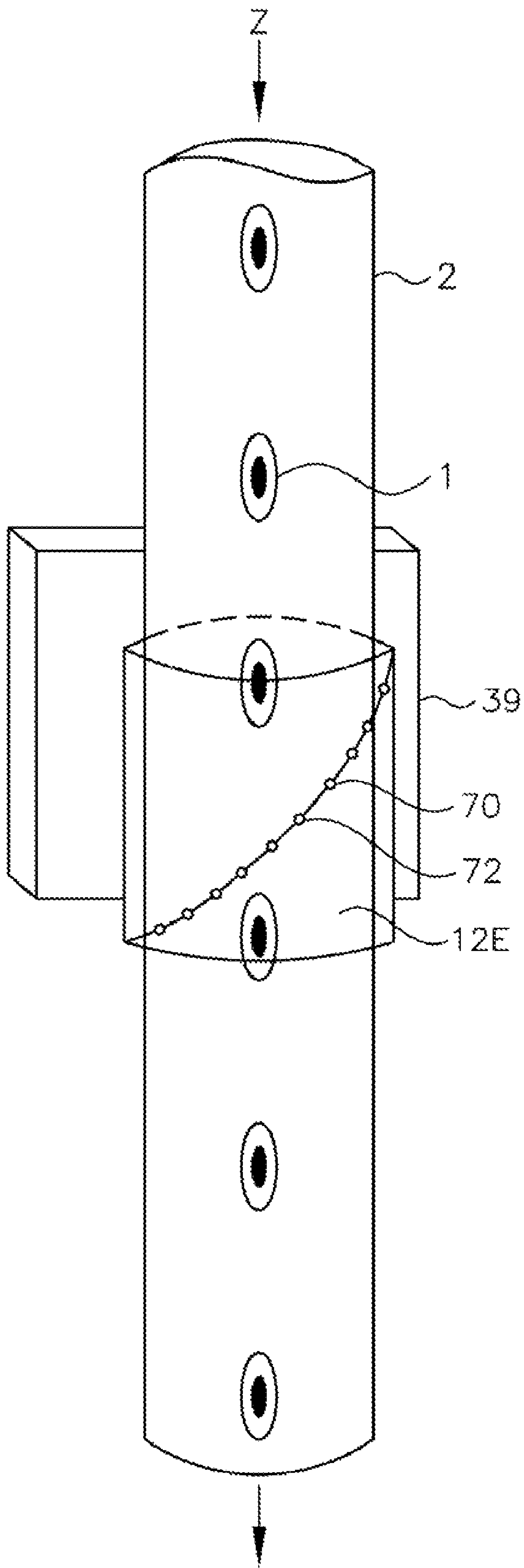


FIG. 8

FIG. 9



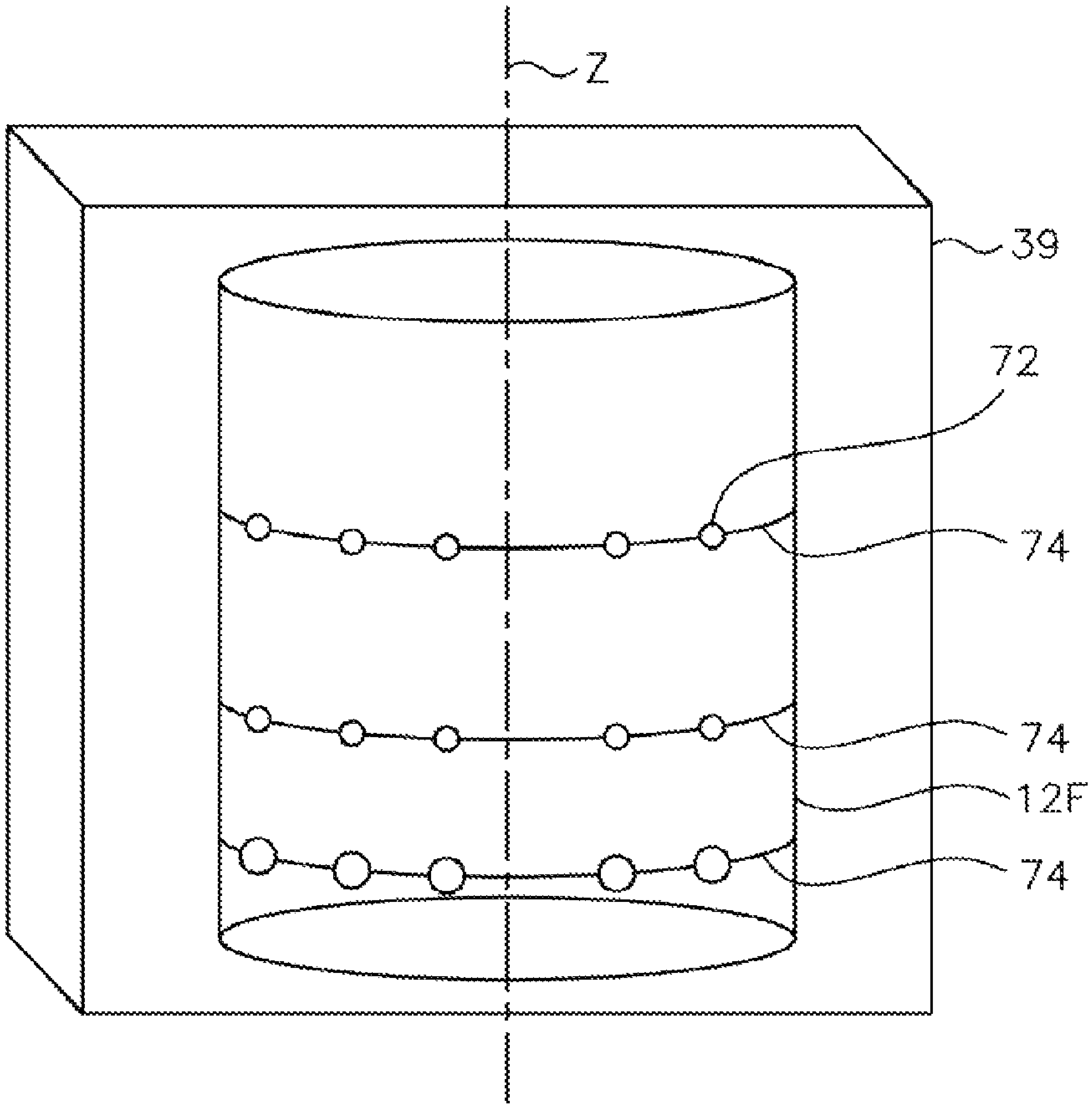


FIG. 10

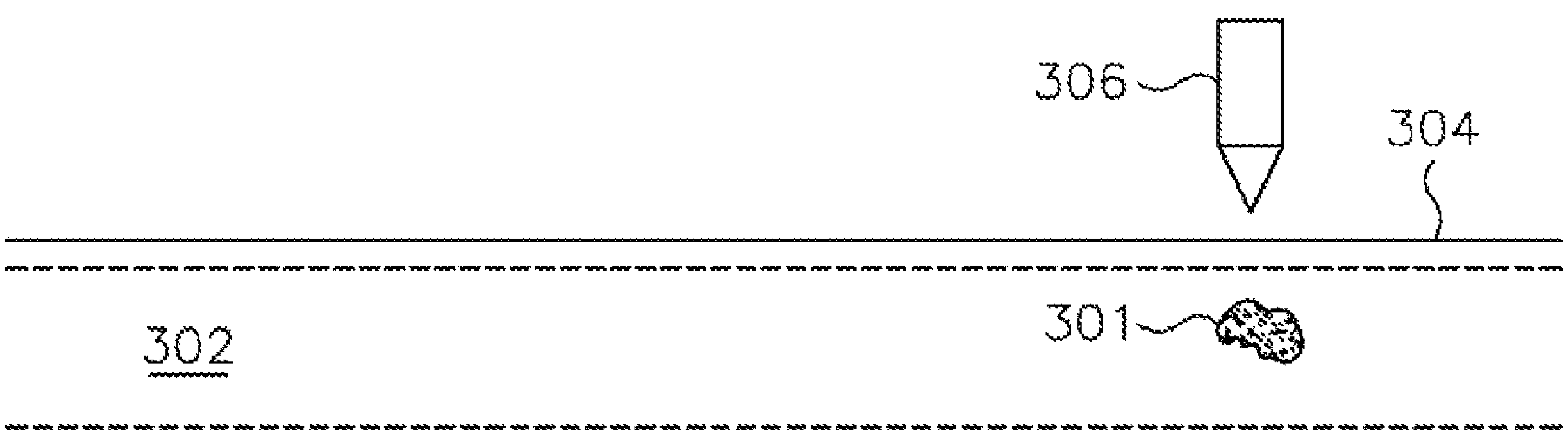


FIG. 11

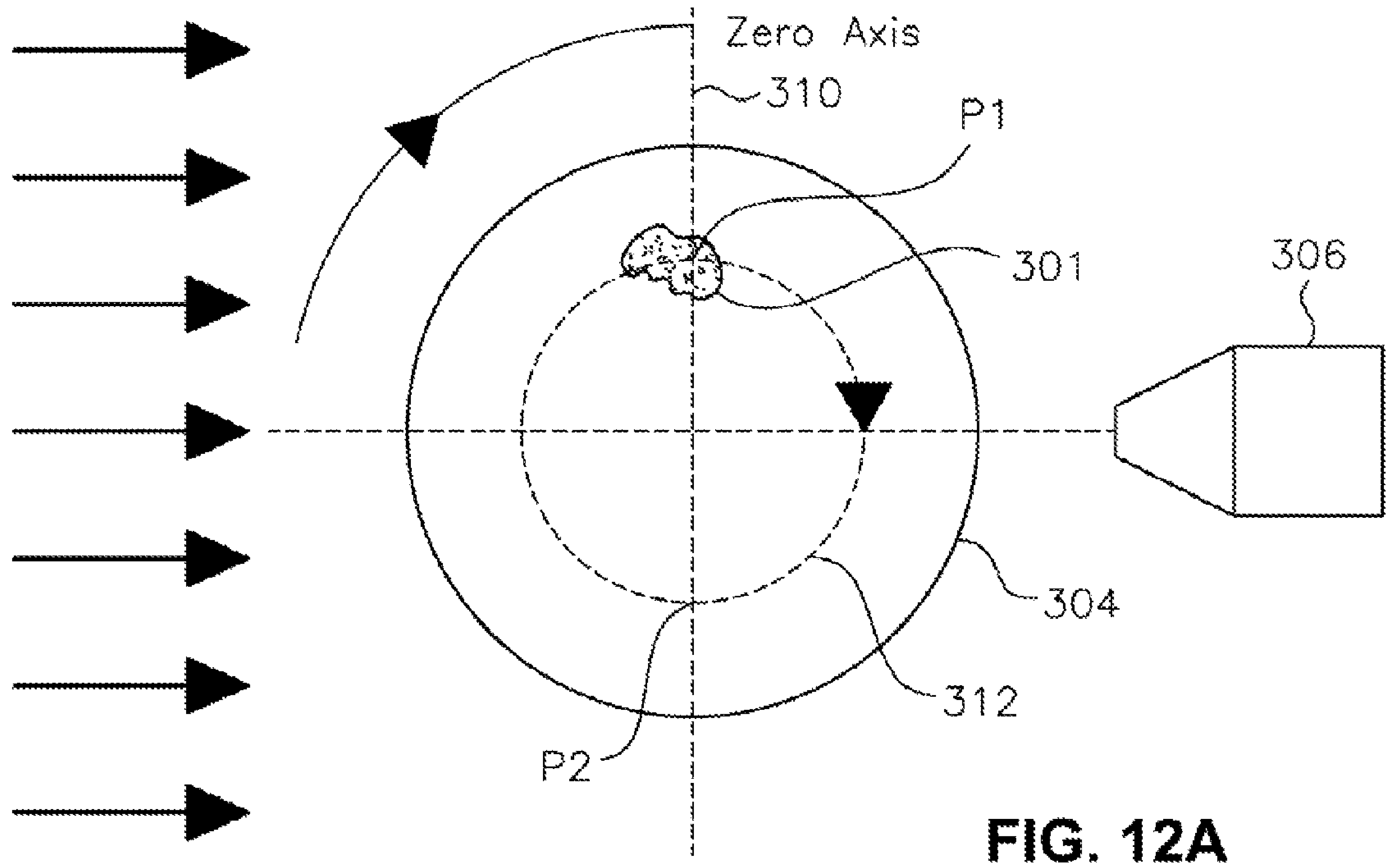


FIG. 12A

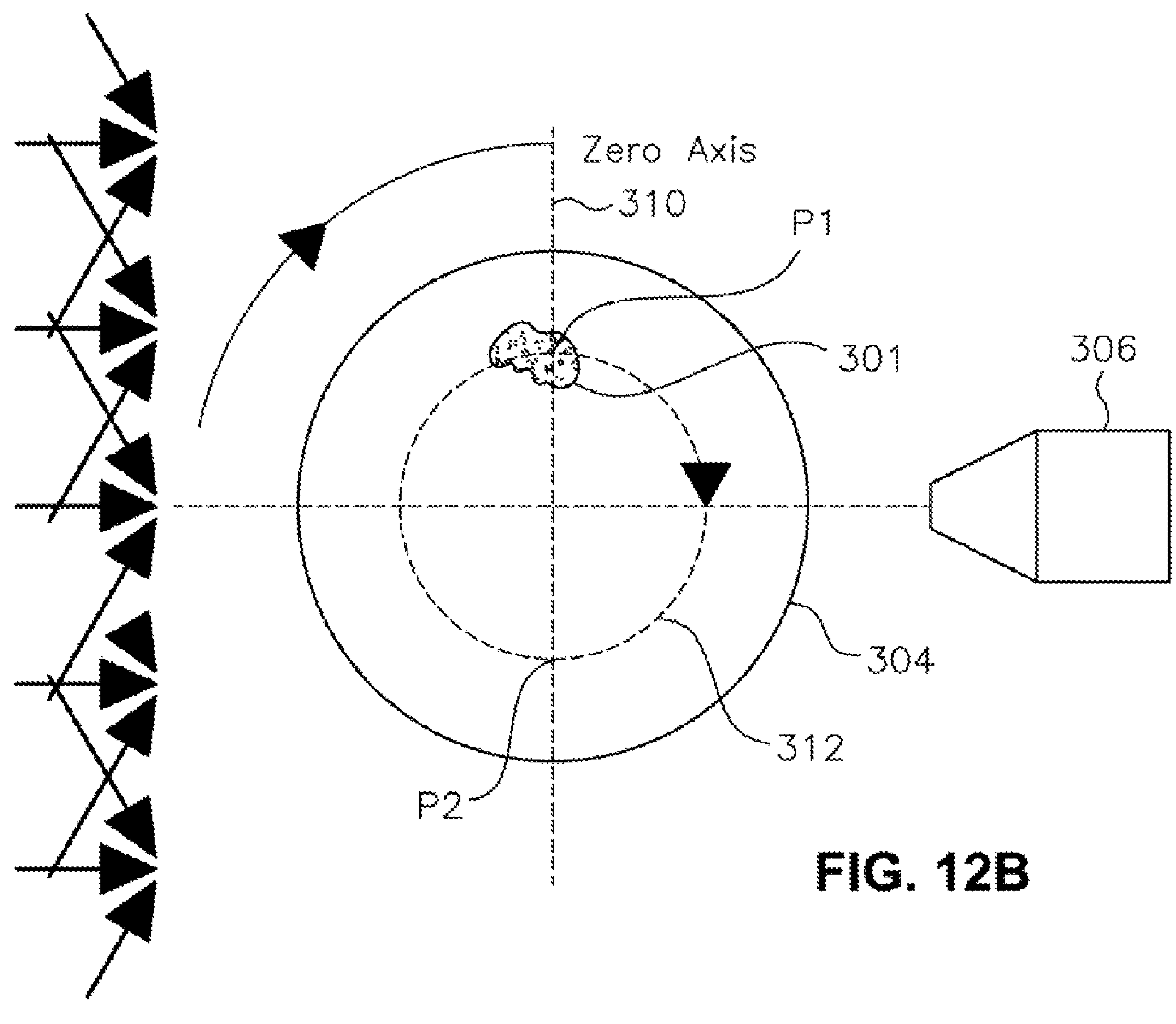
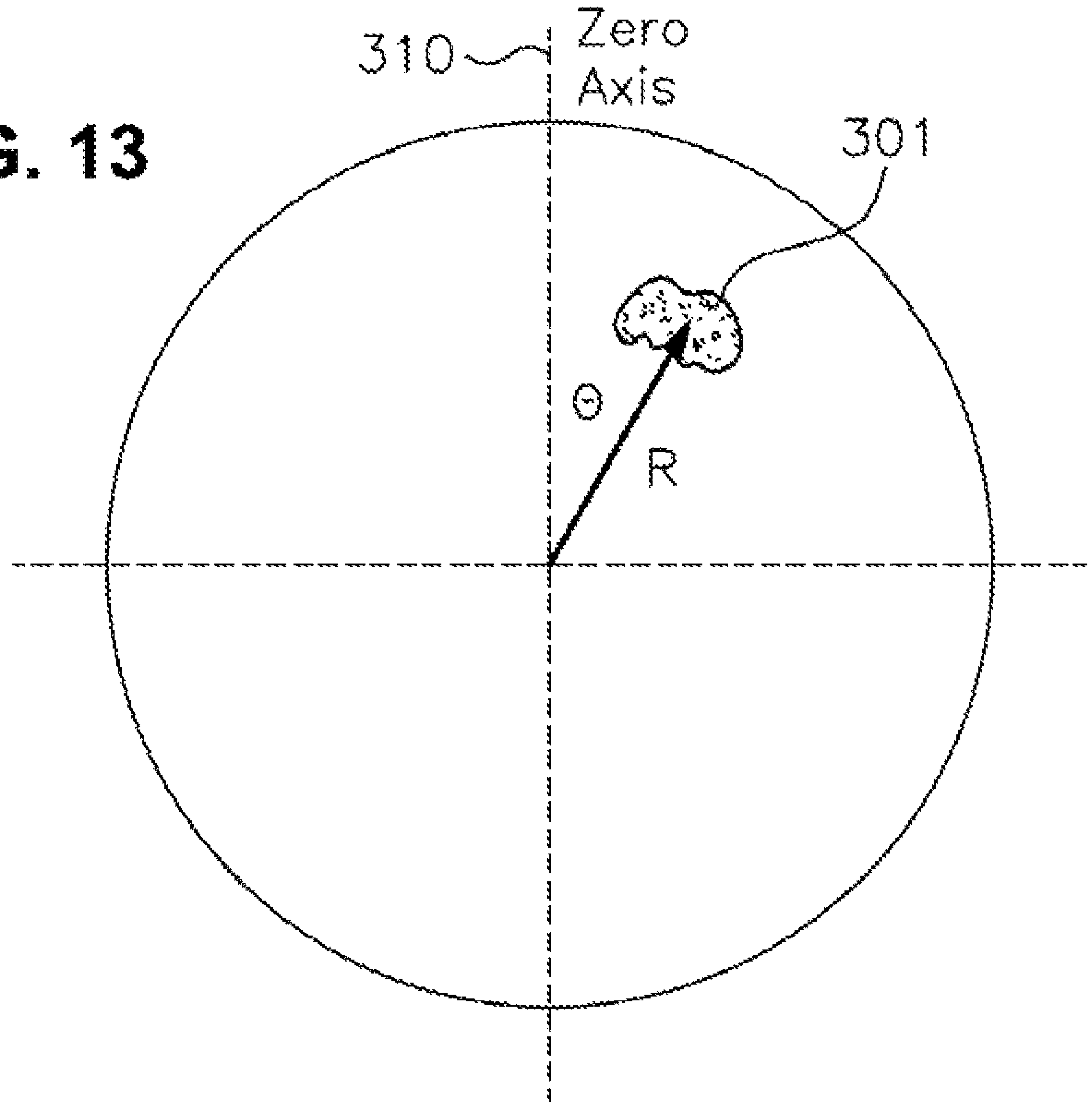


FIG. 12B

FIG. 13



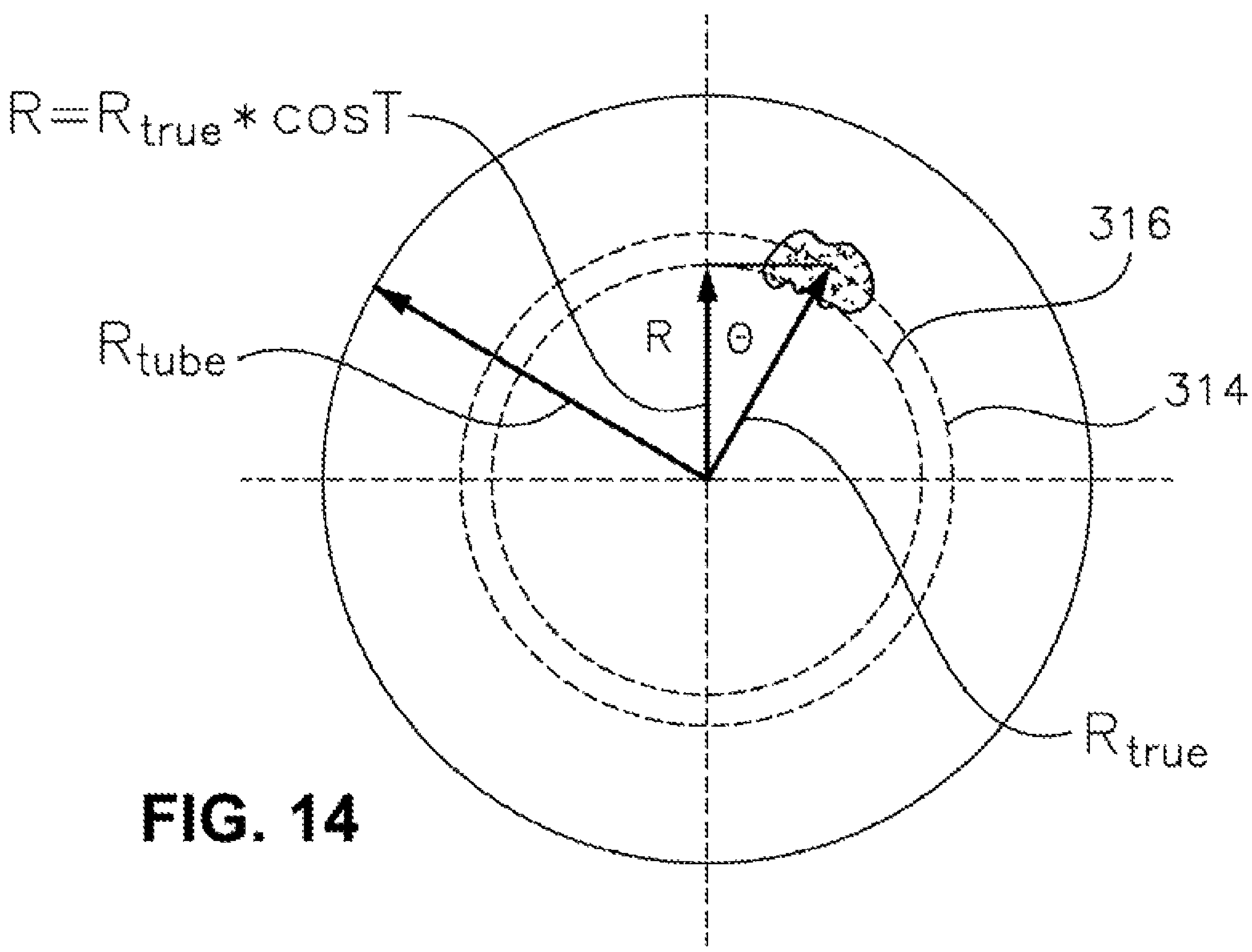


FIG. 14

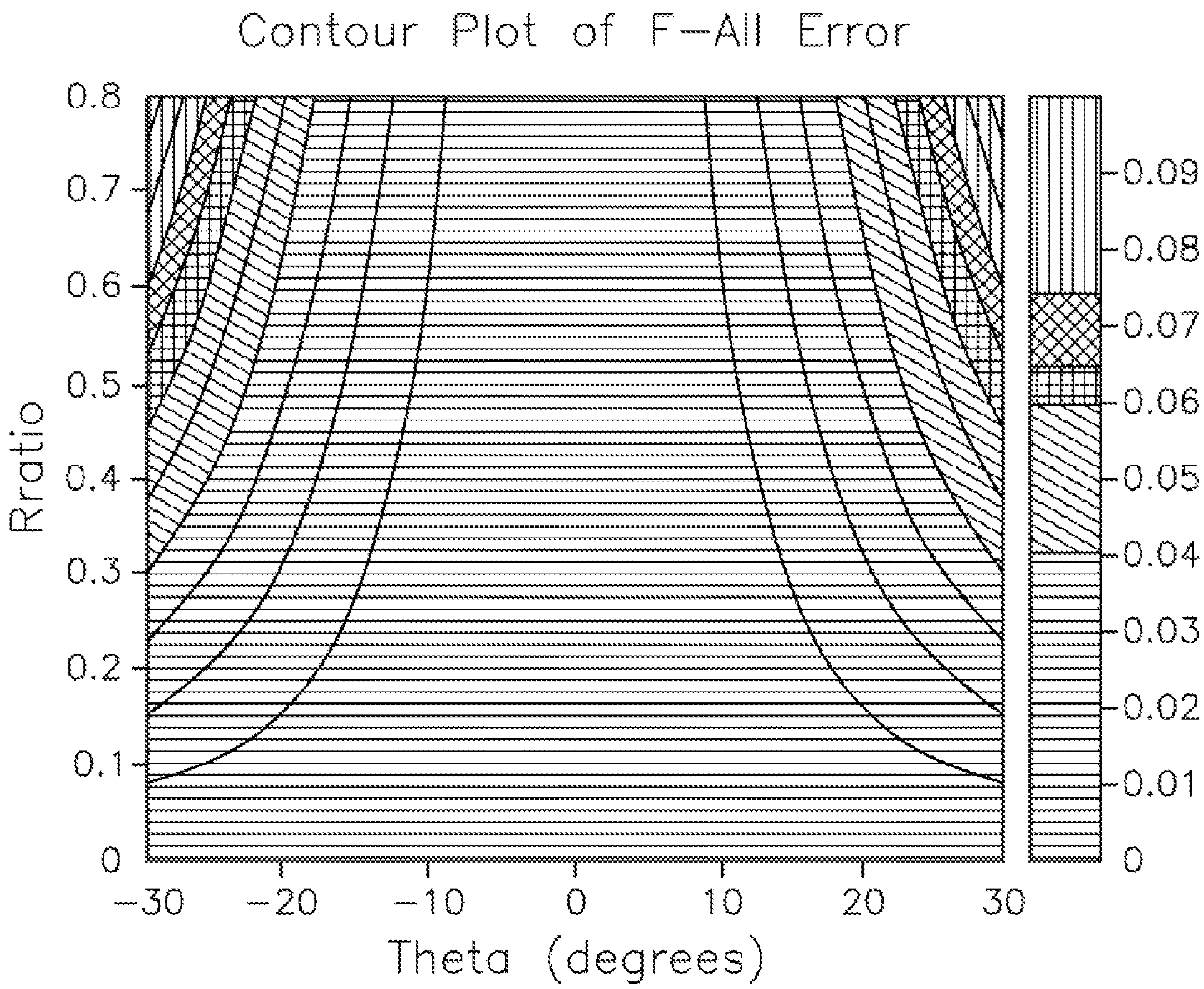
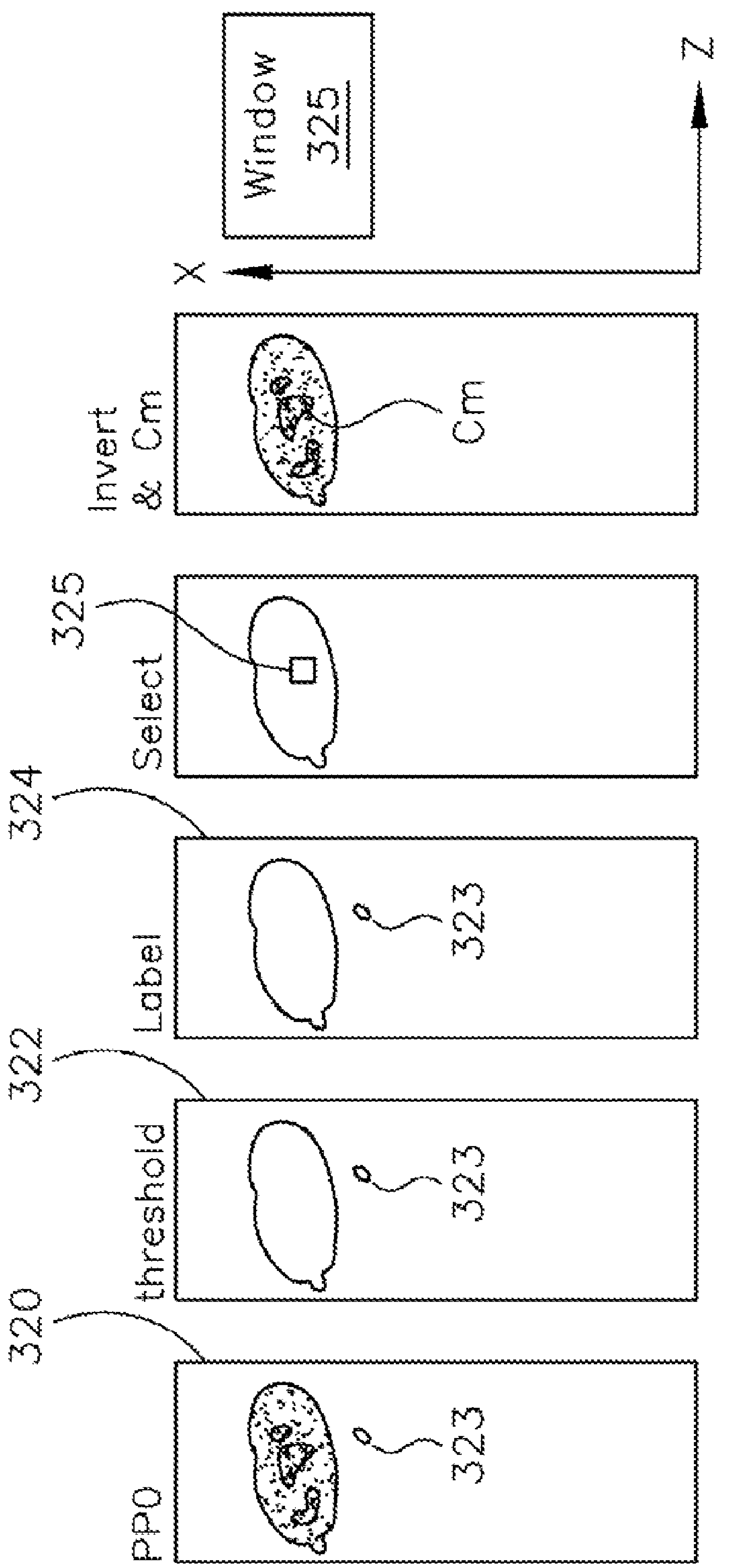

Contour Plot of F-All Error
Rratio
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Theta (degrees)
-30
-20
-10
0
10
20
30
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0

FIG. 15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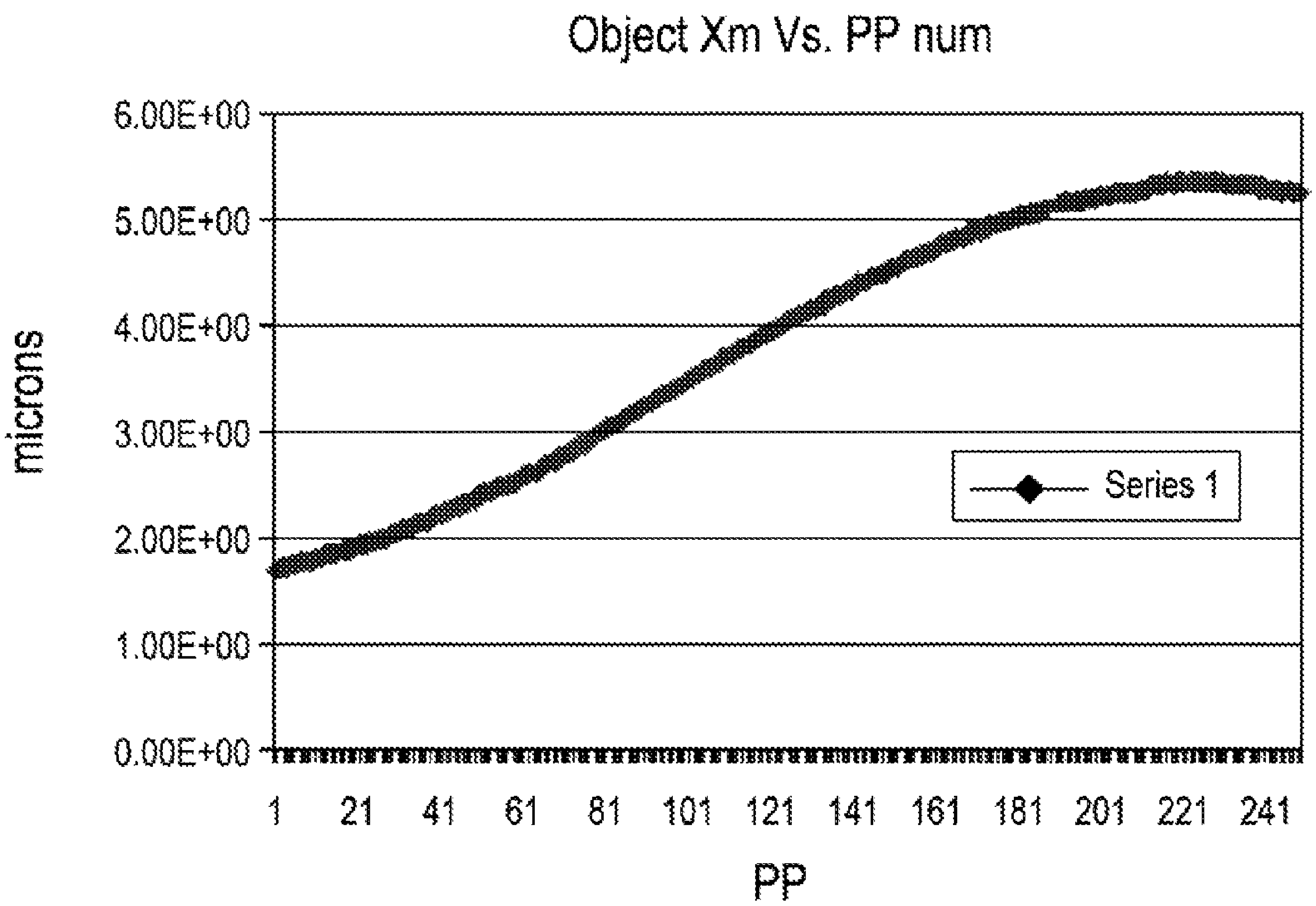
323
Select
325
Invert
& Cm
Cm

FIG. 16


Object Xm Vs. PP num
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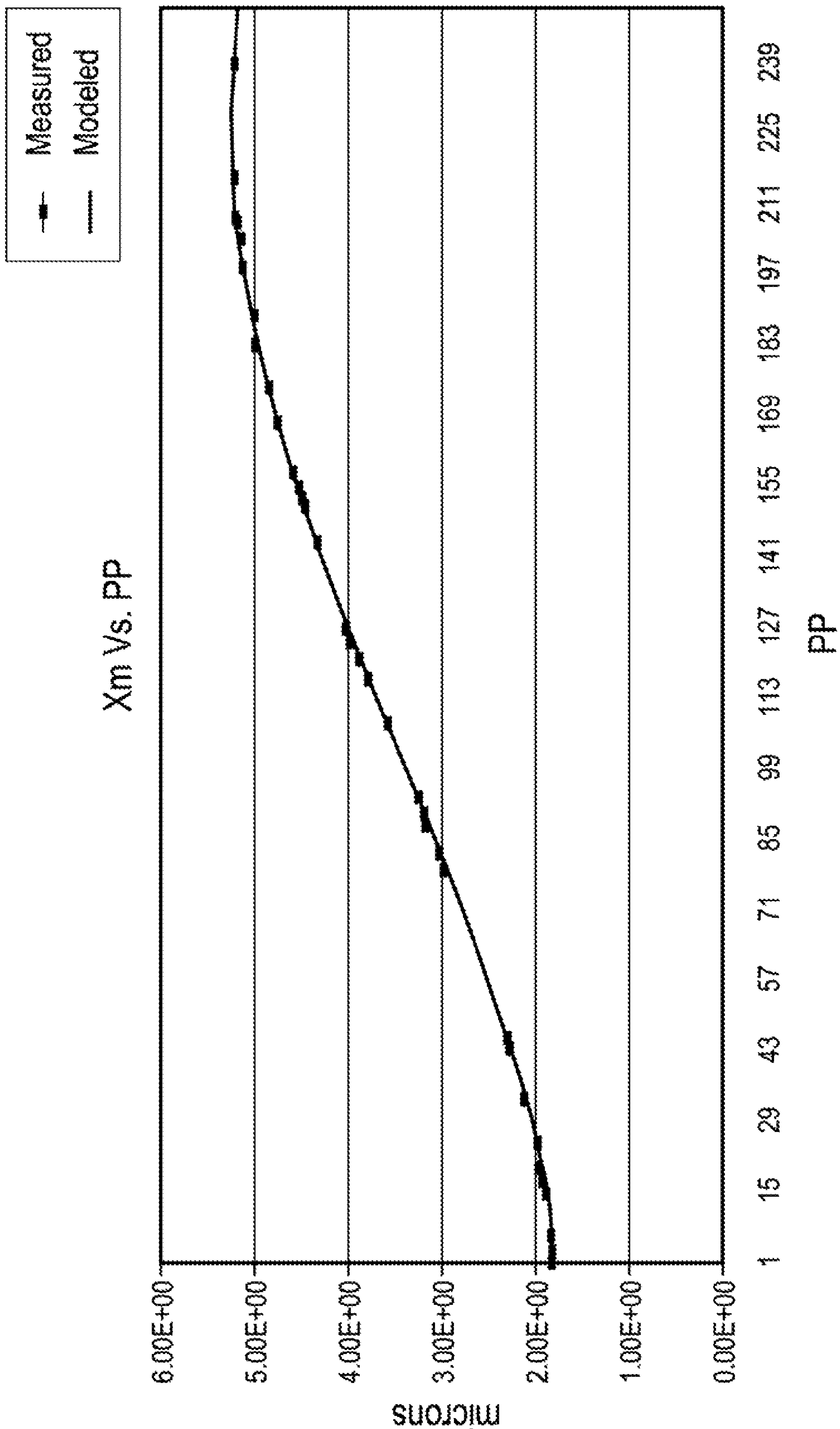
5.00E+00
4.00E+00
3.00E+00
2.00E+00
1.00E+00
0.00E+00
microns
Series 1
1
21
41
61
81
101
121
141
161
181
201
221
241
PP

FIG. 17


Xm Vs. PP
Measured
Modeled
microns
6.00E+00
5.00E+00
4.00E+00
3.00E+00
2.00E+00
1.00E+00
0.00E+00
1
15
29
43
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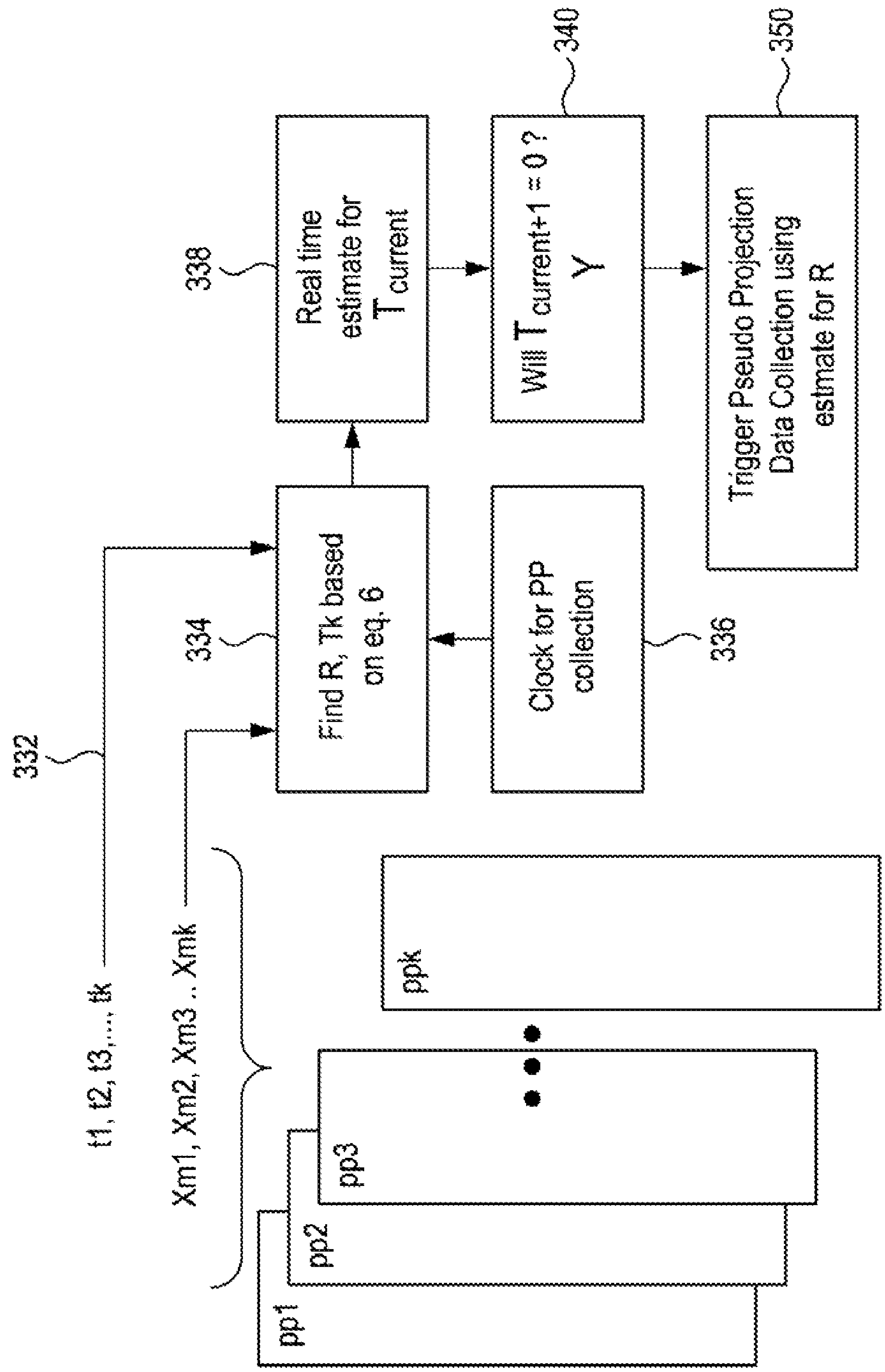
71
85
99
113
127
141
155
169
183
197
211
225
239
PP

FIG. 18



332
t1, t2, t3,..., tk
Xm1, Xm2, Xm3 .. Xmk
334
Find R, Tk based on eq. 6
336
Clock for PP collection
338
Real time estimate for T current
340
Will T current+1 = 0 ?
Y
350
Trigger Pseudo Projection Data Collection using estmate for R
pp1
pp2
pp3
ppk

FIG. 19

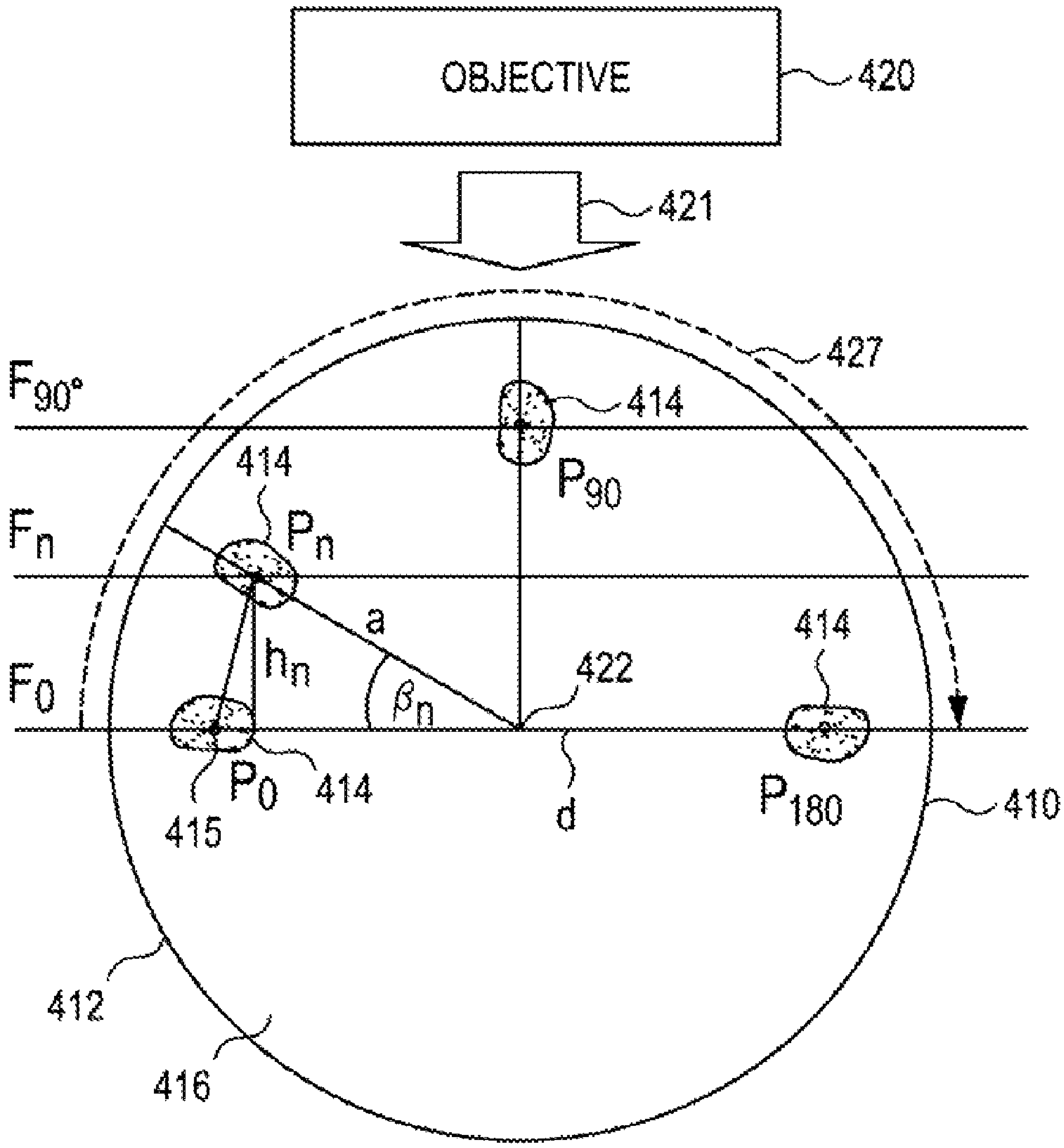

OBJECTIVE
420
421
427
$F_{90°}$
414
P_{90}
414
P_n
F_n
a
h_n
β_n
422
414
F_0
P_0
414
415
d
P_{180}
410
412
416

FIG. 20

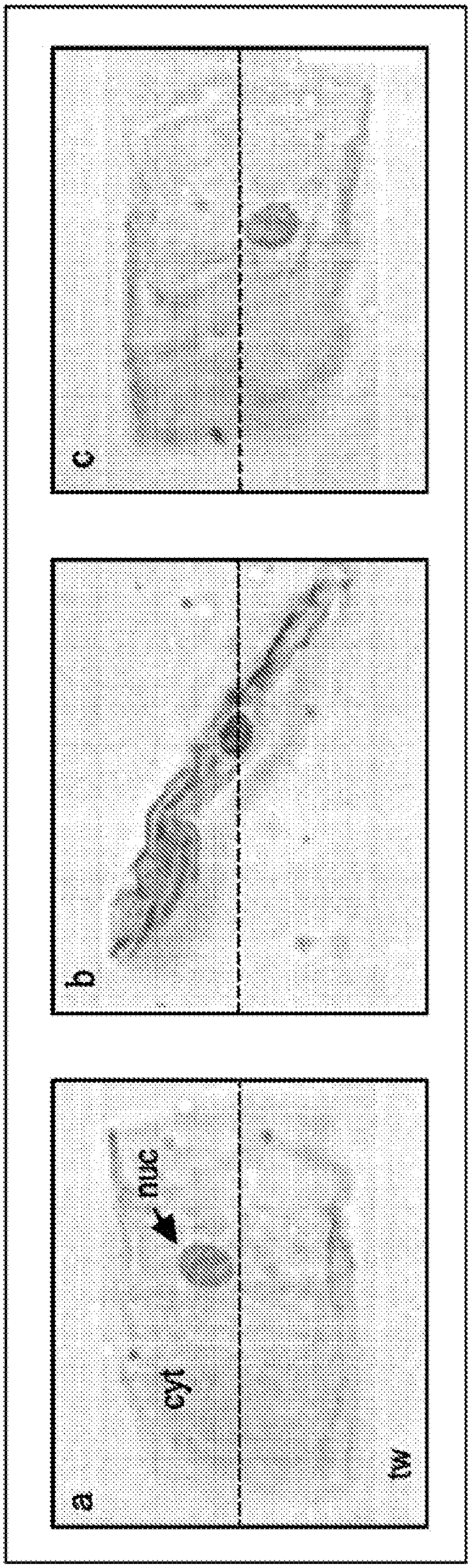


FIG. 21

FOCAL PLANE TRACKING FOR OPTICAL MICROTOMOGRAPHY

RELATED APPLICATION

This application claims priority from and is a continuation-in-part of co-pending U.S. application Ser. No. 11/203,878 of Meyer et al., filed Aug. 15, 2005, entitled "OPTICAL TOMOGRAPHY OF SMALL OBJECTS USING PARALLEL RAY ILLUMINATION AND POST-SPECIMEN OPTICAL MAGNIFICATION." that is in turn a continuation-in-part of U.S. Pat. No. 6,944,322 of Johnson and Nelson, issued Sep. 13, 2005, entitled "OPTICAL TOMOGRAPHY OF SMALL OBJECTS USING PARALLEL RAY ILLUMINATION AND POST-SPECIMEN OPTICAL MAGNIFICATION," that is in turn a continuation-in-part of U.S. Pat. No. 6,522,775 of Alan C. Nelson, issued Feb. 18, 2003, that is in turn related to the provisional application of Alan C. Nelson, Ser. No. 60/279,244, filed Mar. 28, 2001; both entitled "APPARATUS AND METHOD FOR IMAGING SMALL OBJECTS IN A FLOW STREAM USING OPTICAL TOMOGRAPHY." U.S. application Ser. No. 11/203,878 of Meyer et al, is hereby incorporated by reference. U.S. Pat. No. 6,944,322, and U.S. Pat. No. 6,522,775 are also hereby incorporated by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under SBIR Phase I Grant No. HHSN2612004330106 awarded by the National Institute of Health/National Cancer Institute (NIH/NCI). The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to optical tomographic (OT) imaging systems in general, and, more particularly, to microscopic optical tomography where a small object, such as a biological cell, for example, is illuminated by a light beam in the visible or ultraviolet portion of the electromagnetic spectrum, rotated and tracked, and projected images are produced.

BACKGROUND OF THE INVENTION

A patent application of Fauver et al. published as US-2004-0076319-A1 on Apr. 22, 2004, incorporated herein by this reference, discloses a method and apparatus for continuously scanning the focal plane of an optical imaging system along an axis perpendicular to said plane through the thickness of a specimen during a single detector exposure.

One such method is accomplished by moving an objective lens, thereby scanning the focal plane through the thickness of the specimen region, such that the entire specimen thickness is scanned continuously during a single detector exposure interval. A pseudoprojection image is thereby generated whose resolution can depend on the depth of focus of a moving focal plane, as well as on the lateral spatial resolution (i.e., the resolution within the focal plane). The procedure is repeated from several perspectives over an arc of up to 180 degrees, using one or more pairs of light sources and detector arrays simultaneously. The specimen can be rotated and/or translated to acquire additional viewpoints. In this way, a set of pseudoprojections is generated, which can be input to a tomographic image reconstruction algorithm, such as filtered backprojection, to generate a three-dimensional image.

Known techniques work well for a specimen that is positioned in the center of a rotating capillary tube because the specimen will not move out of an initial focal plane during rotation. However, many specimens are positioned off center and will translate out of an initial focal plane. Such offset positions can cause focusing errors and adversely affect post-imaging acquisition reconstruction of the specimen.

SUMMARY OF THE INVENTION

The present invention provides an optical tomography system for imaging an object of interest including a light source for illuminating the object of interest with a plurality of radiation beams. The object of interest is held within an object containing tube such that it is illuminated by the plurality of radiation beams to produce emerging radiation from the object containing tube, a detector array is located to receive the emerging radiation and produce imaging data used by a mechanism for tracking the object of interest.

In one contemplated embodiment, a parallel ray beam radiation source illuminates the object of interest with a plurality of parallel radiation beams. An outer tube has an optically flat input surface for receiving the illumination and a concave output surface, where the concave outer surface acts as a magnifying optic to diverge the radiation emerging from the outer tube after passing through the object of interest. An object containing tube is located within the outer tube, wherein the object of interest is held within the object containing tube. A motor is coupled to rotate and otherwise manipulate the object containing tube to present differing views of the object of interest. A detector array is located to receive the emerging radiation from the concave output surface.

The present invention relates generally to three-dimensional optical tomography using parallel beam projections produced by a laser or other illumination system in conjunction with CCD or CMOS detectors and, more particularly, to three-dimensional tomographic imaging of microscopic objects, including biological cells, in a flow stream or entrained in a rigid medium.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings incorporated in and forming a part of the specification illustrate several aspects of the present invention, and together with the description, serve to explain the principles of the invention. Moreover, in the drawings, like reference numerals designate corresponding parts throughout the several views. In the drawings.

FIG. **1** schematically shows an example illustration of a Parallel Beam Flow Optical Tomography system as contemplated by an embodiment of the present invention.

FIG. **2** schematically shows an example illustration of a Variable Motion Parallel Beam Optical Tomography system as contemplated by an embodiment of the present invention.

FIG. **3** schematically shows an example illustration of a system illumination geometry, including a single source-magnifying concave optic pair as contemplated by one example embodiment of the present invention.

FIG. **4** schematically shows an example illustration of a system illumination geometry, including a single source-magnifying convex optic pair as contemplated by an alternate embodiment of the present invention.

FIG. **4**A schematically shows another example illustration of a system illumination geometry, including a single source-magnifying convex optic pair as contemplated by another alternate embodiment of the present invention.

FIG. 5 schematically shows an example illustration of an illumination geometry and the imaged sample volume with multiple source-magnifying concave optic pairs as contemplated by an embodiment of the present invention.

FIG. 5A schematically shows another example illustration of the illumination geometry and the imaged sample volume with multiple source-magnifying convex optic pairs as contemplated by an embodiment of the present invention.

FIG. 6 is a highly schematic drawing that shows an example illustration of a reconstruction cylinder as contemplated by an embodiment of the present invention.

FIG. 7 schematically shows an example flow diagram illustrating the operation of a TDI image sensor as contemplated by an embodiment of the present invention.

FIG. 8 schematically shows an example illustration of a parallel ray beam light source system as contemplated by an embodiment of the present invention.

FIG. 9 schematically shows an example of a reconstruction cylinder surrounding a flow tube containing flowing object, such as cells, as contemplated by an embodiment of the present invention.

FIG. 10 schematically shows an example of a reconstruction cylinder including a series of partial circumferences arranged along a Z-axis through an object containing tube, wherein each partial circumference may contain more than one source-detector pair.

FIG. 11 schematically shows another example embodiment of the system and method wherein at least one specimen for examination is processed to remove non-diagnostic elements and is fixed and stained as contemplated by an embodiment of the present invention.

FIG. 12A and FIG. 12B schematically show an end view of a micro-capillary tube 304 with parallel beam illumination and non-parallel beam illumination respectively.

FIG. 13 schematically shows an example illustration of tracking parameters describing the placement of the object in a tube as contemplated by one example embodiment of the present invention.

FIG. 14 schematically shows an example illustration of errors in a diagram that characterizes the erroneous identification of R, Θ resulting in a misidentification of the plane of focus for the object of interest.

FIG. 15 schematically shows a contour plot representative of the dependence of F_AllError on Rratio and Θ as contemplated by another alternate embodiment of the present invention.

FIG. 16 schematically shows a diagram for segmenting an object of interest and computing the center of mass for the grey scale pixels associated with a pseudoprojection image PP0 of an object of interest is shown as contemplated by an embodiment of the present invention.

FIG. 17 schematically shows a graphical representation of a trend of an X component of the center of mass from pseudoprojection to pseudoprojection as contemplated by an embodiment of the present invention.

FIG. 18 shows the close correspondence between measured and modeled Xm as contemplated by an embodiment of the present invention.

FIG. 19 schematically shows an example flow diagram illustrating the operation of a focal tracking block diagram of the method of the invention.

FIG. 20 schematically shows a capillary tube during rotation.

FIG. 21*a*, FIG. 21*b* and FIG. 21*c* show images of a single squamous cell during imaging in an optical tomography microscope.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference will now be made in detail to the description of the invention as illustrated in the drawings. While the invention will be described in connection with these drawings, there is no intent to limit it to the embodiment or embodiments disclosed therein. On the contrary, the intent is to cover all alternatives, modifications, and equivalents included within the spirit and scope of the invention as defined by the appended claims.

The invention is further described herein with respect to specific examples relating to biological cells. It will be understood, however, that these examples are for the purpose of illustrating the principals of the invention, and that the invention is not so limited. In one example, constructing a three dimensional distribution of optical densities within a microscopic volume enables the quantification and the determination of the location of structures, molecules or molecular probes of interest. By using tagged molecular probes, the quantity of probes that attach to specific structures in the microscopic object may be measured. For illustrative purposes, an object such as a biological cell may be labeled with at least one stain or tagged molecular probe, and the measured amount and location of this probe may yield important information about the disease state of the cell, including, but not limited to, various cancers such as lung, breast, prostate, cervical and ovarian cancers.

Generally as used herein the following terms have the following meanings when used within the context of optical microscopy processes:

"Capillary tube" has its generally accepted meaning and is intended to include microcapillary tubes and equivalent items with an inside diameter of 100 microns or less. Such microcapillary tubes are manufactured by Polymicro Technologies, LLC., AZ.

"Object" means an individual cell or other entity. One or more objects may comprise a specimen.

"Pseudoprojection" includes a single image representing a sampled volume of extent larger than the native depth-of-field of the optics.

"Specimen" means a complete product obtained from a single test or procedure from an individual patient, (e.g., sputum submitted for analysis, a biopsy, or a nasal swab.) A specimen may be composed of one or more objects. The result of the specimen diagnosis becomes part of the case diagnosis.

"Sample" means a finished cellular preparation that is ready for analysis, including all or part of an aliquot or specimen.

In one example of the present invention, the chosen illumination is parallel, or nearly parallel, until after passage through the object volume that may contain the cell or other specimen or object to be imaged. After passage through the object, a post-specimen optic diverges the emergent pattern of light intensities in order to produce a magnified pattern of light intensities in any plane perpendicular to the system's optical axis and situated downstream from the post-specimen optic. However, the invention is not limited to parallel beam radiation and, in fact, the embodiments described herein are useful for many forms of illumination at venous wavelengths.

Referring to FIG. 1, there schematically shown is an example illustration of a Parallel Beam Flow Optical Tomography (PBOT) system as contemplated by an embodiment of the present invention. The invention provides an apparatus and method for imaging small objects in a flow stream or entrained in a rigid medium using optical point source or parallel beam projections, image sensors, such as, for example, time delay and integration (TDI) image sensors or CCD or CMOS solid state image sensors and the like, and tomographic image reconstruction. The optical tomography (OT) system includes in one example embodiment, a flow cytometer, including a reconstruction cylinder **12**, positioned around object containing tube **2**. The object containing tube **2** may, for example, comprise a cell entrainment tube wherein the cell is held in a gel, or a capillary tube for cell flow, depending on the type of optical tomography system.

The PBOT system **4** is oriented with reference to a coordinate system **40** having coordinates in the X, Y and Z-directions. In operation, an object of interest **1**, such as, for example a cell, including a human cell, is injected into an injection tube **3**. The object containing tube **2** may be wider at an injection end **5** and includes a pressure cap **6**. A sheath fluid **7** is introduced at tube **8** to create laminar flow within the object containing tube **2**. A first source of photons **9***a* and a first photo detector **10***a* work together with a pulse height analyzer **11** to operate as a triggering device. Pulse height analyzer **11** operates to provide a first signal **30***a* for the beginning or leading edge of an object, such as a cell, and a second signal **30***b* for the end or trailing edge of the object as it moves through the tube. The signals **30***a*, **30***b*, **31***a* and **31***b* are represented as a light intensity, "I" versus "TIME" function within pulse height analyzer **11**. The pulse height analyzer **11** may be a conventionally designed electronic circuit or the like. The pulse height analyzer **11** generates a plurality of signals **14** that are sent to a computer **13** which, after a delay related to the velocity of the moving object and distance between the photo detector and the reconstruction cylinder **12**, sends a trigger signal on line **15** to a reconstruction cylinder **12** to initiate and terminate data collection for that particular object of interest. Additionally, a second photon source **9***b* and a second photo detector **10***b* may advantageously be positioned at a known distance downstream from the first set such that an interval between the object triggering a third signal **31***a* and triggering a fourth signal **31***b* may advantageously be used to calculate the velocity of the object and also as a timing signal to synchronize the line transfer rate of a TDI image sensor. The timing signal is transmitted to computer **13** in the plurality of signals **14**. The computer **13**, which may be any useful personal computer or equivalent, in turn sends synchronization signals on line **16** to the reconstruction cylinder **12**. It will be understood that lines **15** and **16** are representative of communication and control lines between the PBOT system and the computer that communicate data, image information, control signals and other signals between the computer and the PBOT system. In this way, for example, the movement of the object along the flow axis **20** may be matched by a rate of transfer of charge from one stage of a TDI sensor to the next, as described and shown in more detail below with reference to FIG. **7**.

Now referring to FIG. **2**, there schematically shown is an example illustration of a Variable Motion Parallel Beam Optical Tomography system as contemplated by one example embodiment of the present invention. A variable motion PBOT system **100** takes advantage of a mechanical positioner to present cells, which are entrained in a rigid medium in a tube, to the imaging system one at a time. As compared to the flow system described with reference to FIG. **1**, in the variable motion PBOT system **100** only one trigger mechanism including a photon source **9** and a photo detector **10** is required since the velocity of the object, such as a human cell, can be precisely controlled to synchronize with the illumination sources and image sensors in the reconstruction cylinder **12**. The trigger here is processed by the pulse height analyzer **11** and the computer **13** and used to start and stop data collection. The pulse height analyzer **11** is an electronic circuit of design similar to pulse height analyzer **11** except that it requires fewer inputs and outputs. As indicated by double arrow line the object containing tube **2** in this embodiment is translated along the z-axis through the reconstruction cylinder **12** by a screw drive **18** driven by a computer controlled motor **17**. The object contained in tube **2** may also be rotated about the z-axis by the computer controlled motor **17**. The computer controlled motor **17** receives control information **19** from the computer **13**. It will be understood by those skilled in the art having the benefit of this disclosure, that any mechanism capable of translating and rotating the object containing tube **2** can be used in place of the screw drive. Signals from the reconstruction cylinder **12** may be analyzed directly or processed using image processing, image analysis and/or computerized tomographic image reconstruction techniques to provide two dimensional or three dimensional information about cells and other objects of interest.

Referring now to FIG. **3**, a system illumination geometry within a reconstruction cylinder **12**A for use in a parallel-beam optical tomography system for imaging an object of interest **1** is shown schematically. The reconstruction cylinder **12**A includes a parallel ray beam radiation source **35** for illuminating the object of interest **1** with a plurality of parallel radiation beams **36**. An outer tube **32** has an optically flat input surface **60** and a concave output surface **29**, where the concave outer surface **29** diverges radiation **61** emerging from the outer tube **32** after passing through the object of interest **1**. An object containing tube **2** is located within the outer tube **32**, wherein the object of interest **1** is held within the object containing tube **2**.

A motor, here indicated schematically as double arrow **34**, is coupled to rotate the object containing tube **2** to present differing views of the object of interest **1**. A detector array **39** is located to receive the emerging radiation **61** from the concave output surface **29**. In one embodiment, the parallel ray beam radiation source **35** comprises a laser. In another example embodiment, the laser may be selected to emit radiation in the visible portion of the electromagnetic spectrum. In yet another example embodiment, the laser may be selected to emit radiation in the ultraviolet portion of the electromagnetic spectrum. The detector array **39** may advantageously comprise a sensor selected from the group consisting of solid state sensors, charge coupled device (CCD) sensors, complementary metal oxide semiconductor (CMOS) sensors and time delay and integration sensors.

In another embodiment of the present invention, a cell or other object to be imaged is present either in a flow tube, capillary tube, linear container, or in an entrainment tube. In one embodiment of the parallel-beam optical tomography system the object of interest **1** comprises a human cell having a nucleus **30**. The cell may also contain subcellular features or constituents. At least one fluorescing or absorbing molecular probe **31** may be bound to one or more cellular constituents.

The object containing tube **2**, for example a flow tube, capillary tube, linear container, or entrainment tube, is located substantially concentrically within the outer tube **32** which has a substantially rectangular outer cross section, and may have either a rectangular or circular inner cross section. Other cross sectional geometries for the outer tube **32** are possible. The curved surface of the object containing tube **2** acts as a cylindrical lens producing a focusing effect that may not be desirable in a projection system. Those skilled in the art having the benefit of this disclosure will appreciate that the bending of photons by the object containing tube **2** can be substantially reduced if the spaces **37** and **33** between the source and the outer tube **32** and between the tube **32** and the detector surfaces **39** are filled with a material having an index of refraction matching that of the object containing tube **2**. Further, the tube can be optically coupled to the space filling material. Such optical coupling may be accomplished with oil or a gel, for example. An index of refraction-matching fluid in space **33**, such as oil, for example, may advantageously be introduced through port **38** to entirely fill the space between the tube **2** in which the cells or other microscopic objects are contained and the outer tube **32**. The index of refraction matching fluid, both tubes **2** and **32**, and any gel or flowing liquid medium surrounding the cells to be imaged have identical, or nearly identical indices of refraction. The object contained within tube **2** may be rotated and/or translated within the index of refraction matching fluid and outer tube **32** with both axial and rotational motions under computer control.

In operation, a laser or other light source **35** produces parallel illuminating beams **36**, which impinge on the outer tube **32**, optionally delivered by an index of refraction-matched coupling element **37**. In the absence of scatter, the light traverses parallel ray paths through both tubes **2** and **32**. Since the refractive indices of all materials in the light path are matched, the rays traversing the index of refraction matching fluid and the object space within the volume to be imaged are parallel. Both tubes **2** and **32** comprise transparent, or nearly transparent material with respect to the illuminating wavelength. Both tubes **2** and **32** may comprise fused silica, glass or other similar optical material.

The exit face **29** of the outer, rectangular tube **32** may advantageously be provided with a diverging or magnifying optic, which, in one contemplated embodiment, may be a circularly symmetric polished depression, or dimple, in the fused silica or other optical material. The dimple acts as a plano-concave lens, causing the light ray paths **61** to become divergent at its exit surface **29**. Such a dimple or any other optical element or combination of optical elements, including multiplets, or other equivalent elements, designed to perform the same function is referred to herein as a post-specimen optic. The post-specimen optic comprises, generally, a magnifying optic.

Using known optical design principles, the radius of curvature of the post-specimen optic may be determined and designed to impart the desired degree of divergence to the exiting light ray paths **61**. The degree of divergence, together with the distance between the post-specimen optic and the TDI, CCD, CMOS or other image sensor **39**, determines the magnification of the projection images. The magnification required is determined by the relationship between the desired spatial resolution of the projection images and the detector pixel size, and it is advantageous for the magnification to be much larger than twice the quotient of the pixel size and the desired spatial resolution of the projection.

For example, in one contemplated embodiment of the present invention, if the desired spatial resolution in the projections is 0.5 micron and the detector pixel size is 10 microns, it is advantageous for the magnification to be significantly larger than 40 times. In this example, it may be desirable for the magnification to be 80 times, 100 times, or even more.

For a contemplated embodiment of the current invention in which the post-specimen optic is a circularly symmetric polished dimple on the exit face **29** of the outer tube **32**, and in which this post-specimen optic functions as a plano-concave diverging lens, the front focal plane of the lens is at infinity. There is no back focal plane. Thus, a magnified projection image, pseudoprojection image, or shadowgram containing information about the absorption of the illumination as it passed through the cell or other object to be imaged **1**, can be produced by capturing this emergent pattern of transmitted light intensities on a TDI, CCD or CMOS detector or other digital imaging detector **39**. The photo-conversion surface of the detector can be situated in any plane perpendicular to the system's optical axis and downstream from the post-specimen optic. Furthermore, the magnification can be chosen by the placement of the detector plane: the further the detector plane is downstream from the object, the greater the magnification.

In embodiments of the present invention such as those depicted schematically in FIG. **3** and FIG. **4**, having a single source-detector pair, two-dimensional or three-dimensional tomographic imaging of the cell or other microscopic object is performed by obtaining images from varying angles of view. After obtaining a first projection with the object containing tube **2** held stationary at a first rotational angle with respect to the optical axis, the object containing tube **2** may be rotated by a discrete angle about an axis as indicated by the double arrow **34**. A useful axis is identified as the Z axis in FIG. **2**, and/or pointing out of the page in FIG. **3** and FIG. **4**, that is perpendicular to the system's optical axis in order to orient the cell or other object **1** at a second rotational angle with respect to the optical axis. A subsequent transmitted projection image may be obtained after rotation of the object containing tube **2**. The process of rotating and imaging may be repeated with the object containing tube **2** repeatedly rotated in discrete increments. A two-dimensional projection image is recorded at each angle until a sufficient number of projections are obtained to produce a three-dimensional image of the cell or other object **1**, or portion thereof, or to produce two-dimensional images depicting slices of the absorption pattern in the imaged object's interior.

Three-dimensional reconstructions are produced by image processing of the plurality of two-dimensional projection images with known three-dimensional image reconstruction algorithms. Two-dimensional images of transverse slices through the imaged object are produced by processing lines of data extracted from the plurality of projections, where these lines of data are oriented parallel to rotated versions of the X and Y axes as depicted in FIG. **1** and FIG. **2**. The lines of data are generally referred to as rows of detector data. The ability to reconstruct transaxial slices through the cell or other object from rows of detected projection data is an advantage of the method described in the present invention relative to cone beam geometry, in which many lines of detector data would contribute to each transverse image plane through object space.

Referring now to FIG. **4**, there shown schematically is an alternate embodiment of a system illumination geometry within a reconstruction cylinder **12**B as contemplated by the present invention, where a cell or other object to be imaged **1** may be present in a flow tube or entrainment tube **2**. The reconstruction cylinder **12**B includes a parallel ray beam radiation source **35** for illuminating the object of interest **1** with a plurality of parallel radiation beams **36**. An outer tube **32**A has an optically flat input surface **60** and a convex output surface **28**, where the convex outer surface **28** focuses radiation emerging from the outer tube **32**A after passing through the object of interest **1**. As in the above embodiment described with respect to FIG. **3**, an object containing tube **2** is located within the outer tube **32**A, wherein the object of interest **1** is held within or flows through the object containing tube **2**. A motor, indicated schematically by double arrow **34**, may advantageously be coupled to rotate and/or translate the object containing tube **2** so as to present differing views of the object of interest **1**. A pinhole aperture **127** is located at the focal point **128** of the convex lens and arranged to produce a cone beam of emergent radiation **125**. As described above, a detector array **39** is located to receive the cone beam of emergent radiation **125** from the pinhole aperture **127**. In one example embodiment, the outer tube **32**A may advantageously have a port **38** and the space **33** around the object containing tube **2** is filled with a fluid such as optical oil having the same index of refraction as the outer tube **32**A and the object containing tube **2**.

Referring now to FIG. **4**A, there shown schematically is another alternate embodiment of a system illumination geometry within a reconstruction cylinder **12**D as contemplated by the present invention, where a cell or other object to be imaged **1** may be present in a flow tube or entrainment tube **2**. The reconstruction cylinder **12**D includes all of the elements as in the above embodiment described with respect to FIG. **4**, with the addition of an optical element **126**. The optical element **126** may advantageously comprise a plano-concave or other diverging or magnifying optic located between the pinhole aperture **127** and the sensor array **39**. As in FIG. **4**, a pinhole aperture **127** is located at the focal point **128** of the convex lens **28** and arranged to produce a cone beam of emergent radiation **125**. The emergent radiation **125** is received by the plano-concave optical element **126**, whereby it is further diverged into radiation beams **225**. As described above, a detector array **39** is located to receive a cone beam of emergent radiation **225** from the pinhole aperture **127**.

FIG. **5** schematically shows an example illustration of illumination geometry and imaged sample volume with multiple source-magnifying concave optic pairs as contemplated by another embodiment of the present invention. A parallel-beam optical tomography system for imaging an object of interest **1** generally includes the illumination geometry described above with reference to FIG. **3** and a plurality of parallel ray beam radiation sources **1**-N **35**, where N is at least two, for illuminating the object of interest **1**. Each of the plurality of parallel ray beam radiation sources **1**-N **35** generates a plurality of parallel radiation beams at a differing angle of view with respect to the object of interest **1**. Each of the plurality of parallel ray beam radiation sources **1**-N **35** may be an individual light source, such as a laser, or at least one laser with light routed through one or more optical fibers or optical fiber bundles, as described herein below with respect to FIG. **8**. An outer tube **41** has a plurality of optically flat input surfaces **63** and a plurality of corresponding concave output surfaces **65**, where the plurality of corresponding concave output surfaces **65** cause the radiation emerging from the outer tube **41** to diverge after passing through the object of interest **1**, so as to produce magnified projection images of the object **1**. Alternatively, as described above with reference to FIG. **3**, the post-specimen optic may comprise any magnifying optical element or combination of elements, including lens multiplets or other equivalents.

As in the other examples described herein, an object containing tube **2** is located within the outer tube **41** wherein the object of interest **1** is held within the object containing tube **2**, and a plurality of detector arrays **1**-N **39** are disposed to receive emerging radiation **36**. Each of the plurality of detector arrays **1**-N **39** is located to receive the emerging radiation **36** from one or more of the plurality of concave output surfaces **65**.

FIG. **5**A schematically shows another example illustration of illumination geometry and imaged sample volume with multiple source-magnifying convex optic pairs as contemplated by an embodiment of the present invention. FIG. **5**A is constructed substantially similar to FIG. **5**, with the exceptions that an outer tube **41**A has a plurality of optically flat input surfaces **66** and a plurality of corresponding convex output surfaces **67**, where the plurality of corresponding convex output surfaces **67** focus radiation **68** emerging from the outer tube **41**A after passing through the object of interest **1**. An object containing tube **2** is located within the outer tube **41**A, wherein the object of interest **1** is held within the object containing tube **2**. A plurality of pinhole apertures **127** are located at the respective focal points **69** of the convex output surfaces **67** where each of the plurality of pinhole apertures **127** receives radiation from one of the plurality of corresponding convex output surfaces **67** so as to produce an emergent cone beam **70**.

A plurality of detector arrays **1**-N **39** are disposed to receive the cone beams **70**. Each of the plurality of detector arrays **1**-N **39** is constructed as described hereinabove and located to receive the emerging radiation from one or more of the plurality of pinhole apertures **127**.

Referring to FIG. **6**, there shown is a useful design of a reconstruction cylinder **12**C as contemplated by an embodiment of this invention. Here, a ring of point sources **27** is disposed about the object containing tube **2** and a ring of image sensors **25** is placed in a plane situated above, at or below the plane containing the point sources **27**. While only four point sources and four sensors are shown in the illustration, it will be understood that the rings of sources and image sensors may advantageously comprise a greater number, that being enough to enable tomographic reconstruction of imaged objects. The image sensors can be below or above or in the plane of the point sources. By placing the point sources **27** and image sensors **25** on separate planes, point sources on opposing sides of the cylinder will not physically interfere with other illumination beams. Each of the point sources may advantageously generate a parallel ray beam **135** which may be magnified after passing through the imaged object as described herein above with reference to FIGS. **3**, **4**, **4**A, **5** and **5**A.

During the course of moving through the reconstruction cylinder, the cell **1** passes through at least one photon point source. A central feature of the present invention is that a number of photon point sources **27** of selectable wavelength are disposed around and concentric with the object containing tube. The photon point sources operate in conjunction with opposing CCD, CMOS, TDI or other image sensors **25** that are sensitive to selectable portions of the light spectrum, thus allowing the acquisition of projections **21** of the light transmitted through the cell **1**. In this manner, a set of projection rays **135** can be generated where the projection rays can be described as the straight line connecting the source point to an individual sensing element. The difference between the number of photons leaving the source point along a particular projection ray and the number of photons received at the particular sensing element is related to the number of photons lost or attenuated due to interactions with the cell and other contents of the object containing tube **2** along the projection ray path.

However, complications may arise from light scatter, photon energy shifts, imperfect geometry and poor collimation, and photons from different sources may arrive at a particular sensing element when multiple source points are energized simultaneously. With careful construction of the reconstruction cylinder, for example by judicious choice of the geometry for the pattern of point sources and their opposing detectors as described herein, and by proper timing or multiplexing of activation of the multiple point sources and readout of the sensor arrays, the photon contamination due to these issues can be minimized.

Photon contamination can be partially accounted for by calibration of the system, for example, with no cells present. That is, each light source may be illuminated in turn and its effects on each of the sensors can be measured, thereby providing offset data for use in normalizing the system. An additional calibration step may entail, for example, imaging latex polymer beads or other microspheres or oblate spheroids whose optical properties are known and span the density range of interest for cellular imaging.

Now referring to FIG. **7**, there schematically shown is an example of a flow diagram **50** illustrating the operation of a TDI image sensor. Charge corresponding to an image element of the cell is transferred down a column of pixel elements **51** of the TDI sensor in synchrony with the image. The charge transfer occurs sequentially until the accumulated charge from the column is read out at the bottom register of the sensor **26**.

In one embodiment of the optical tomography system contemplated by the invention, a plurality of TDI sensors **25** are oriented such that each sensor has a direction of line transfer **52** that is parallel to that of cell movement **20** along the z-axis. The TDI image sensor line transfer rate is synchronized to the velocity of the cells by timing or clocking signals from the computer **13**.

The flow diagram of FIG. **7** shows a moving cell **1** and its location with respect to a TDI sensor **25** at various times along a time line **34**. At time=0 the cell **1** is just above the TDI sensor **25** and no image is sensed. At time=1 the cell **1** is partially imaged by the TDI sensor **25**. A shadowgram **51** of the cell **1** is imaged one line at a time. Electrical charges **22** corresponding to each image line are transferred to the next line of sensor pixel elements **23** in synchrony with the movement of that image line down the TDI image sensor from time=0 to time=5. In this way, electrical charge corresponding to each pixel is accumulated down each column **24** of the TDI detector **25** until it is read out at the bottom register **26** at time=5.

The TDI sensors are oriented such that the direction of line transfer **52** is the parallel to that of cell movement **20** along the z-axis. The TDI image sensor line transfer rate is synchronized to the velocity of the cells. Depending on the number of lines or stages in the TDI image sensor, additional photogenerated charge is accumulated and the signal is boosted (e.g., up to 96 fold with a 96 stage TDI sensor such as the Dalsa IL-E2 sensor).

Light Source.

Referring now to FIG. **8**, an example illustration of a parallel ray beam light source as contemplated by an embodiment of the present invention is schematically shown. In this example, the parallel ray beam light source includes a laser **105** coupled to optical fibers **110**. The optical fibers **110** may comprise individual fibers or optical fiber bundles or the equivalent. In operation the plurality of optical fibers **110** receive laser beams **107** and deliver parallel radiation beams **36** to source positions surrounding the flow tube or capillary tube. In this way, the number of lasers needed for multiple light source systems, such as, for example, described with respect to FIG. **5** and FIG. **5**A above, may advantageously be reduced by routing light beams from a single laser through a number of optical fibers. Optical elements such as lenses and/or mirrors may be incorporated at the input or output, or both, of the optical fibers **110**.

In operation, each laser beam diameter may be on the order of one-half to several millimeters, allowing a single laser to couple many optical fibers having openings ranging from about thirty microns to one hundred-micron fibers out of each laser source.

Each source may have the same general characteristics, preferably:

- it may approximate a small circular point source,
- it may be a laser, laser diode or light emitting diode,
- it may be bright with known spectral content,
- the photons emitted from the source may form a beam of a known geometry such as a pencil beam where all photon rays are parallel.

Each source creates data for one projection angle. In an example data collection geometry, a plurality of sources arranged along a helix whose axis is the center axis of the object containing tube creates data from multiple projection angles as the cell moves through the module. Depending on the sensor geometry, several point sources could be disposed about the same circumference with angular separation such that the projections do not overlap at the sensor. The desired number of sources is a function of the needed resolution within each planar reconstruction (the x-y plane) or volumetric reconstruction. Further, the wavelength of the sources is selectable either by use of various diode or other lasers or by bandpass filtering of a white or other broadband source, for example a mercury or xenon arc lamp. There are several options that can be employed to create optical source points, such as:

- a laser or laser diode,
- a laser-fiber bundle combination,
- an aperture in front of a laser or other high intensity photon source,
- an aperture utilizing surface plasmon focusing of photons on both the entry and exit sides of the pinhole,
- an optical fiber with a small cross-section,
- a virtual point source from a short focal length lens in front of a photon source,
- an electron beam that irradiates a point on a phosphor surface (a form of CRT), and
- various combinations of the above.

The geometry using a diverging beam of light is such that, the closer the point source to the object of interest **1** (e.g. a cell), the higher the magnification due to the wider geometric angle that is subtended by an object closer to the source. Magnification in a simple projection system is approximately $M=(A+B)/A$, where A is the distance between the point source and the object (cell) and B is the distance between the object and the detector. Conversely, if the required resolution is known in advance of the system design, then the geometry can be optimized for that particular resolution. For background, those skilled in the art are directed to Blass, M., editor-in-chief, *Handbook of Optics: Fiber Optics and Nonlinear Optics,* 2^{nd} ed., Vol. IV, Mcgraw-Hill, 2001.

Referring now to FIG. **9**, there shown schematically is an example of a reconstruction cylinder **12**E, surrounding flow tube **2** containing flowing objects **1**, such as cells, as contemplated by an embodiment of the present invention. A reconstruction cylinder **12**E includes, for example, a helix **70** including a plurality of parallel ray beam sources **72** disposed at a predetermined helical pitch. Sensing elements **39** are disposed to receive light from the point sources, after it passes through the cell or other object of interest **1** and is magnified by post-specimen optical elements as described above with reference to FIGS. **3**, **4**, **4**A, **5** and **5**A.

While the arrangement of the plurality of parallel ray beam sources **72** is helical, an array of parallel ray beam sources used in a reconstruction cylinder as contemplated by the present invention may take on a wide variety of geometric patterns, depending in part on the speed of the electronics, the cell velocity and the geometry that achieves non-overlapping projection signals at the sensor (detector).

For example, with reference to FIG. **10**, there shown is a reconstruction cylinder **12**F including a series of partial circumferences **74** arranged along a Z-axis through the object containing tube **2**, wherein each partial circumference **74** may contain more than one source-detector pair.

The fixed optical point sources **72**, in conjunction with opposing detectors **39** mounted around a circumference of the tube can sample multiple projection angles through the entire cell as it flows past the sources. By timing of the emission or readout, or both, of the light source and attenuated transmitted and/or scattered and/or emitted light, each detected signal will coincide with a specific, known position along the axis in the z-direction of the flowing cell. In this manner, a cell flowing with known velocity along a known axis perpendicular to a light source that is caused to emit or be detected in a synchronized fashion can be optically sectioned with projections through the cell that can be reconstructed to form a 2D slice in the x-y plane. By stacking or mathematically combining sequential slices, a 3D picture of the cell will emerge. It is also possible to combine the cell motion with the positioning of the light source (or sources) around the flow axis to generate data that can be reconstructed, for example, in a helical manner to create a 3D picture of the cell. Three dimensional reconstruction can be done either by stacking contiguous planar images reconstructed from linear (1D) projections, or from planar (2D) projections directly. The 3D picture of the cell can yield quantitative measures of sub-cellular structures and the location and amount of tagged molecular probes that provide diagnostic information.

Focal Plane and Object Tracking

An optical tomography system for imaging an object of interest is further contemplated by the invention as described herein. The optical tomography system includes a light source for illuminating the object of interest with a plurality of radiation beams, an object containing tube, wherein the object of interest is held within the object containing tube such that it is illuminated by the plurality of radiation beams to produce emerging radiation from the object containing tube. A detector array located to receive the emerging radiation and produce imaging data. Means for tracking the object of interest is coupled to receive and respond to the imaging data.

The image of the object of interest may comprise a projection image or a pseudoprojection image. A pseudoprojection image is typically produced by integrating a series of images from a series of focal planes integrated along an optical axis. The focal planes are preferable arranged back-to-back. The tracking means as described herein may include means for tracking a pseudoprojection image center, means for tracking a projection image center, or means for tracking a focal plane.

Referring now to FIG. **11**, there shown is another example embodiment of the shadowgram optical tomography system of the invention wherein at least one specimen **301** for examination, as for example, a cell or plurality of cells, is processed to remove non-diagnostic elements and is fixed and stained. The specimen **301** is then suspended in a gel medium **302**. The cells in gel mixture are then inserted into a glass micro-capillary tube **304** of approximately 40 μm-60 μm inner diameter. In one implementation, pressure is applied to the gel to move a specimen **301** into the optical path of a high-magnification microscope, represented here by objective **306**. In an alternative embodiment, the tube may be translated relatively to the objective while the specimen remains stationary relatively to the tube.

Once the specimens are in place the tube **304** is rotated to permit capture of a plurality of high resolution images of the desired object taken over a predetermined range of tube rotation. In one useful embodiment about 250 images are obtained over a tube rotation range of 180 degrees. When integrated along the optical axis the images form a pseudoprojection image. The images are typically processed using filtered back projection to yield a 3-D tomographic representation of the specimen. Based on the tomographic reconstruction, features may be computed and used to detect cells with the characteristics of cancer and its precursors. These features are used in a classifier whose output designates the likelihood that object under investigate is a cancer cell. Among other things, good quality reconstruction and classification depends on good focus for all images taken in step three. The present invention provides a method to establish good focus across all pseudoprojections taken during processing as described herein.

Referring now to FIG. **12**A and FIG. **12**B, an end view of a micro-capillary tube **304** is shown with parallel beam illumination and non-parallel beam illumination respectively. In either case, to minimize diffraction of light after it has left an object of interest, such as specimen **301**, it is advantageous to turn the tube so as to minimize the distance between the object and the objective lens, integrated over the duration of an image capture cycle. Thus the image capture must be initiated when the object of interest, specimen **301**, is at position P**1** located within zero axis **310**, where the zero axis **310** runs transverse to and preferably perpendicular to the optical axis of the objective **306**. The object of interest is then rotated as shown by the dashed line indicating the path of travel **312**, and end at position P**2**. Note that in so doing the plane of focus for the system must be varied to correspond to the path of travel **312**.

In one useful embodiment, a focal tracking system incorporated into the optical tomography system and method of the invention and operates to trigger capture of pseudoprojection images when the object center is aligned with the zero axis **310**. The focal tracking system also operates to adjust the focus so that it tracks the object center as it rotates around the tube. Note that the tracking system as described herein may be employed in an optical tomography system that uses any suitable form of illumination or optics, including parallel beam illumination or optics, fan beam, point light sources and other equivalent light sources known to those skilled in the art.

Referring now to FIG. **13**, tracking parameters describing the placement of the object in the tube are schematically shown, including:

R—The Radius from the tube center to the object center.

Θ—Theta, the angular placement of the object relative to the 0 degree axis or angular error value when measured at initiation of image capture. (Image capture is most preferably initiated when Θ is 0, so any other value at initiation of image capture is an indication of angular error.)

Referring now to FIG. **14**, errors are schematically illustrated in a diagram that characterizes the erroneous identification of R, Θ resulting in a misidentification of the plane of focus for the object of interest. Since the object travels on a circular path, image capture should be initiated with R correctly identified to the object center and when the object center is aligned with the zero axis. Errors arise when the object is assumed to be positioned on the zero axis, but is actually offset from the zero axis by Θ. Where Θ is other than zero (0), there is a difference between the true path of travel **314** for the object and the assumed path of travel **316**. In such cases, R is also undervalued as indicated by the relationship $R=R_{true}\cos(\Theta)$. Although at the point when image capture is initiated the object is in focus, if no adjustment is made for the

Θ offset as the object is rotated through 180°, an increasing error develops between the object center and the focal plane assigned by the tracking system.

The plane of focus F for the object may be modeled as:

$$F=F_{tube\ center}-R_{true}\cos(\Theta)\sin(\pi PP/249)\text{ where } PP \text{ is the image number:} \quad \text{Equation 1}$$

PP=0, 1, 2, . . . , 249

This path corresponds to the true and desired path of the object when R is the true value (R_{true}) and Θ=0. This trajectory may be modeled as in eqn. 2.

$$F_{true}=F_{tube\ center}-R_{true}\sin(\pi PP/249) \quad \text{Equation 2}$$

The error in focus F_{error} may be modeled as the difference ($F-F_{true}$) using eqns. 1 & 2.

$$F_{error}=R_{true}\sin(\pi PP/249)(1-\cos(\Theta)) \quad \text{Equation 3}$$

A metric for assessing the overall severity of the focus error may be found by integrating eqn. 3 over all PP.

$$F_{AllError}=(2\pi * R_{true}/249)*(1-\cos(\Theta)) \quad \text{Equation 4}$$

Taking $R_{true}/R_{tube}=R_{ratio}$, the second half of this equation is represented as a contour plot over $-30° \leqq \Theta \leqq 30°$ and $0 \leqq R_{ratio} \leqq 0.8$. This is represented in FIG. **15** and gives a sense for the dependence of $F_{AllError}$ on R_{ratio} and Θ. Note that for the purposes of this example 249 represents the case where 250 pseudoprojection images are acquired. If a different number of pseudoprojection images are acquired the constant 249 must be adjusted accordingly.

Estimation of R, Θ by visual examination is an error prone enterprise since a fairly large Θ error is needed before an appreciable translation of the object is observed. On the other hand it can be difficult to render the distance to the true object center without certainty in Θ. Therefore it is the aim of the present invention to provide a method for

1. estimating R, and
2. establishing a means to trigger image capture so that data is taken as the object center passes through the zero axis **310**.

Referring now to FIG. **16**, a diagram for segmenting the object of interest and computing the center of mass for the grey scale pixels associated with the a pseudoprojection image PP**0** of an object of interest is shown. The first thing needed to estimate R is to find the object center of mass. This is accomplished by segmenting the object of interest and computing the center of mass for the grey scale pixels associated with the object of interest.

1. Threshold: A threshold for the pseudoprojection PP**0** is found by finding the average light level in box region **320**.
2. A connected components algorithm is applied to the thresholded image **322** in order to segment objects where all non-zero pixels are connected. This process yields the labeled image **324**. Note that extraneous non-connected features, as for example feature **323**, have been substantially removed and/or darkened by the threshold and connected components algorithms.
3. The component corresponding to the object of interest is selected based on identifying a pixel **325** in the object of interest.
4. Selection of the object **326** yields a mask that is then applied to the original grey value image. The object center is found by computing the center of mass C_m based on inverted grey values.

In one example embodiment, the average light level is determined by measuring an average light level using a box region including the first 75 pixels from the top left corner moving down 75 pixels and over to the opposite edge. The threshold is set at approximately 85% of the average grey value of the pixels in the box region. Of course the invention is not so limited and those skilled in the art may use equivalent threshold-setting methods.

The step of selecting the object of interest may be based on a user input, for example, a user activating a pixel on a computer display screen or automatically with pattern recognition algorithms or equivalent software algorithms. Once the object of interest has been selected during acquisition of the first pseudoprojection, a window **325** through the capillary tube may be established, where the window is made larger than the object of interest in order to provide a view of the entire object, but not the part of the image containing uninteresting information. Then, during subsequent image acquisitions, it is only necessary to view the object through the window and the selection step can be skipped.

Referring now to FIG. **17**, a graphical representation of the trend of X component of the center of mass from pseudoprojection to pseudoprojection is shown. The center of mass for a single pseudoprojection image is found as according to the method described hereinabove. Computing R and the Θ of the object, at the time image capture is initiated, may be made by analyzing the trend in the X component of the center of mass X_m from pseudoprojection to pseudoprojection. Since the path of movement of the object is circular the translation of the object center with rotation may be described by a cosine function when the movement is viewed from the perspective of the objective lens.

The trend in X_m data may be modeled as X'_m:

$$X'_m=R*\cos(\pi PP(1+\zeta)/249+\pi+\Theta)+34.7+A+B*PP+C*PP^2 \quad \text{Equation 5}$$

In Eqn. 5 the parameters of the model have the significance as shown in Table 1.

TABLE 1

Model Parameter Descriptions

Model Parameter	Description
R	Distance between the micro-capillary tube center and object center
Θ	Angular error
ζ	Controller error. ζ will be a value other than 0 when the controller rotates the object of through some other value than 180°
PP	Pseudoprojection Number: 0,1, . . . ,249
34.7	Half of the pseudoprojection frame height in microns. The micro-capillary tube walls should be centered about this value.
A	The average offset (all PP) of the micro capillary tube around the tube center
B	The linear translation of the micro-capillary tube as it rotates
C	The second order translation of the micro-capillary tube as it rotates.

Focal Track Parameter Solution

The parameters of Table 1 may be solved for by minimizing the RMS error between the X_m and X'_m for all 250 pseudoprojections in accordance with the following equation.

$$\text{Error}=\sqrt{\Sigma(X_m-X'_m)^2/250} \quad \text{Equation 6}$$

In eqn. 6 Boldface X_m is used to represent the ensemble of X_m over all PP. For the case of FIG. **17** a search was done that yielded the following parameters for the model. FIG. **18** shows the close correspondence between measured X_m and modeled X'_m.

TABLE 2

Model Parameter Values	
Model Parameter	Parameter Value
R	18.58μ
Θ	18.48°
ζ	−0.035
A	1μ
B	−0 .004 μ/PP
C	−1.49e-5 μ/PP^2

For this solution a total RMS error of 3.35e-3 was achieved. Note that parameters B and C may be left out of the equation (or set to 0) without substantially affecting the results. Thus, useful implementations of the method of the invention may be carried out without consideration of parameters B and C.

Focal Tracking Implementation

Referring now to FIG. **19**, a focal tracking block diagram of the method of the invention is shown. The analysis of the previous section shows that the parameters for proper focal tracking may be estimated with small error by fitting measured values for X_m with the model of eqn. 5. In the optical tomography system as contemplated by the present invention, when a desired object comes into view it is necessary to find R and to estimate when the object center passes through the zero axis so that image capture for reconstruction may be initiated. A set of k images pp**1**-ppk are collected at step **330** just after the object is identified for capture, where k may be any number of images useful for reconstructing a 3-D image. The set of k images are collected with an initial estimate for R. It is not necessary to collect the set of k images when the object is placed in any specific way since the set of k images will be used to estimate the true value of R and establish the trigger point for collecting the pseudoprojection images to be used for reconstruction. Center of mass values for X components X_{m1}, X_{m2}, X_{m3} . . . X_{mk} are found for the object in pseudoprojections pp**1**-ppk and the time of collection t**1**, t**2**, t**3**, . . . , t_k for each image is also recorded at step **332**. R and the value of Θ at time t_k are computed at step **334**. Based on this data and the clock for PP collection **336** the real time value of Θ is estimated at step **338**. This value is tested for proximity to the value 0 at step **340**. When Θ is anticipated to be 0 on the next clock cycle the trigger for capture of the 250 set of pseudoprojections is enabled at step **350**.

Proper functioning of the controller that rotates the micro capillary tube may be checked for by comparing the value ζ against a criterion. ζ in excess of the criterion initiates a service call and causes data to be discarded.

Parameter A gives the average error for micro-capillary tube centration. This value may be compared against a specification for it. A value of A in excess of the specification stops data collection and alerts the user that the tube needs to be re-centered.

Referring now to FIG. **20** a schematic of capillary tube during rotation is shown for the purpose of illustrating yet another embodiment of the invention. The capillary tube **410** has inner wall **412** with a diameter d and center of rotation **422** which is the center of the capillary tube.

A specimen including an object **414** is held in place with respect to the capillary tube inner wall **412** by a suitable gel **16** or equivalent substance substantially filling the capillary tube **410**. An objective lens **420** is positioned to view the object **414**. While not so limited, the object **414** may comprise for example, a biological specimen including a cell, or more particularly a structure of interest within a cell, such as a cell nucleus stained with absorptive dye.

The object **414** is shown at a plurality of positions P_0, P_n, P_{90}, and P_{180}, where each of the plurality of positions illustrates a position at different times during rotation of the capillary tube **410**. For example, position P_0 represents the position where the center of mass of object **414** is coincident with the focal plane that bisects the capillary tube. Focal plane F_0 may advantageously be located in a plane perpendicular to an optical axis of the objective lens **420**. In contrast, position P_{90} lies in a plane along the optical axis of objective lens **420**, or at an angle of 90° relative to focal plane F_0. The distance h between F_0 and F_n is largest at 90°, where it equals value a. Position P_n corresponds to a position at an angle β_n relative to focal plane F_0. Only the inner walls of the capillary tube are shown. The path of the specimen depends on its distance to the center of rotation **422**.

In one useful example embodiment of the process of the invention for adjusting the focal-plane tracking, the focus is first set to F_0, which is achieved by focusing on the inner tube walls at the section were they are spaced apart the farthest. An optional method for determining F_0 is to find the optical contrast reversal zero-crossing at the inner tube wall. Optimal focus may be achieved by an operator looking through the lens, or, more preferably, by machine vision algorithms locating the sharpest edge within the image while focus is adjusted by computer control. The tube is then rotated until the specimen is at a maximum distance to the center **422** and in the same focal plane F_0 as the tube walls at the widest separation. A centroid of the structure of interest is located and marked with a cursor or using standard machine visions algorithms. A distance of the centroid to the center of rotation is measured using available machine vision tools. Useful machine vision tools may be constructed from languages such as, for example, Labview™ software from National Instruments of Austin, Tex. The measured distance value is used for calculating a change of focal plane (h_n) at a corresponding rotation angle (β_n), using the equation $F_n = F_0 + (a \sin(\beta_n))$. h_n is then converted into a signal which is sent to the piezoelectric objective positioner, that moves the objective in a sinusoidal function according to the translating centroid of the specimen.

For example, if a is measured to be 10 μm, the specimen will move 0.174 μm out of the focal plane during 1° of rotation. At 90°, F_n will be 10 μm apart from F_0, and at 180° F_0 and F_n, will be equal.

Referring now to FIG. **21***a*, FIG. **21***b* and FIG. **21***c* where images of a single squamous cell during imaging in the Optical Projection Tomography Microscope are shown. The cell includes a nucleus nuc, surrounded by cytoplasm cyt within capillary tube wall tw. FIG. **21***a* shows a cell in a starting position where the cell is rotated to a position where its nucleus is in maximum distance to the center of the tube. FIG. **21***b* shows a cell after 90° rotation. The nucleus appears now in the center of the tube. FIG. **21***c* shows a cell where the position of the nucleus is at the end of the 180° rotation cycle. The tube walls tw are shown as horizontal lines above and below the cell in FIG. **21***a* to FIG. **21***c*. The fragmented line symbolizes the center of the capillary tube (tw=tube wall, cyt=cytoplasm, nuc=nucleus).

A method of testing the focal-plane tracking is to bring the object **414** into the starting position P_0. A centroid **415** is marked, and the specimen is then rotated until it is positioned exactly in the middle between the two tube walls, without changing the focus. In one implementation, data acquisition is started before rotation of the capillary tube begins. Upon rotating the tube the object should come into focus at the middle of a cycle. If pseudoprojections are obtained, the object should come into focus at number 125 out of 250 pseudoprojections.

Variables such as direction of object motion and object velocity during rotation can also aid in determining the object radius and angle. Typically an extended depth of field image, as for example, pseudoprojection, obtained by scanning objective lens during single exposure onto camera, is used to create the images because no prior knowledge of object location is required. The maximum depth of field of the image is the inner diameter of the tube.

Since overscanning often leads to loss of resolution and contrast in an extended depth-of-field image, it is advantageous to optimize the depth-of-field extension so that it encompasses the object without significant overscanning. An optimized depth-of-field extension may be determined by measuring the extent of the object. The extent of the object may then be used to minimize the range of scanning to create the extended depth-of-field pseudoprojection image. Using the image data acquired during calibration for object tracking (or additional image data can be acquired if desired), the extent of the object may be determined. By determining the extent of the object along the direction perpendicular to the tube axis for at least two angles, the minimum scan range can be found. The two viewing angles chosen must be 90 degrees apart.

For example, by finding the object extent at the 0 degree position, the minimum scan range is found by rotating the object to the 90 degree position. Likewise, by measuring the object extent when the object is at the 90 degree position, the minimum scan range at 0 and 180 degrees may be determined. Short of taking many images through a minimum rotation of 90 degrees to determine the largest extent of the object, two extended depth-of-field measurements of the object extent may be taken at a first rotation angle θ and a second rotation angle θ+90° and a worst case value for object extent may be calculated according to the relationship:

$$\text{object\_extent}=\sqrt{((\text{extent}_0)^2+(\text{extent}_{\theta+90°})^2)}.$$

Reducing the objective lens scanning range may be required to increase image quality either for calibration accuracy, or for contrast-preservation in pseudoprojection used for 3D reconstruction. In this case the range of scanning is subdivided into parts, and multiple extended depth-of-field images acquired.

In another embodiment of the method of the invention the control signals move the objective lens sinusoidally according to a translating centroid of the object. Where the tube has a rotation cycle, the distance value and a set of angle values may be used to compute a proportional sinusoidal function for objective lens position. The sinusoidal function will have a wavelength proportional to the rotational cycle.

In yet another embodiment of the method of the invention the step of creating a projection image of the object being rotated comprises centering the projection image within a circle of reconstruction during tomographic reconstruction. The sinusoidal function may be modulated by an additional function, such as, for example, a derivative of the sinusoidal function, to further control the scanning of the objective lens to form a pseudoprojection. Using a secondary function, such as a derivative, operates to more precisely preserve higher spatial frequencies and image contrast in resultant images of an object, cell, structure or other items of interest during rotation.

Other variations of method of the invention generally recognize that movement of the focal plane may not be equivalent to movement of the objective lens. More particularly, it is the movement of a focal plane through an object that matters for optical projection tomographic imaging. Since imaging errors could be due to first order spherical aberrations, in one example variation, basic sine wave function focal plane adjustments as described above are pre-distorted with pre-compensation values to correct for axial shifts in best focus across the entire field.

In yet another example, a pre-compensation look-up table for adjusting the focal plane is performed using isolated microspheres located at different regions of the field. In yet another example, a pre-compensation calibration using a specific capillary tube sandwich is performed before scanning each sample. In still another example, a pre-compensation for adjusting the focal plane is performed while the tube is rotating rather than using the static tube in the sandwich to account for eccentricities of the tube. In yet another example, the focal plane is pre-compensation for thickness variations of gel as cell is rotated.

The invention has been described herein in considerable detail in order to comply with the Patent Statutes and to provide those skilled in the art with the information needed to apply the novel principles of the present invention, and to construct and use such exemplary and specialized components as are required. However, it is to be understood that the invention may be carried out by specifically different equipment, and devices and reconstruction algorithms, and that various modifications, both as to the equipment details and operating procedures, may be accomplished without departing from the true spirit and scope of the present invention.

What is claimed is:

1. An optical tomography system comprising:

a light source for illuminating an object of interest with a plurality of radiation beams;

an object containing tube, wherein when the object of interest is held within the object containing tube it is illuminated by the plurality of radiation beams to produce emerging radiation from the object containing tube;

an objective lens, having an optical axis, for scanning the object at a set of viewing angles to generate a set of pseudoprojection images from the emerging radiation, where each pseudoprojection image is produced by integrating a series of images from a series of focal planes integrated along the optical axis for each angle;

a detector array located to receive the set of pseudoprojection images; and

means for tracking the object of interest responsively to the imaging data, wherein the tracking means comprises means for tracking a pseudoprojection image center.

2. The system of claim **1** wherein the tracking means further comprises means for tracking a focal plane.

3. The optical tomography system of claim **1** wherein the plurality of radiation beams comprise a plurality of parallel radiation beams.

* * * * *